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New Analytical Methods For The Determination of Pesticides in Food and Environmental Samples with Gas Chromatography - Mass Spectrometry and Liquid Chromatography - Mass Spectrometry

DISSERTATION

zur Erlangung des Doktorgrades
an der Universität für Bodenkultur Wien

Eingereicht von ***MSc. Céline Lesueur***
Unter der Betreuung von Ao.Univ.-Prof. Dipl.-Ing. Dr. Maria Fürhacker

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Summary

With the worldwide increased foodstuff demand and the detection of the properties of DDT in the mid 1940's, the intensive use of pesticides in the agriculture increased 50-fold within the last 50 years of the 20th century. In contrary to other contaminants, pesticides are deliberately introduced by human beings in their food. Since pesticides, that are intended to prevent, destroy, repel or mitigate any pest, are possibly persistent, accumulate in the food chain and are potentially harmful to humans, their meticulous monitoring and regulation as residues in foodstuff and environmental samples is necessary.

Ideally the detection of pesticides in food and environmental samples should be conducted with multiresidue methods, which allow the detection of several substances simultaneously. Pesticides, especially at trace concentrations, are to be monitored with selective and sensitive devices like gas chromatography (GC) or liquid chromatography (HPLC) coupled to mass selective (MS) detectors but the extraction methods applied until now, like the European Guideline DIN EN 12393 for instances, are quite time-consuming, tedious, solvent-consuming and not adapted for emerging pesticides.

The goals of this study were to simplify, broaden and validate the actual methods for the extraction and determination of pesticides in food and environmental samples with GC-MS and HPLC-MS and the combination of different devices as a thermodesoption system. In another step, a new GC-MS software enabling the simultaneous qualitative and quantitative analyses of more than 100 pesticides was applied to 3,300 fruit and vegetable samples.

The extraction methods used in this work were the DIN EN 12393, based on the German Guideline DFG S19 (extraction with acetone, partitioning with ethylacetate/cyclohexane and a clean-up by gel permeation chromatography), the QuECheRS method (extraction with acetonitrile and a clean-up with the dispersive sorbent Bondesil-PSA), a pressurised liquid extraction (PLE) and a ultrasonic solvent extration (USE) methods (extraction with a water/acetonitrile (1:2, v/v) solution). The QueCheRS method was validated with grape, lemon, onion, tomato and leaf samples and applied to food and environmental matrices. The DIN EN 12393 was used with food and environmental matrices. Soil samples were in addition extracted with PLE and USE methods. The new GC-MS software used permitted the simultaneous full scan and SIM data acquisition and allowed the qualitative and quantitative analysis of more than 100 pesticides within a single run. Moreover, polar pesticides were analysed with ion trap (IT) mass spectrometry detector in

combination with HPLC. Consequently, the validation of the QuECheRS method for the analysis of pesticides in foodstuff and environmental samples with GC-MS and HPLC-MS was carried out according to the SANCO Guidelines. For the simplification of the methods, a thermodesorption system was coupled to a GC-MS for the analysis of apolar and middle polar pesticides. 3,300 conventional and organic farming foodstuff were evaluated in comparison to guidelines and a hit list of contaminated food was compiled.

As results the SANCO Guidelines' requirements for almost all the substances and the matrices (grape, lemon, onion, tomato and leaf) could be met with lower recoveries for the two difficult matrices onion and lemon. All the selected GC-amenable substances showed a linear range in the selected ion monitoring (SIM) mode. Although most of the LC-amenable substances presented a linear range, four substances (furathiocarb, indoxacarb, oxycarboxim, and pyraclostrobin) showed non-linear correlation with quadratic functions The limits of detection (LODs) were ranging from 1 to 400 µg.kg-1 fresh weight and not yet meeting the authorised values of 10 µg.kg-1 fresh weight required for organic farming samples for all the substances.

The analysis of 24 herbicides and insecticides from soil samples showed that the best extraction method was the QuECheRS method (median recovery of 72.7 %) followed by the European Guideline DIN EN 12393 (median recovery of 65.7 %) and the PLE (median recovery of 63.5 %) whereas the USE was less good (median recovery of 57.0 %). Substances like carbendazim, metamitron or monolinuron were recovered from the spiked samples only with some of the extraction methods. Neither the DIN EN 12393 nor the PLE gave sufficient results for polar pesticides and are therefore not applicable for their extraction.

The thermodesorption injection for the analysis of pesticide residues with GC-MS showed a carry-over for the less volatile substances, which could be reduced with a higher ratio of purge flow to split vent but at the same time the LOD decreased.

Finally, the comparison of measured data from 3,300 samples with the DIN EN 12393 indicated that the number of conventional farming samples exceeding LODs or maximum residue levels (MRLs) decreased between 2004 and 2005, but the number of organic farming samples with residues exceeding the assigned value of 10 µg.kg-1 doubled. A significant number of conventional farming samples were found with multiresidue contaminations up to 13 pesticides. It still remains alarming that some samples presented very high contamination exceeding the authorized for MRLs several times.

Keywords: pesticides; gas chromatography; liquid chromatography; mass spectrometry; foodstuff; environmental samples

Zusammenfassung

Mit zunehmenden Bedarf an Lebensmitteln und der Entdeckung der Eigenschaften von DDT in den Mitte 40ern ist die intensive Anwendung von Pestiziden in der Landwirtschaft in den letzten 50 Jahren des 20. Jahrhunderts um das 50-fache angestiegen. Im Gegensatz zu anderen Schadstoffen werden Pestizide bewusst bei der Lebensmittelproduktion eingesetzt. Pestizide werden eingesetzt, um Krankheitserreger zu zerstören und Krankheiten zu verhindern. Aufgrund ihrer möglichen Persistenz und Bioakkumulation ist aber ein konsequentes Monitoring und eine entsprechende gesetzliche Regulierung in Lebensmitteln und Umweltproben notwendig.

Idealerweise sollte der Nachweis von Pflanzenschutzmitteln in Lebensmittel und Umweltproben mithilfe von Multirückstandsmethoden durchgeführt werden, die die Detektion von mehreren Substanzen gleichzeitig erlauben. Speziell bei Spurenkonzentrationen sind Pestizide mittels selektiven und sensitiven Methoden wie Gaschromatografie (GC) oder Flüssigchromatografie (HPLC) gekoppelt mit massenselektiven (MS) Detektoren nachzuweisen.

Die Ziele dieser Arbeit waren die Vereinfachung und Ergänzung bestehender Methoden für die Analyse von Pestiziden in Umwelt- und Lebensmittelproben mittels GC-MS und HPLC-MS. Weiters wurde eine neue GC-MS- Software, die eine simultane Qualifizierung und Quantifizierung von über 100 Pflanzenschutzmitteln erlaubte, an mehr als 3.300 Proben angewendet.

Die in der Arbeit angewendeten Extraktionsmethoden waren die auf der deutschen DFG S19 Guideline basierende DIN EN 12393 (Extraktion mit Aceton, Trennung mit Ethylacetat/Cyclohexan und Clean-Up mit Gel-Permeation-Chromatografie), die QuECheRS-Methode (Extraktion mit Acetonitril und Clean-Up mit dem dispersiven Sorbent Bondesil-PSA), eine beschleunigte Lösungsmittel-Extraktions-Methode (PLE) und eine Ultraschallextraktionsmethode (beide beruhen auf einer Extraktion mit einer Wasser/Acetonitril (1:2, v/v)- Lösung). Die QuECheRS Methode wurde sowohl mit Tomaten-, Trauben-, Zitronen-, Zwiebel- und Blätterproben validiert als auch an Lebensmittel- und Umweltmatrizen angewendet; zusätzlich wurden Bodenproben mit PLE und USE extrahiert. Die neue angewendete GC-MS- Software erlaubte die Full-Scan-simultan zur SIM- Datengewinnung und sorgte für die gleichzeitige Qualifizierung und Quantifizierung von mehr als 100 Pestiziden während eines Laufs. Weiters wurden polare

Pestizide mittels Ionenfallenmassenselektiven („ion trap“) Detektoren in Kombination mit HPLC analysiert. Damit wurde auch die Validierung der QuECheRS- Methode für die Pestizidanalyse in Lebensmitteln und Umweltproben nach den SANCO- Richtlinien möglich. Für die Vereinfachung der Methoden wurde ein Thermodesorptionsverfahren für die GC-MS-Anwendung für apolare und mittelpolare Substanzen adaptiert. 3.300 konventionell und biologisch angebaute Proben wurden analysiert bzw. mit Richtlinien verglichen und eine Liste von Pestizidkontaminierten Lebensmittel wurde erstellt.

Als Ergebnisse könnten die SANCO- Richtlinien für fast alle Substanzen und Matrizen (Trauben, Zitronen, Zwiebeln, Tomaten und Blätter) eingehalten werden, mit geringerer Wiederfindung für die zwei schwierigen Matrizen Zwiebeln und Zitrone. Alle GC- tauglichen Substanzen zeigten eine lineare Korrelation im SIM- Betrieb (R^2>0,99). Obwohl für die meisten der LC- tauglichen Substanzen lineare Korrelationsbereiche ermittelt wurden, wiesen vier Substanzen (Furathiocarb, Indoxacarb, Oxycarboxim und Pyraclostrobin) nichtlineare Korrelationen mit quadratischen Funktionen auf. Die Nachweis- und Bestimmungsgrenzen, die mit GC-MS und HPLC-MS erreicht wurden, lagen zwischen 1 und 400 µg.kg-1 Frischgewicht und die geforderte Nachweisgrenze von 10 µg.kg-1 Frischgewicht für biologischen Anbau konnte noch nicht für alle Substanzen erreicht werden.

Die Analysen von 24 Herbiziden und Insektiziden in Bodenproben zeigten, dass sich die QuECheRS- Methode (Median der Wiederfindung 72,7%) gefolgt von der DIN EN 12393-Methode (Median der Wiederfindung 65,7%) und der PLE- Methode (Median der Wiederfindung 63,5%) als die beste, während die USE- Methode mit einem Median der Wiederfindung von 57,0% als weniger geeignet herausstellte. Substanzen wie Carbendazim, Metamitron oder Monolinuron wurden nur von einigen Extraktionsmethoden ausreichend wiedergefunden. Weder die Extraktion nach DIN EN 12393 noch die PLE ergaben ausreichende Wiederfindungen und stellten sich daher für den Nachweis von polaren Pestiziden als nicht angepasst heraus.

Die Thermodesorption für die Analyse von Pestizidrückständen mit GC-MS zeigte eine Verschleppung für schwachflüchtige Substanzen. Dies konnte durch einen höheren Spülfluss zum Split-Ventil reduziert werden, führte aber zu einer wesentlichen Reduktion der Nachweisgrenze.

Bei Vergleich der Untersuchungsergebnisse von 3.300 Proben mit Grenzwerten wurde eine rückläufige Anzahl von Proben aus konventionellem Anbau mit Nachweisgrenzen- als auch Höchstwertüberschreitung zwischen 2004 und 2005 festgestellt.

Die Anzahl der Proben aus biologischem Anbau mit Grenzwertüberschreitung (10 µg.kg-1)) verdoppelte sich jedoch in dieser Zeit. Bei einer signifikanten Anzahl von Proben aus konventionellem Anbau wurden Mulitrückstände mit bis zu 13 Pestiziden nachgewiesen. Es war alarmierend, wie viele Proben hoch kontaminiert waren und die Grenzwerte mehrfach überschritten haben.

Schlüsselworte: Pflanzenschutzmittel; Gaschromatografie (GC); Flüssigchromatografie (HPLC); Massspectrometrie; Lebensmittel; Umweltproben

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List of abbreviations

Alex: automated liner exchange
DRS: deconvolution reporting software
EU : European Union
GC : gas chromatography
GPC: gel permeation chromatography
HPLC : high performance liquid chromatography
IARC : International Agency for Research on Cancer
IT : ion trap
LLE : liquid liquid extraction
LOD : limit of detection
LOQ : limit of quantification
LSE : liquid solid extraction
m/z: mass to charge ratio
MAE : microwave assisted extraction
MS : mass spectrometry
PAN UK: Pesticide Action Network United Kingdom
PLE: pressurised liquid extraction
QuECheRS : quick easy cheap rugged and safe
R.T.: retention time
S/N: signal to noise ratio
SBSE : stir-bar sorptive extraction
SFE : supercritical fluid extraction
SIM : selected ion monitoring
SPDE : solid phase dynamic extraction
SPE : solid phase extraction
SPME : solid-phase microextraction
SQ : single quadrupole
SRM : selected reaction monitoring
TDAS : thermal desorption system
TIC : total ion chromatogram
TOF : time-of-flight
TPP : triphenylphosphate
TQ : triple quadrupole
USE: ultrasonic solvent extraction

US EPA : American Environmental Protection Agency

WHO : World Health Organisation

1 Introduction

Since the discovery of the high efficient properties and the introduction of DDT in the early 20th century, the worldwide utilisation of pesticides has incredibly increased. The pesticide use, which was already 50,000 tons a year around 1945 [WIMMER, 2004], has raised by approximately a 50-fold to reach a total annual world pesticide use estimated at 2.5 million tons nowadays [PIMENTEL, 1997].

Pesticides are defined by the US Environmental Protection Agency (EPA) [US EPA] as *"any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest"*. Pesticide is a general acronym for insecticide, herbicide, fungicide, acaricide or nematicide. Insecticides kill or prevent the growth of insects. Herbicides control or destroy plants. Fungicides control or destroy fungi. Acaricides control or destroy mites. Nematicides control or destroy nematodes.

In the last years, pesticides became an increasingly issue since some of them are persistent, accumulate in the food chain and contaminate the environment. Moreover, they are potentially harmful to humans as stated by the US EPA [US EPA], the World Health Organisation (WHO) [WHO] and the Pesticide Action Network (PAN) UK [PAN UK]. In 1973, the WHO established a classification of pesticides that distinguishes between the more and the less hazardous forms of each pesticide, which is ever since yearly up-dated [WHO, 2004]. In 1993, the US EPA, in coordination with the International Agency for Research on Cancer (IARC) and the European Union (EU) listed 70 possible carcinogenic pesticides [US EPA, 2006]. In 2005 this list was extended to 160 potential carcinogen pesticides and published in a briefing paper of the PAN UK [PAN UK, 2005]. Furthermore, some pesticides are suspected of being endocrine disruptors. These chemicals affect parts of the hormonal system, and can lead to birth defects, sexual abnormalities and reproductive failure. The EU issued "the implementation of the Community Strategy for Endocrine Disruptors - a range of substances suspected of interfering with the hormone systems of humans and wildlife" [COMMISSION OF THE EUROPEAN COMMUNITY, 1999].

Therefore, the European Community published directives and Maximum Residue Levels (MRLs). The European Directive 91/414/EEC [COMMISSION OF THE EUROPEAN COMMUNITY, 1991a] established the selection criteria for the placing of plant protection products on the market. The European Directive 90/642/EEC [COMMISSION OF THE EUROPEAN COMMUNITY, 1990], which has been implemented under the

Bundesgesetzblatt BGBl. II 441/2002 in Austria, [ÖSTERREICHISCHES BUNDESGESETZBLATT, 2002] establishes MRLs in foodstuff. MRLs are based on the assumption that good agricultural practice is applied in farming i.e. that the product has been used in an appropriate manner and suitable withdrawal periods have been permitted; they are depending on the pesticide and the foodstuff. For water, the European Directive 2000/60/EC [COMMISSION OF THE EUROPEAN COMMUNITY, 2000] is far less complete and sets MRLs only for few pesticides. Nevertheless, soils and plants themselves should also be taken into consideration since pesticides can on the one hand run-off in soil and on the other hand be up-taken directly from the leaves to the fruits or vegetables during the vegetation and florescence time. Although, there are no MRLs available for plants, pesticides authorised for agricultural use in Austria are listed in § 11 and § 12 of the pesticide national law [FEDERAL MINISTRY OF LIFE] where residue concentrations in leaf samples lower than 100 $\mu g.kg^{-1}$ fresh weight are defined as non-used pesticides. Since residues of pesticides may be metabolised in tissues, MRLs are expressed either in terms of parent compound or toxic metabolites.

To minimise the uptake of pesticides, people, who are becoming more and more aware of potential health problems, prefer products of a new way of farming known as “organic farming”. Organic farming is defined in the Directive 2092/91/EEC [COMMISSION OF THE EUROPEAN COMMUNITY, 1991b] and states that *“only products composed of substances mentioned in Annex I and Annex II of the Directive 2092/91/EEC i.e. not chemically synthesised substances may be used as plant protection products, fertilisers or soil conditioners”*. Organic farming is assigned to an absence of non natural pesticides defined in praxis as a pesticide residue concentration lower than 10 $\mu g.kg^{-1}$ product for any synthetic pesticide.

It is obvious that food and environmental samples have to be analysed in order to primarily control the use of pesticides in the agriculture, and, indeed to limit the risk of pesticide residue uptake by humans through the consumption of pesticide-contaminated products, and, secondly to verify the pollution of the environment through pesticides. Consequently, with the monitoring of pesticide residues in foodstuff, soils and plants during the growing time of the cultures, the pesticides used by farmers can be verified and controlled. Finally, an investigation of the pesticide contamination in soil is required since some pesticides are persistent in soil and can consequently be absorbed by the roots of the plants many years after their last use.

2 Background

2.1 Analyses

Concerning the analysis of pesticides in food and environmental samples nowadays mainly two detection methods are used. With the interdiction of organochlorine pesticides and their replacement by more degradable and polar pesticides, a wide class of pesticides with diversified physico-chemical properties, polarities, etc… is to be monitored nowadays. Traditionally apolar and middle polar pesticides are analysed with gas chromatography (GC) [ANDREU et al., 2004; AGÜERA et al., 2004a; ANASTASSIADES et al., 2003a; 2003b; BÖRJESSON et al., 2000; BOUAID et al., 2001; CONCHA-GRAÑA et al., 2006; DABROWSKA et al., 2003; DAGNAC et al., 2005; DFG-METHODE S19; DIN EN 12393 ; GONÇALVES et al., 2005; LAMBROPOULOU et al., 2004; LEANDRO et al., 2005; LEÓN et al., 2003; LESUEUR et al., 2005; 2007a; 2007c; 2007d; LIU et al., 2006; NAVARRO et al., 2000; POPP et al., 1997; RISSATO et al., 2007; SHEN et al., 2003; ŠTAJNBAHER et al., 2003; STAN, 2000; TOR et al., 2006; VAN DER HOFF et al., 1999; WALORCZYK et al., 2006; WIMMER et al., 2005; ZHU et al., 2000], whereas more recent polar pesticides are in most of the cases appraised with high-performance liquid chromatography (HPLC) [ANDREU et al., 2004; AGÜERA et al., 2004b; BELMONTE-VEGA et al., 2005; BLASCO et al., 2003; 2004; 2005; CHAN SEO et al., 2002; DAGNAC et al., 2005; DFG-METHODE 704; EVANS et al., 2000a; 2000b; 2001; FERNÁNDEZ et al., 2001; FERRER et al., 2003; 2005a; 2005b; 2005c; GRANBY et al., 2004; GREULICH et al., 2006; HENRIKSEN et al., 2002; HERNÁNDEZ et al., 2004; 2006; HETHERTON et al., 2004; HOGENBOOM et al., 1996; 1998; 2000; IBÁNEZ et al., 2004; INGELSE et al., 2001; JANSSON et al., JEANNOT et al., 2000; JUAN-GARCÍA et al., 2004; KLEIN et al., 2003; LACASSIE et al., 1999; LESUEUR et al., 2007b; 2007c; 2007d; MARCHESE et al., 2001; MOL et al., 2003; NOGUEIRA et al., 2004; ORTELLI et al., 2004; PAPADAKIS et al., 2002; PICÓ et al., 2000; POZO et al., 2001; SANCHO et al., 2003; 2006; SANNINO et al., 2004; SOLER et al., 2004a; 2004b; 2006; SUN et al., 2003; TAYLOR et al., 2002; THURMAN et al., 2002; 2005a; 2005b; ZAMORA et al., 2004; ZROSTLÍKOVÁ et al., 2002; 2003].

GC, coupled to single quadrupole (SQ) mass spectrometry (MS) detectors [ANASTASSIADES et al., 2003a; 2003b; BÖRJESSON et al., 2000; BOUAID et al., 2001; CONCHA-GRAÑA et al., 2006; DABROWSKA et al., 2003; DFG-METHODE S19; DIN EN 12393 ; GONÇALVES et al., 2005; LAMBROPOULOU et al., 2004; LEÓN et al., 2003; LESUEUR et al., 2005; 2007a; 2007c; 2007d; LIU et al., 2006; NAVARRO et al., 2000; POPP et al., 1997; RISSATO et al., 2007; SHEN et al., 2003; ŠTAJNBAHER et al., 2003;] or

in less extend to triple quadrupole (TQ) MS detectors [AGÜERA et al., 2004a; DAGNAC et al., 2005; LEANDRO et al., 2005; WALORCZYK et al., 2006; WIMMER et al., 2004; 2005; ZHU et al., 2000], has been for a long time the detection of choice for pesticides on the ground of their physico-chemical properties. Up to now, when working with GC-SQ/MS systems, it was common practice to screen the samples without any target list by acquiring a total ion chromatogram (TIC) in full scan mode before repeating the measurement in selected ion monitoring (SIM) mode to quantify the samples. For samples with pesticide residues, this meant a minimum of 2 measurements, which was twice more time- and money-consuming. In 2005, due to a new software capable of acquiring full scan and SIM data in parallel, the development of analytical methods allowing the simultaneous screening and quantification of samples as well as their confirmation through a coupled systematic deconvolution reporting software (DRS) was possible [LESUEUR et al., 2005].

With the emergence of new pesticides and due to their physico-chemical properties making them more amenable for HPLC, a need for new detection methods emerged. Until recently, SQ detectors coupled to HPLC were the most common systems on the market and often applied for the detection of pesticides in food and environmental samples [BELMONTE-VEGA et al., 2005; BLASCO et al., 2003; DAGNAC et al., 2005; FERNÁNDEZ et al., 2001; JUAN-GARCÍA et al., 2004; LACASSIE et al., 1999; MARCHESE et al., 2001; NOGUEIRA et al., 2004; PAPADAKIS et al., 2002; PICÓ et al., 2000; SUN et al., 2003]. These detectors are easy to use, stable and cheap but they do not offer a possibility for confirmation and can support false positive results. More elaborated mass spectrometers like TQ, time-of-flight (TOF) and ion-trap (IT) detectors allow this confirmation since they can practice a tandem spectrometry. TQ detectors were until now the detectors of choice for the quantification of pesticide multiresidues in environmental and food samples due to their stability, sensitivity, selectivity and linearity [AGÜERA et al., 2004b; DAGNAC et al., 2005; DFG-METHODE 704; GRANBY et al., 2004; HENRIKSEN et al., 2002; HERNÁNDEZ et al., 2004; 2006; HETHERTON et al., 2004; HOGENBOOM et al., 1996; 1998; 2000; INGELSE et al., 2001; JANSSON et al., 2004; GREULICH et al., 2006; KLEIN et al., 2003; MOL et al., 2003; ORTELLI et al., 2004; POZO et al., 2001; SANCHO et al., 2003; 2006; SANNINO et al., 2004; SOLER et al., 2004a; TAYLOR et al., 2002; ZAMORA et al., 2004]. These detectors are also expensive and relatively not sensitive in full scan. The other MS detectors on the market were until now principally reputed as confirmation and identification systems because they can measure exact masses (TOF) or operate in MS^n mode (IT) but are less sensitive and linear. TOF has been studied in the recent years as identification tool to discover unknown pesticides in environmental and food samples, as intensively published by the research groups of among others Thurman and Hernández [CHAN SEO et al., 2002;

HERNÁNDEZ et al., 2004; FERRER et al., 2005a; 2005b; 2005c; IBÁNEZ et al., 2004; THURMAN et al., 2002; 2005a; 2005b]. Regarding IT, some works have been published on the detection of up to 14 pesticides in water [HOGENBOOM et al., 1998; JEANNOT et al., 2000; SUN et al., 2003], detection of quaternary ammonium pesticides in foodstuff [EVANS et al., 2000a; 2000b; 2001] and detection of up to 17 pesticides in food samples and especially citrus fruits [BLASCO et al., 2004; 2005; 2006; SOLER et al., 2004a; 2006; ZROSTLÍKOVÁ et al., 2002; 2003]. Still, there are no multiresidue methods available for a wider range of substances.

2.2 Sample preparation

Concerning the sample preparation of pesticides from foodstuff matrices, several extraction techniques have been applied like liquid-liquid extraction (LLE) with acetone [BLASCO et al., 20003; ZAMORA et al., 2004], ethyl acetate [AGÜERA et al., 2004a; BLASCO et al., 2004; FERRER et al., 2005a; ORTELLI et al., 2004; SOLER et al., 2004a; ZROSTLÍKOVÁ et al., 2002], methanol [BELMONTE-VEGA et al., 2005; GRANBY et al., 2004; SANCHO et al., 2006] or acetonitrile [HETHERTON et al., 2004; ZROSTLÍKOVÁ et al., 2003]. Some research groups also tried solid phase extraction (SPE) [BELMONTE-VEGA et al., 2005; NOGUEIRA et al., 2004; HERNÁNDEZ et al., 2004], solid phase dynamic extraction (SPDE) [WIMMER et al., 2005] or stir-bar sorptive extraction (SBSE) [JUAN-GARCIA et al., 2004] as simultaneous extraction/clean-up methods.

Nevertheless, the foodstuff sample preparation and determination of apolar and middle polar pesticides in non fatty products is regulated by the European Guideline DIN EN 12393 part 1 to 3 [DIN EN 12393] adapted from the German Guideline DFG S19 [DFG-METHODE S19] and largely applied [STAN, 2000].

The pesticide measurement with the European Guideline DIN EN 12393 follows a customarily sample preparation after liquid extraction, partitioning and clean-up with gel permeation chromatography (GPC), which entails several steps and serves to the clean-up and the enrichment of the samples. Although well recognised and optimised, this method is still highly tedious, very laborious, environmentally-unfriendly and last but not least time- and money-consuming.

For the determination of polar pesticides in food samples, an extraction method with methanol followed by a clean-up on ChemElut and an analysis with HPLC-TQ/MS has been

developed as draft for a European Guideline [DFG-METHODE 704; GREULICH et al., 2006; KLEIN et al., 2003].

One of the latest successful foodstuff preparation methods for the analysis of pesticide multiresidues in food matrices to our knowledge is the QuECheRS (quick, easy, cheap, rugged and safe) method [ANASTASSIADES et al., 2003a], which can interestingly be combined not only with GC but also with HPLC. It was already applied to GC-MS for the determination of pesticides from fruit and vegetable matrices, which are difficult to detect and quantify [ANASTASSIADES et al., 2003a; 2003b; MASTOVSKA et al., 2004] and to post-harvest fungicides and their metabolites in citrus fruits with HPLC-MS [THURMAN et al., 2005b].

The QuECheRS method principally relies on the extraction of pesticides from the matrices with acetonitrile followed by a salting-out effect with magnesium sulphate ($MgSO_4$) and sodium chloride (NaCl). The actual clean-up step of the matrices is achieved through the addition of a dispersive sorbent in the bulk liquid followed by its take off. The QuECheRS method, on contrary to the European Guideline DIN EN 12393, is very quick, sure, solvent-saving and clean. The problem still remains the fouling of the devices through the limited clean-up of the food matrixes and the high limits of detection (LODs) and limits of quantification (LOQs) in the samples due to the reduced enrichment of the samples. With HPLC-MS/MS, these drawbacks can be by-passed through the higher selectivity and sensitivity of the devices.

The sample preparation methods for the monitoring of multiresidues of pesticides in soil are scarce. This is overall explainable on the ground of the wide physico-chemical property range of the pesticides of interest that implies a comprehensive extraction and furthermore an expensive equipment such as GC-MS and HPLC-MS [ANDREU et al., 2004]. Traditionally, pesticide analyses in soil were prepared with the Soxhlet extraction, which is time- and solvent-consuming and, as a consequence, very expensive and not easy to use [US EPA METHODE 3540]. Due to its drastic conditions, this method is more and more replaced by more environmentally-friendly procedures including shake-flask extraction [BÖRJESSON et al., 2000; DABROWSKA et al., 2003; POZO et al., 2001], pressurised liquid extraction (PLE) [CONCHA-GRAÑA et al., 2006; DAGNAC et al., 2005; HENRIKSEN et al., 2002; LUQUE-GARCÍA et al., 2002; MARCHESE et al., 2001; ZHU et al., 2000], ultrasonic solvent extraction (USE) [BABIĆ et al., 1998; BELMONTE-VEGA et al., 2005; BOUAID et al., 2001; GONÇALVES et al., 2005; LAMBROPOULOU et al., 2004; POPP et al., 1997; TOR et al., 2006], microwave assisted extraction (MAE) [PAPADAKIS et al., 2002; SHEN et al.,

2003; SUN et al., 2003] or supercritical fluid extraction (SFE) [SUN et al., 2003] followed in some cases by a clean-up step with SPE [BELMONTE-VEGA et al., 2005; DABROWSKA et al., 2003, MARCHESE et al., 2001; PAPADAKIS et al., 2002; POZO et al., 2001] or solid-phase microextraction (SPME) [LAMBROPOULOU et al., 2004; SHEN et al., 2003].

Liquid-solid extraction (LSE) for soil sample preparation has the disadvantage of being time-, solvent-consuming and tedious. The new strategies are more environmentally-friendly with advantages like rapidity, automation, selectivity and low consumption of solvents but lack sensitivity and selectivity [ANDREU et al., 2004]. Among them the USE of contaminants from solid samples is becoming more and more favoured. PLE and SFE are sample-volume restricted; in addition the recoveries of polar and/or thermolabile pesticides from soil samples can be critical [LUDVIGSEN et al., 2006]. USE and MAE are reported to improve the extraction efficiency but due to their limited selectivity and simultaneous co-extraction of soil and sediment components together with the target analytes, they often require a further clean-up step [DABROWSKA et al., 2003; GONÇALVES et al., 2005]. Usually, USE are operated with ultrasonic baths [BABIĆ et al., 1998; BELMONTE-VEGA et al., 2005; BOUAID et al., 2001; GONÇALVES et al., 2005; LAMBROPOULOU et al., 2004; POPP et al., 1997; TOR et al., 2006] but a recently developed and more efficient system using a cylindrical ultrasonic probe for the sonication of soil samples was described and applied to the dispersion of soils [MAYER et al., 2002; MENTLER et al., 2004].

2.3 Injection systems

With GC-MS, matrix interferences and dilution effects can be partially short-circuited through an automatic liner exchange (Alex) system [DEUSSING, 2004] or through a direct thermodesorption system (TDAS) [CHROMTECH, 2004]. The Alex system allows the automatic liner exchange of the injector after a given number of injections. The advantage is a gain of time compared to the traditional and up-to-now classical manual exchange of the liner. One drawback is the possible simultaneous introduction of air in the system, which disturbs any GC-MS analysis. In a direct TDAS system, an aliquot of the samples after extraction is absorbed on glass wool in a TDAS vial, evaporated till dryness and fully-automatised brought to the TDAS oven where the substances absorbed on glass wool are thermally desorbed/extracted and injected in the GC-MS. The advantage of a TDAS system is the coupled increase of the injected volume, multiplying the enrichment of the sample, and reduction of the influence of the matrix, whose components stay on the glass wool.

3 Goal of the study

The goal of this study was to simplify and broaden the actual methods for the determination of pesticides in environmental and food samples with GC-MS and HPLC-MS. This was to be achieved by firstly improving the analytical methods i.e. working on device combinations and parameters and by changing the sample preparation.

At first, a new GC-MS software permitting the simultaneous full scan and SIM data acquisition allowed the optimisation of the analysis of apolar and middle polar pesticides with GC-MS within a single run saving time during the analysis of samples and expanding the number of monitored pesticides. The goal was to develop a simultaneous full scan and SIM data acquisition method for more than 100 pesticides in a concentration range between 50 and 10,000 $\mu g.l^{-1}$.

Recent hardware developments on HPLC-MS systems allowed the consideration of an IT/MS detector in combination with HPLC for the analysis of polar pesticides. We aimed to apply and validate the QuECheRS method for the extraction of pesticides from leaf samples and to develop a HPLC-MS multiresidue method for the analysis of around 50 polar pesticides. The linearity, sensitivity, selectivity and accuracy of an IT/MS detector coupled to HPLC for the qualitiative and quantitative analysis of pesticides in foodstuff matrixes were tested.

Consequently, the implementation of the QuEChERS method brought the possibility of analysing apolar, middle polar and polar pesticides in food and environmental samples with GC-MS and HPLC-MS. At this point the goal was to validate the QuECheRS method for the analysis of more than 100 pesticides with GC-MS and around 50 pesticides with HPLC-MS in fruit and vegetable matrices. We tested the methods for their sensitivity, selectivity and accuracy following the SANCO Guidelines' requirements.

Furthermore different sample preparation methods among which the European Guideline DIN EN 12393, the QuECheRS method, a PLE method and an USE method were considered and compared for the analysis of pesticides in soil samples. 24 pesticides were analysed with GC-MS and HPLC-MS in three soils with different properties in order to determine the best extraction method for the analysis of pesticide multiresidues owing very different physico-chemical properties.

Moreover, to recover the sensitivity lost during the extraction of samples, a TDAS system for GC coupled to SQ/MS detector for the analysis of apolar and middle polar pesticides was considered. The goal of this work was to get rid of the matrix interferences and to increase the injection volume in order to reduce the fouling of the GC-MS injector and gain on a concentration factor for the analysis of pesticides with big differences in volatility.

Finally, the results of the analysis of more than 3,300 samples of 2 years of pesticide residue investigations in fruit and vegetable samples in Austria were discussed.

All the experiments could be carried out in the laboratory of the Gartner & LVA Analytik GmbH under the supervision of Dr. Michael Gartner thanks to a funding of the Austrian Research Promotion Agency (FFG) for the project number 910393, „Innovative Rückstandsanalytiklösungen für sichere Lebensmittel“.

Chapter 1 introduces the subject of the study. Chapter 2 deals with the necessary background concerning the up-to-now foodstuff and environmental sample preparation and analyses. Chapter 3 presents the goals of the study. Chapter 4 gathers the material and method part with details concerning the instruments used for the analysis of pesticides, the sample preparation, the selection of pesticides and the validation of the methods used for the analysis of foodstuff and environmental samples. Chapter 5 focuses on the results of the work for the development of a simultaneous full scan and SIM data acquisition method for the analysis of pesticides with GC-MS, the validation of the QuECheRS method for the analysis of pesticides in foodstuff and leaf samples with GC-MS and HPLC-MS, the comparison of four extraction methods for the analysis of pesticides in soil samples with GC-MS and HPLC-MS, the development of an innovative thermodesorption system for the analysis of pesticides with GC-MS and finally the analytical results of 2 years of pesticide investigations in fruit and vegetable samples. Chapter 6 presents the conclusions of the study and Chapter 7 the acknowledgments for the granting of this work. Finally, Chapter 8 is dedicated to the references and the Appendix presents the printed publications related to the present study.

4 Material and methods

4.1 Reagents and chemicals

The pesticides have been selected on the base of their occurrence and relevance as residue in foodstuff and environmental samples as well as according to their analytical feasibility with GC and HPLC. Each analyte was provided either from Sigma-Aldrich, Ehrenstorfer or Riedel-de Haën with the highest available purity.

Single standard stock solutions were prepared dissolving 10 mg of solid standard in 10 ml acetone and acetonitrile for the GC-MS and the HPLC-MS analyses, respectively, and further diluted down to 10 $\mu g.ml^{-1}$. Multicompound standard stock solutions were prepared dissolving 10 mg of each standard in 1000 ml acetone and acetonitrile for the GC-MS and the HPLC-MS analyses, respectively, reaching 10 $\mu g.ml^{-1}$ and further diluted to achieve concentrations of 5 $\mu g.ml^{-1}$, 2 $\mu g.ml^{-1}$, 0.5 $\mu g.ml^{-1}$, 0.2 $\mu g.ml^{-1}$ and 0.05 $\mu g.ml^{-1}$. Matrix-matched standards were prepared dissolving 10 mg of each standard in 1000 ml blank matrix extracted the QuECheRS method reaching 10 $\mu g.ml^{-1}$ and further diluted with the matrix extract to achieve concentrations of 5 $\mu g.ml^{-1}$, 2 $\mu g.ml^{-1}$, 0.5 $\mu g.ml^{-1}$, 0.2 $\mu g.ml^{-1}$ and 0.05 $\mu g.ml^{-1}$. The single and multicompound standards were stored at 4 °C in the dark.

Ultra-residue reagent acetone, ultra-residue reagent ethyl acetate, ultra-residue reagent cyclohexane, ultra-residue reagent acetonitrile, HPLC-MS grade methanol, ultra HPLC-MS grade water and HPLC-MS grade formic acid were purchased from J.T.Baker.

Sodium chloride (NaCl), sodium sulphate anhydrous ($NaSO_4$), magnesium sulphate anhydrous ($MgSO_4$), sodium chloride (NaCl) and sodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$) salts were purchased from J.T.Baker, di-sodium hydrogen citrate sesquihydrate ($C_6H_6Na_2O_7.1.5H_2O$) salt was provided from Fluka and Bondesil-PSA 40 µm was from Varian.

The folded filters were from Whatman and the 0.45 µm PTFE-membrane filters were from Sartorius.

The internal standards aldrin and triphenylphosphate (TPP) were from Riedel-de Haën and Ehrenstorfer, respectively.

4.2 Apparatus

For the European Guideline DIN EN 12393 and the QuECheRS method, the ultrasound baths were a Sonorex super RK 106 and a Sonorex RK 510S from Bandelin. The shaking device was a 3019 from GFL. The rotary evaporators were Rotavapors R 200 from Büchi. The GPC was home-made and composed of an Autosampler G2260A (Agilent Technologies), an Isopump G1310A (Agilent Technologies), an Automatic Sample Collector

G1364A (Agilent Technologies) and a GPC column filled with Bio-Beads S-X3 from Bio-Rad. The centrifuge was a EBA 21 from Hettich. The nitrogen device was a Visidry from Supelco.

The pressurised liquid extractor used for the soil experiments was an ASE 100 from Dionex. In preliminary experiments, the best set up for the extraction with water/acetonitrile (1:2, v/v) was found to be 110 bars and 140 °C during 20 min with 3 PLE cycles. The ultrasonic extraction of soil samples was operated with a Sonoplus HD 2200 from Bandelin equipped with a cylindrical probe US 70 T with a diameter of 12.7 mm. The sonication took place for 2 minutes at 20 kHz; the vibration amplitude was 35 µm; the energy value was 7.8 $J.ml^{-1}$ and the insertion depth 10 mm.

The GC-MS analyses were performed on 3 Hewlett-Packard (Agilent Technologies, Waldbronn) GC-MS Model 6890N Series gas chromatography coupled to 5973N and 5975 mass selective detectors. Details concerning the devices and operating conditions have already been published in Lesueur and Gartner (2005) also presented in **Appendix 1**. The Agilent Chemstation Software G1701DA version D.02.00.237 was used for data analysis. The samples were at first analysed in the full scan mode before quantification in the SIM mode.

The TDAS system was a PAL TDAS 2000 from Chromtech and set at 320°C for 4 min. For the thermal desorption samples, 20 µl of standards were injected on glass wool in thermal desorption vials and evaporated till dryness. The thermal desorption vials were then placed in the autosampler and fully-automatised brought to the injector. The purge flow to split vent varied from 5 to 70 $ml.min^{-1}$ and from at 0 to at 5 min. The column flow was set at 4.5 $ml.min^{-1}$ for 5 min reduced to 3.3 $ml.min^{-1}$ for the rest of the run as explained in **Appendix 5**.

The HPLC-MS analyses were performed on an Agilent Technologies HP-1100 Series (Agilent Technologies, Waldbronn) coupled to an Agilent Technologies mass spectrometer LC/MSD trap XCT Plus (Agilent Technologies, Waldbronn) equipped with an electrospray ionisation (ESI) interface operated in positive mode. Details concerning the devices and operating conditions are to be found in Lesueur et al. (2007b) also presented in **Appendix 3**. The Agilent Technologies LC/MSD trap software 5.3 was used for data analysis. The samples were at first analysed in full scan mode (MS mode) before confirmation in selected reaction monitoring (SRM) mode (MS^2 mode). For that purpose, precursor ions were isolated and fragmented with an amplitude of 0.6 V to produce a first set of fragmentation ions (SRM).

4.3 Sample preparation

Around 500 g of unwashed, unpealed fruit, vegetable or leaf samples were homogenised as purchased with a chopper. 50 g of the mixed samples were extracted according to the European Guideline DIN EN 12393 whereas 10 g of mixed samples were prepared with the QuECheRS method. For soil samples, the weight of the samples was reduced to 25 g with the European Guideline DIN EN 12393.

The conventional European Guideline DIN EN 12393 [DIN EN 12393] consisted of 9 steps: (1) add the needed amount of water to reach 50g in consideration of the natural water amount of the sample and 100 ml of acetone to the sample for the extraction step and extract 15 min with an ultrasound bath and 30 min on a shaking device; (2) filter the solid phase from the liquid phase on a folded filter in a 500 ml separating funnel; (3) add 35 g sodium chloride (NaCl) salt and shake for 3 minutes before addition of 50 ml of ethylacetate/cyclohexane (1:1, v/v) for the partitioning of the water and organic layers; (4) separate and collect the organic phase and add 30 g sodium sulphate anhydrous ($NaSO_4$) salt for its dewatering; (5) filter sodium sulphate salt from the organic layer on a folded filter in a rotary flask; (6) concentrate the organic phase to dryness with rotary evaporator and redissolve it in 10 ml ethylacetate/cyclohexane (1:1, v/v); (7) filter 1.5 ml of the organic phase through a 0.45 µm membrane filter and clean-up by GPC; (8) concentrate the purified organic phase to 1.5 ml with rotary evaporator; (9) evaporate the 1.5 ml to dryness under a gentle stream of nitrogen and redissolve it in 400 µl acetone/ethyl acetate (1:1, v/v) for its analysis with GC-MS. Aldrin, which is forbidden to use since over 20 years, was used as internal standard spiked at the partitioning step to reach a 2 $\mu g.ml^{-1}$-concentration in the final extract. The extraction step provides enriched and purified samples concentrated by a factor 20 through the different extraction and cleaning steps.

The QuECheRS method [ANASTASSIADES et al., 2003a] consisted of 9 steps: (1) add 10 ml acetonitrile (ACN) and shake the sample vigorously for 1 min using a vortex mixer for the extraction step; (2) add 4 g magnesium sulfate anhydrous ($MgSO_4$), 1 g sodium chloride (NaCl), 1g sodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$) and 0.5 g di-sodium hydrogen citrate sesquihydrate ($C_6H_6Na_2O_7.1.5H_2O$) salts and vortex immediately for 1 min for the partitioning; (3) for acidic sample add 600 µl of a 6N NaOH solution to reach a pH value between 5 and 5.5, (4) centrifuge the extracts for 3 min at 5000 rpm; (5) transfer a 6 ml aliquot of the organic phase (upper layer) into a 15 ml teflon centrifuge tube containing 150 mg Bondesil-PSA and 950 mg magnesium sulfate anhydrous ($MgSO_4$) salt as clean-up; (6) centrifuge the extracts for 3 min at 5000 rpm; (7) filter through 0.45 µm filter; (8) transfer 1.5 ml of the extract into 2

autosampler vials containing 15 µl of a 5% formic acid solution for GC-MS and HPLC-MS analyses; (9) for the analysis with GC-MS, evaporate the extract to dryness before dilution in 150 µl acetone/ethylacetate (1:1, v/v).
Triphenylphosphate (TPP) was used as internal standard and spiked before extraction to reach a 1 $\mu g.ml^{-1}$-concentration in the final extract for fruits and vegetables or a 0.5 $\mu g.ml^{-1}$-concentration for leaf and soil samples. For leaf and soil samples, the volume of acetonitrile for the extraction was increased to 20 ml because of the higher volume of the samples.

For the PLE experiments, 5 g of sample was mixed with 1 g silica gel, introduced in a 10 ml steel column and extracted with a water/acetonitrile (1:2, v/v) solution. The collected extract (ca. 40 ml) was evaporated to dryness with rotary evaporator at 40°C, further dissolved in 10 ml acetonitrile/acetone (1:1, v/v), filtrated through 0.45 µm and 1.5 ml were filled in 2 autosampler vials for GC-MS and HPLC-MS analysis. TPP was used as internal standard and spiked before extraction to reach a concentration of 0.5 $\mu g.ml^{-1}$ in the final extract.

USE experiments were carried out with 20 g of sample extracted with 60 ml of a water/acetonitrile (1:2, v/v) solution in a 200 ml glass beaker. The samples were homogenised with a small magnetic stirring bar during the ultrasonic extraction. The extract was filtered through 0.45 µm and 1.8 ml aliquot was evaporated under a gentle stream of nitrogen and collected in 400 µl acetonitrile/acetone (1:1, v/v) for the soil samples and 700 µl acetonitrile/acetone (1:1, v/v) for the sea sand samples, respectively. Triphenylphosphate (TPP) was used as internal standard and spiked before the extraction to reach a concentration of 0.5 $\mu g.ml^{-1}$ in the final extract.

4.4 Blank samples, real samples, proficiency tests and comparison samples

The blank samples used for matrix-matched standards and recovery experiments were uncontaminated samples verified to present pesticide residue below LOD.

As explained in Lesueur et al. (2007a) and in **Appendix 6**, the foodstuff samples were samples purchased on the Austrian market. They represented a total of around 3,300 organic farming and conventional farming fruit and vegetable products for a period of over two years (2004 and 2005) and were analysed according to the European Guideline DIN EN 12393. They were distributed as follows: 1,500 samples in 2004 meaning 600 organic farming products and 900 conventional farming products, and 1,800 samples in 2005 implying 450 organic farming samples and 1,350 conventional farming samples. More than

600 of these samples were extracted according to the European Guideline DIN EN 12393 and the QuECheRS method for the validation of the QuECheRS method as quality control samples.

As published in Lesueur et al. (2007b) and presented in **Appendix 3**, the leaf samples represented a total of 1,400 samples over a period of 3 months from production sites in Austria originating from organic farming (22 %) and conventional farming (88 %). They consisted of apple tree leaves (42 %), grape tree leaves (38 %), cornstalks (9 %), potato plant leaves (7 %), vegetable plant leaves (cabbage, cucumber, pumpkin) (3 %), hop plant leaves (1 %) and strawberry bush leaves (1 %).

As presented in Lesueur et al. (2007c) and **Appendix 2**, nine proficiency tests were analysed with the QuECheRS method during the time of the study: eight for GC-amenable pesticides (one date, two flour, two melon, two tomato and one wine) and one for HPLC-amenable pesticides (lemon) from the FAPAS proficiency institute.

The soil reference materials, as detailed in Lesueur et al. (2007d) and **Appendix 4**, were the European reference material EUROSOIL 7 and its subsoil SO26 from the European Commission Environment Institute, Joint Research Center, ISPRA as well as a sea sand, purified by acid and calcinated, from Merck. These soils have been selected since they represent 24 % of the arable land in Austria [WEISSTEINER et al., 1999].

Proficiency tests and comparison samples were reviewed on the basis of z-score calculation. The tested value (i.e. the QuECheRS result) and the assigned value (i.e. the proficiency test institute average value for proficiency tests and the European Guideline DIN EN 12393 result for comparison samples) were considered as not significantly different as long as their difference was smaller than 2 times the standard deviation (i.e. –2 < z-score < 2). The standard deviation and z-score were calculated as shown in equations 1 to 4.

The standard deviation depends on the assigned value concentration range:

- ✓ for assigned value concentrations < 120 µg.kg^{-1},

$$\sigma_P = \frac{0.22c}{mr} \qquad \text{(equation 1)}$$

- ✓ for assigned value concentrations ≥ 120 µg.kg^{-1} and ≤ 13.8 g.kg^{-1},

$$\sigma_P = \frac{0.22c^{0.8495}}{mr} \qquad \text{(equation 2)}$$

- ✓ for assigned value concentrations > 13.8 g.kg^{-1},

$$\sigma_P = \frac{0.01c^{0.5}}{mr} \qquad \text{(equation 3)}$$

Finally the z-score values could be calculated:

$$z = \frac{(x-c)}{\sigma_P} \qquad \text{(equation 4)}$$

with:

σ_P: target value for standard deviation

z: z-score

c: assigned value (concentration in MS mode)

x: tested value (concentration in MS^2 mode)

mr: dimensionless mass ratio

4.5 Pesticide selection

4.5.1 Pesticides analysed with GC-MS

The European Guideline DIN EN 12393 was validated in the Gartner & LVA Analytik GmbH the laboratory in 2003 for more than 300 apolar and middle polar pesticides. Among this list, 112 pesticides were found in 2004 and 2005 in foodstuff samples and were consequently selected for a statistical study of the results [LESUEUR et al., 2007a] also presented in **Appendix 6**. These were 47 insecticides, 45 fungicides, eight acaricides, eight herbicides, two chitin synthesis inhibitors, one growth retardant inhibitor and one synergist.

As presented in Lesueur et al. (2007c) and in **Appendix 2**, a total of 105 GC-amenable pesticides were selected for the validation of the QuECheRS method with foodstuff representing 44 fungicides, 41 insecticides, nine acaricides, five herbicides, two chitin synthesis inhibitors, one plant growth regulator, one growth retardant inhibitor, one synergist and one pesticide metabolite (of atrazine).

Additionally, twelve GC-amenable pesticides i.e. six insecticides, three herbicides, two pesticide metobiltes (of atrazine) and 1 fungicide were selected for the soil experiments as explained in Lesueur et al. (2007d) and in **Appendix 4**. Nine of the pesticides selected for the soil experiments were already figuring in the pesticide list for the QuECheRS experiments (atrazine-desethyl, chlorfenvinphos, chlopyrifos, chlorpyrifos-methyl,

deltamethrin, dieldrin, lindane, trifluralin and vinclozoline). Additionally atrazine, atrazine-desisopropyl and simazine were monitored.

For the TDAS experiments [LESUEUR et al., 2006] summarised in **Appendix 5**, we selected 46 pesticides made of 39 of the 105 pesticides for the validation of the QuECheRS method and 7 additional pesticides representing 2 insecticides, 3 pesticide metobiltes (of endosulfan) and 2 fungicides (acephate, alpha-endosulfan, beta-endosulfan, captan, endosulfan sulfate, folpet and lambda-cyhalothrin) were selected to match the Fapas Institute proficiency test list.

Consequently, a selection of 116 selected GC-amenable pesticides is presented in **Table 1** together with their retention time (R.T.), target and qualifier ions defined as mass/ to charge ratio (m/z).

Table 1: Pesticides selected for the GC-MS analyses

Analyte	Pesticide class	R.T. (min)	Target ion *(m/z)*	Qualifier ion *(m/z)*	Qualifier ion *(m/z)*	Qualifier ion *(m/z)*
Acepahte	Insecticide	7.9	136	112	142	94
Alpha-endosulfan	Metabolite of Endosulfan	22.8	241	207	195	
Atrazine	Herbicide	13.5	200	215	173	202
Atrazine-desethyl	Metabolite of Atrazine	11.5	172	187	145	174
Atrazine-desisopropyl	Metabolite of Atrazine	11.5	158	173	145	175
Azoxystrobin	Fungicide	37.0	344	388	345	75
Benalaxyl	Fungicide	26.8	148	91	206	234
Beta-endosulfan	Metabolite of Endosulfan	25.6	195	237	241	207
Bifenthrin	Acaricide	28.8	181	165	166	182
Biphenyl	Fungicide	6.9	154	153	152	
Bitertanol	Fungicide	31.6	170	152	141	112
Bromopropylate	Acaricide	29.0	341	339	343	183

Bupirimate Analyte	Fungicide Pesticide class	24.8 R.T. (min)	273 Target ion *(m/z)*	316 Qualifier ion *(m/z)*	208 Qualifier ion *(m/z)*	 Qualifier ion *(m/z)*
Buprofezin	Chitin synthesis inhibitor	24.6	105	172	106	249
Captan	Fungicide	21.9	79	149	80	77
Carbaryl	Acaricide	9.1	144	115	116	145
Carboxin	Fungicide	24.8	143	235		
Chlorfenvinphos	Acaricide	21.4	267	269	323	325
Chlorothalonil	Fungicide	16.1	266	264	268	
Chlorpropham	Plant growth regulator	11.1	127	213	171	153
Chlorpyriphos	Insecticide	19.1	314	197	97	258
Chlorpyriphos methyl	Insecticide	16.5	286	288	323	290
Chlorthaldimethyl	Herbicide	19.3	301	332	223	
Cyfluthrin	Insecticide	32.6	163	165	206	226
Cypermethrin	Insecticide	33.2	181	209	163	
Cyproconazole	Fungicide	25.7	222	224		
Cyprodinil	Fungicide	21.2	224	225		
Cyromazine	Chitin synthesis inhibitor	13.8	151	165	166	
Deltamethrin	Insecticide	36.5	181	253	251	255
Diazinon	Acaricide	13.8	304	276	199	
Dichlorvos	Insecticide	5.5	185	109	220	
Dicloran	Fungicide	13.0	206	176	124	208
Dicofol	Acaricide	19.9	139	111	141	75
Dieldrin	Insecticide	24.0	279	277	237	345
Difenoconazole	Fungicide	35.8	232	265	325	267
Dimethoate	Insecticide	12.6	87	93	125	143
Dimethomorph	Fungicide	37.4	301	303	387	165
Diphenylamine	Acaricide	10.6	169	168	167	170

Analyte	Pesticide class	R.T. (min)	Target ion *(m/z)*	Qualifier ion *(m/z)*	Qualifier ion *(m/z)*	Qualifier ion *(m/z)*
Endosulfan sulfate	Metabolite of Endosulfan	27.0	387	272	237	261
Ethion	Insecticide	25.9	231	153	97	125
Ethoprophos	Insecticide	10.3	158	200	242	
Etofenprox	Insecticide	33.2	163	181	147	165
Fenarimol	Fungicide	31.0	139	219	330	251
Fenbuconazole	Fungicide	32.7	129	198		
Fenhexamid	Fungicide	26.8	97	177	266	301
Fenitrothion	Insecticide	18.1	277	260		
Fenoxycarb	Insecticide	29.1	116	186	88	207
Fenpropathrin	Insecticide	29.1	181	209	265	
Fenvalerate	Insecticide	34.7	167	125	181	225
Fludioxonil	Fungicide	25.2	248	127	154	182
Fluquinconazole	Fungicide	32.0	340	313	286	108
Flusilazole	Fungicide	24.9	233	315	206	
Fluvalinate-tau	Insecticide	34.9	250	252	251	209
Folpet	Fungicide	23.2	260	295	262	
Heptachlor-exo-epoxid	Insecticide	20.7	353	355	351	357
Heptachlor-endo-epoxid	Insecticide	21.0	183	253	289	353
Heptenophos	Insecticide	9.2	124	200	215	
Imazalil	Fungicide	24.3	215	173	217	175
Iprodione	Fungicide	27.0	187	189	244	246
Kresoxim-methyl	Fungicide	24.8	131	282	106	
Lambda-cyhalothrin	Insecticide	30.4	181	197	208	209
Lindane	Insecticide	13.8	219	254	181	
Malathion	Insecticide	18.2	173	158	125	
Mecarbam	Insecticide	21.4	131	97	159	329
Metalaxyl	Fungicide	16.9	206	160	132	249
Metconazole	Fungicide	29.7	125	70	250	319

Methamidophos Analyte	Insecticide Pesticide class	5.7 R.T. (min)	94 Target ion *(m/z)*	95 Qualifier ion *(m/z)*	141 Qualifier ion *(m/z)*	64 Qualifier ion *(m/z)*
Methidathion	Insecticide	22.5	145	85	93	302
Methiocarb	Insecticide	9.5	168	153	109	
Monocrotophos	Insecticide	11.6	127	67	192	97
Myclobutanil	Fungicide	25.1	179	150	206	288
o,p'-DDT	Fungicide	26.0	235	237	165	236
Ofurace	Insecticide	27.0	132	160	232	281
Omethoate	Fungicide	9.8	156	110	109	79
o-phenyphenol	Fungicide	8.8	170	141	115	
Oxadixyl	Insecticide	26.1	163	105	132	278
p,p'-DDD	Insecticide	26.4	235	237	165	236
p,p'-DDE	Insecticide	24.4	246	318	248	316
p,p'-DDT	Insecticide	27.5	235	237	165	236
Paclobutrazol	Growth retardant regulator	23.0	236	125	238	167
Parathion	Insecticide	19.3	291	109	97	235
Parathion-methyl	Insecticide	16.6	263	125	109	79
Penconazole	Fungicide	21.6	248	159	161	250
Pendimethanil	Herbicide	20.9	252	281		
Permethrin	Insecticide	31.7	183	163	165	184
Phosalone	Insecticide	30.1	182	121	184	367
Piperonyl butoxide	Synergist	28.0	176	177	178	
Pirimicarb	Insecticide	15.3	166	72	238	167
Primiphos-methyl	Insecticide	17.9	290	276	305	125
Prochloraz	Fungicide	32.2	180	70	308	310
Procymidone	Fungicide	22.4	96	283	285	67
Propamocarb	Fungicide	6.8	188	58	143	
Propargite	Acaricide	27.8	135	173	355	350
Propiconazole	Fungicide	27.4	259	191	173	
Propyzamide	Herbicide	14.2	173	175	240	255
Pyridaben	Insecticide	32.0	147	364	309	
Pyrimethanil	Fungicide	14.5	198	199	200	

Pyriproxyfen Analyte	Insecticide Pesticide class	30.2 R.T. (min)	136 Target ion *(m/z)*	226 Qualifier ion *(m/z)*	96 Qualifier ion *(m/z)*	 Qualifier ion *(m/z)*
Quinalphos	Insecticide	21.8	146	157	241	298
Quinoxyfen	Fungicide	27.5	237	272	307	238
Quintozene	Fungicide	13.9	295	265	249	
Simazine	Herbicide	13.3	186	201	173	158
Tebuconazole	Fungicide	28.1	125	250	70	207
Tecnazene	Fungicide	10.2	261	215	203	
Terbutryne	Herbicide	18.2	226	185	241	170
Tetradifon	Acaricide	30.0	159	111	356	229
Tetramethrin	Insecticide	28.9	164	123	165	81
Thiabendazole	Fungicide	22.5	201	174		
Tolclofos-methyl	Fungicide	16.8	265	267	125	266
Tolylfluanid	Fungicide	21.2	181	238	137	
Triadimefon	Fungicide	19.5	208	181	128	
Triadimenol	Fungicide	22.3	112	128	168	
Triazophos	Insecticide	26.7	161	162	172	285
Trifloxystrobin	Fungicide	27.1	116	132	186	222
Trifluralin	Herbicide	11.1	306	264	290	307
Vinclozoline	Fungicide	16.8	285	198	189	241

4.5.2 Pesticides analysed with HPLC-MS

We selected a total of 49 HPLC-amenable pesticides representing 24 insecticides, eleven herbicides, six fungicides, five chitin synthesis inhibitor, two mite growth inhibitors and one acaricide for the experiments with foodstuff as explained in Lesueur et al. (2007b) and detailed in **Appendix 3** . After the first experiments, the list was reduced to 46 pesticides as stated in Lesueur et al. (2007b) (**Appendix 3**) since benomyl was easily converted to carbendazim, acephate was thermally labile, pH-labile and very polar and not retained on the column and finally chlorfluazuron could hardly be detected at a 10 µg.ml^{-1} level due to its very high LOD and LOQ in full scan of 10.3 µg.ml^{-1} and 3.09 µg.ml^{-1}, respectively. This resulted in a multiresidue method for 46 pesticides.

Additionally 12 HPLC-amenable substances were thought for the soil experiments as presented in Lesueur et al. (2007d) and in **Appendix 4**. Since ten pesticides were already present in the foodstuff experiments (carbendazim, diuron, flufenoxuron, linuron, metamitron, methabnezthiazuron, metobromuron, metoxuron, monolinuron and pencycuron), only two substances were added: chloroxuron and isoproturon.

Table 2 presents the 51 HPLC-amenable pesticides selected for the experiments as well as their retention time, precursor ion and fragmentation ions.

Table 2: Pesticides selected for the HPLC-MS analyses

Name	Pesticide class	R.T. (min)	Precursor ion *(m/z)*	Fragmentation ion *(m/z)*	Fragmentation ion *(m/z)*
Acephate	Insecticide	0.7	183.8	143.1	
Acetamiprid	Insecticide	8.6	222.9	126.1	196.1
Aldicarb	Insecticide	9.3	212.9	212.9	98.0
Avermectin B1a	Insecticide	16.7	895.5	751.5	607.5
Bendiocarb	Insecticide	9.8	223.9	167.1	109.2
Benomyl	Fungicide	12.5	291.0	192.1	
Butocarboxim	Insecticide	9.3	212.9	212.9	155.8
Carbaryl	Insecticide	10.1	201.9	145.1	
Carbendazim	Fungicide	6.6	191.9	159.9	192.9
Carbofuran	Insecticide	9.8	221.9	165.1	123.2
Chlorfluazuron	Chitin synthesis inhibitor	14.6	539.9	382.9	383.8
Chloroxuron	Herbicide	11.5	291.1	163.9	
Clomazone	Herbicide	11	239.9	124.9	127.9
Clothianidin	Insecticide	8.1	249.9	169.1	168.1
Cyromazine	Chitin synthesis inhibitor	1.4	166.9	125.2	85.4
Demeton-S	Insecticide	11	280.9	253.0	281.1
Dictrotophos	Insecticide	7.8	237.9	193.1	112.3
Diflubenzuron	Chitin synthesis inhibitor	11.7	311.0	157.9	140.9
Dimethoate	Insecticide	8.4	229.8	199.0	
Diuron	Herbicide	10.7	232.9	232.9	72.3
Ethiofencarb	Insecticide	10.3	225.9	168.9	107.0

Name	Pesticide class	R.T. (min)	Precursor ion *(m/z)*	Fragmentation ion *(m/z)*	Fragmentation ion *(m/z)*
Ethirimol	Fungicide	8.8	210.0	210.0	211.0
Fenamiphos	Insecticide	11.8	304.1	262.1	234.1
Fenpyroximate	Acaricide	14.5	422.3	366.3	
Fenthion	Insecticide	12.4	278.9	247.0	169.0
Flufenoxuron	Chitin synthesis inhibitor	13.4	489.1	158.1	141.1
Furathiocarb	Insecticide	13.4	383.2	194.8	251.9
Hexythiazox	Mite growth regulator	13.4	353.1	227.8	270.8
Imazalil	Fungicide	10.26	296.9	255.1	201.0
Imidacloprid	Insecticide	8.01	255.9	209.1	175.1
Indoxacarb	Insecticide	12.5	528.0	496.2	249.1
Isoproturon	Herbicide	10.6	207.1	165.1	72.2
Linuron	Herbicide	11.1	248.9	182.0	160.0
Metamitron	Herbicide	8.4	202.9	202.9	
Methabenzthiazuron	Herbicide	10.7	221.9	164.9	
Metobromuron	Herbicide	10.5	260.9	147.9	171.8
Metoxuron	Herbicide	9.3	228.9	228.9	229.8
Monocrotophos	Insecticide	7.4	223.9	193.0	98.3
Monolinuron	Herbicide	10.3	214.9	214.8	147.9
Omethoate	Insecticide	5.4	213.9	183.0	196.1
Oxycarboxim	Fungicide	8.7	267.9	175.0	120.2
Paclobutrazol	Mite growth regulator	11.3	294.0	294.0	206.9
Pencycuron	Herbicide	11.8	329.1	329.1	124.8
Propamocarb	Herbicide	4.8	188.9	144.2	102.3
Pyraclostrobin	Herbicide	12.2	388.1	194.1	296.1
Rotenone	Insecticide	11.9	395.1	395.1	213.1
Tebufenozide	Insecticide	11.9	297.1	132.9	
Thiabendazole	Fungicide	7.5	201.9	175.0	205.1
Thiamethoxam	Insecticide	7.2	291.9	211.1	210.1
Thiofanox	Insecticide	10.3	240.9	183.8	
Triflumuron	Chitin synthesis inhibitor	12.1	359.2	156.0	139.1

4.5.3 Pesticides analysed with GC-MS and HPLC-MS

Nine thermolabile and/or highly polar pesticides (carbaryl, cyromazine, dimethoate, imazalil, monocrotophos, omethoate, paclobutrazol, propamocarb and thiabendazole) were analysed with both GC-MS and HPLC-MS since they were amenable on both devices with comparable quality. Experiments during this study were meant to conclude as to their consequent analysis with GC-MS or HPLC-MS.

4.6 Validation study

The validation of the QuECheRS method for foodstuff analysis was done based on the European SANCO Guideline [COMMISSION OF THE EUROPEAN COMMUNITIES, 2006] testing the method for sensitivity, recovery and precision.

The reference matrixes selected for the validation of the QuECheRS method with foodstuff were tomato (high water content), lemon (high acid content), grape (high sugar content) and onion (high sulphur content) as recommended by the European SANCO Guideline [COMMISSION OF THE EUROPEAN COMMUNITIES, 2006] as well as plant leaf.

4.6.1 GC-MS

The validation of the QuECheRS method with GC-MS was done with 105 substances in the SIM mode [LESUEUR et al., 2007c] as presented in **Appendix 2**. On the same way, the experiments with the twelve substances in soil samples were carried out in the simultaneous full scan/SIM mode with quantification from the SIM mode [LESUEUR et al., 2007d] as shown in **Appendix 4**.

The linearity was studied for standards in acetone/ethyl acetate. The repeatability within-day and between-days were not tested because SQ/MS detector are known to be unstable within long sequences. As a consequence each sequence was run with three to six standards and only seven samples. The GC-MS were daily up-dated, tuned and relocked. The accuracy expressed as coefficient of variation (%) was determined by analysing six concentration levels between 0.05 and 10 $\mu g.ml^{-1}$ of the standards in the reference matrices.

LODs and LOQs were estimated in the solvent and matrix-matched standards for the ion with a m/z ratio at the highest intensity as the lowest concentration injected that yielded to a signal to noise (S/N) ratio of 3 and 10, respectively.

The recovery experiments were performed with seven replicates of reference matrix blank samples spiked at ten times the LOQ (10*LOQ) i.e. 500 µg.kg^{-1} fresh weight for foodstuff and 1,000 µg.kg^{-1} fresh weight for leaf samples. The spiked samples stood for 30 min before extraction to allow the pesticides to penetrate into the matrix.

4.6.2 HPLC-MS

The validation of the QuECheRS method for foodstuff was done for the 46 substances in the full scan mode and for ten selected substances (i.e. those found in leaf samples) in the SRM mode [LESUEUR et al., 2007b; 2007c] (**Appendix 2** and **Appendix 3**) based on the European SANCO Guideline [COMMISSION OF THE EUROPEAN COMMUNITIES, 2006] testing the method for sensitivity, recovery and precision. Experiments with soil samples were carried out and quantified in the SRM mode [LESUEUR et al., 2007d] (**Appendix 4**).

Linearity was studied for the standards in acetonitrile and in the foodstuff reference matrices by analysing in quintuplicate six concentration levels between 0.05 and 10 µg.ml^{-1}.

LODs and LOQs were estimated in the solvent and matrix-matched standards in the full scan mode for the precursor ion with a m/z at the highest intensity as the lowest concentration injected that yielded to a S/N ratio of 3 and 10, respectively. Additionally, LODs and LOQs were estimated in the SRM mode for the leaf and soil matrices as explained for the full scan mode but with the fragmentation ion presenting the highest response.

The recovery experiments were performed with seven replicates of reference matrix blank samples spiked at the LOQ (i.e. 50 µg.kg^{-1} fresh weight for foodstuff and 100 µg.kg^{-1} fresh weight for leaf samples) and ten times the LOQ (10*LOQ) (i.e. 500 µg.kg^{-1} fresh weight for foodstuff and 1,000 µg.kg^{-1} fresh weight for leaf samples). The spiked samples stood for 30 min before extraction to allow the pesticides to penetrate into the matrix.

Repeatability within-day, repeatability between-days and accuracy expressed as coefficient of variation (%) were determined by analysing in quintuplicate six concentration levels between 0.05 and 10 µg.ml^{-1} of the standards in the reference matrices.

5 Results and discussion

5.1 Simultaneous fullscan and SIM data acquisition method for GC-MS measurements

The first consideration was to reduce the analysis time and costs with GC-MS by adapting the software developed by Agilent Technologies in 2005 capable of qualification, quantification and confirmation through a coupled systematic DRS within a single run. This was applied to the most encountered GC-amenable pesticides in the Gartner & LVA Analytik GmbH laboratory for the monitoring of 113 pesticides in the full scan mode and quantification of 68 pesticides in the SIM mode extracted with the European Guideline DIN EN 12393 as published in Lesueur and Gartner (2005) and presented in **Appendix 1**.

As explained in this study, we obtained a linearity of the calibration curves in the SIM mode over 2 orders of magnitude (LOQ-10 $\mu g.ml^{-1}$) with correlation factor in any case higher than 0.99. Furthermore, in full scan mode, LODs between 0.005 and 0.62 $\mu g.ml^{-1}$ injection volume and LOQs between 0.015 and 2.06 $\mu g.ml^{-1}$ injection volume were achieved. This corresponds in samples extracted according to the European Guideline DIN EN 12393 to LODs and LOQs between 0.02 and 31 $\mu g.kg^{-1}$ fresh weight product and between 0.8 and 10 $\mu g.kg^{-1}$ fresh weight product, respectively. Although relatively high, the LOQs in full scan were relatively unimportant since the quantification was achieved in the SIM mode. Important in full scan mode were the LODs, which should be, in the best case, below the assigned maximum value of 10 $\mu g.kg^{-1}$ fresh weight product for organic farming products. For six substances, this limit was not achieved but closely approched (alpha endosulfan, LOD = 12 $\mu g.kg^{-1}$, beta endosulfan LOD = 15 $\mu g.kg^{-1}$, cyfluthrin, LOD = 31 $\mu g.kg^{-1}$, cypermethrin, LOD = 18 $\mu g.kg^{-1}$, diphenylamine, LOD = 18 $\mu g.kg^{-1}$, fenhexamid, LOD = 14 $\mu g.kg^{-1}$). As reported in numerous studies, these substances are thermolabile and/or very polar implying a difficult detection with GC-MS.

We reached with the present method LODs in SIM mode as low as 0.0004 $\mu g.ml^{-1}$ injection volume and LOQs as low as 0.001 $\mu g.ml^{-1}$ injection volume for pyrimethanil. The highest LOD and LOQ values in SIM mode were obtained for fenvalerate and reached 0.089 and 0.30 $\mu g.ml^{-1}$ injection volume, respectively. This corresponds for samples extracted with the European Guideline DIN EN 12393 to a maximal LOD of 4 $\mu g.kg^{-1}$ fresh weight product i.e. a value below the organic farming assigned value of 10 $\mu g.kg^{-1}$ fresh weight product. Since only three substances had a LOQ in sample higher than 10 $\mu g.kg^{-1}$ fresh weight product and highly approching this threshold (fenvalerate: LOQ = 15 $\mu g.kg^{-1}$, fluvalinate-tau: LOQ = 14

$\mu g.kg^{-1}$, mecarbam: LOQ = 14 $\mu g.kg^{-1}$), it could be considered that the method allowed a quantification of the selected substances in conventional farming and organic farming products.

More details about the linearity of the simultaneous full scan/SIM mode method, the LODs and LOQs are available in Lesueur and Gartner (2005) and in **Appendix 1**.

Finally, each simultaneous full scan and SIM acquisition was verified with a DRS as shown in Lesueur and Gartner (2005) (**Appendix 1**) for a potato sample. The pesticides proposed by the DRS are confirmed or denied with the full scan monitoring and the AMDIS and NIST database match of the DRS. Practically, the total ion chromatogram (TIC) is used by the DRS for library search, the full scan is required for the qualification of the sample for the selected pesticides and the SIM data are necessary for the quantification of the analytes in the sample.

5.2 Validation of the QuECheRS method for the measurement of apolar and middle polar pesticides with GC-MS and polar pesticides with HPLC-MS/MS in foodstuff and leaf samples

We validated the QuECheRS method for the analysis of apolar and middle polar pesticides with GC-MS in foodstuff [LESUEUR et al., 2007c] and leaf samples as well as to polar pesticides with HPLC-MS in foodstuff [LESUEUR et al., 2007c] and leaf samples [LESUEUR et al., 2007b] as shown in **Appendix 2** and **Appendix 3**.

As published in these studies, with one single extraction, we successfully developed a multiresidue method for the qualitative and quantitative analysis of 105 pesticides with GC-MS equipped with electron impact ionization and for 46 pesticides with HPLC-IT/MS equipped with an electrospray ionization in positive mode.

5.2.1 Linearity of the method

Based on the simultaneous full scan and SIM mode acquisition presented in Lesueur and Gartner (2005) (**Appendix 1**), two simultaneous full scan/SIM mode methods (with each 50 and 55 analytes in the SIM mode) were developed for the analysis of 105 pesticides in foodstuff and leaf samples. In LESUEUR et al. (2007c) (**Appendix 2**), it was shown that the 105 GC-amenable substances were linear in the SIM mode over a 0.050-10 $\mu g.ml^{-1}$ concentration range with correlation factors R^2 higher than 0.99 for the standards in acetonitrile.

As stated in Lesueur et al. (2007b; 2007c) (**Appendix 2** and **Appendix 3**), most of the 46 HPLC-amenable substances were linear over a range between 0.05-5 $\mu g.ml^{-1}$ or 0.05-2 $\mu g.ml^{-1}$ with correlation factors R^2 higher than 0.99. Avermectin B1a, which had a higher LOQ, was linear between 0.2 and 10 $\mu g.ml^{-1}$ with R^2 higher than 0.99. Four substances (furathiocarb, indoxacarb, oxycarboxim and pyraclostrobin) did not show any linear range but quadratic functions with correlation factor R^2 higher than 0.99. It could be distinguished between the matrices with a trend to limited influence (lower than 10 %) on the response like lemon and tomato and the matrices with a high influence. Onion for instances tended to a signal suppression with most of the slope ratios lower than 0.9 whereas grape showed a trend to signal enhancement with most of the substances with a slope ratio higher than 1.1. For the substances with a high reactivity to matrix influence (like propamocarb), the use of matrix-matched standards is naturally recommended.

5.2.2 LODs and LOQs for GC-MS and HPLC-MS/MS

As explained in both studies, LODs and LOQs were always expressed in terms of $\mu g.kg^{-1}$ product (fresh weight). **Table 3** and **Table 4** present the achieved LOQs in food and leaf samples for the selected 105 GC-amenable in SIM mode and 46 HPLC-amenable substances in full scan, respectively. The LOQs were obtained from the LODs by multiplying the values by a factor 3.

Table 3: LOQs for GC-amenable analytes in SIM mode

Substances	Grape LOQ ($\mu g.kg^{-1}$)	Lemon LOQ ($\mu g.kg^{-1}$)	Onion LOQ ($\mu g.kg^{-1}$)	Tomato LOQ ($\mu g.kg^{-1}$)	Leaf LOQ ($\mu g.kg^{-1}$)
Atrazin desethyl	19	29	14	11	79
Azoxystrobin	22	7.6	26	26	26
Benalaxyl	11	19	11	21	14
Bifenthrin	8.3	14	6.8	7.2	9.4
Biphenyl	28	6.4	14	34	38
Bitertanol	54	8.4	10	4.5	71
Bromopropylate	27	18	15	11	66
Bupirimate	24	21	20	32	29
Buprofezin	18	38	31	15	62
Carbaryl	48	34	36	22	12
Carboxin	63	16	33	11	67

Substances	Grape LOQ ($\mu g.kg^{-1}$)	Lemon LOQ ($\mu g.kg^{-1}$)	Onion LOQ ($\mu g.kg^{-1}$)	Tomato LOQ ($\mu g.kg^{-1}$)	Leaf LOQ ($\mu g.kg^{-1}$)
Chlorfenvinphos	39	21	19	31	75
Chlorothalonil	5.8	16	8.8	11	41
Chlorpropham	50	35	17	26	29
Chlorpyrifos	9.2	20	8.6	11	31
Chlorpyrifos-methyl	46	12	7.3	20	7.6
Chlorthaldimethyl	16	10	10	29	19
Cyfluthrin	134	109	61	30	65
Cypermethrin	34	72	66	111	100
Cyproconazole	35	17	19	10	48
Cyprodinil	43	33	5.7	3.3	14
Cyromazine	29	35	40	24	71
Deltamethrin	40	48	31	49	78
Diazinon	18	16	7.2	54	35
Dichlorvos	24	12	17	28	89
Dicloran	8.4	19	23	7.8	36
Dicofol	9.6	29	44	28	54
Dieldrin	18	30	30	28	77
Difenoconazole	38	24	25	12	35
Dimethoate	60	16	34	13	61
Dimethomorph	31	31	35	22	53
Diphenylamine	40	8.0	3.7	11	39
Ethion	19	14	11	8.5	30
Ethoprophos	40	6.7	44	12	10
Fenarimol	62	31	26	51	38
Fenbuconazole	80	17	61	22	61
Fenhexamid	46	37	43	40	77
Fenithrothion	57	37	28	31	45
Fenoxycarb	35	31	35	25	33
Fenpropathrin	24	11	22	18	65
Fenvalerate	42	9.5	18	18	99
Fludioxonil	89	6.2	13	56	42
Fluquinconazole	40	19	8.7	19	52
Flusilazole	15	29	9.5	47	34

Substances	Grape LOQ ($\mu g.kg^{-1}$)	Lemon LOQ ($\mu g.kg^{-1}$)	Onion LOQ ($\mu g.kg^{-1}$)	Tomato LOQ ($\mu g.kg^{-1}$)	Leaf LOQ ($\mu g.kg^{-1}$)
Fluvalinate-tau	7.0	3.4	7.2	11	72
Heptachlor-exo-epoxid	31	41	38	49	45
Heptaclor-endo-epoxid	1.2	37	6.3	23	52
Heptenophos	3.3	16	30	3.4	36
Imazalil	99	33	8.5	31	48
Iprodione	46	32	35	32	62
Kresoxim-methyl	46	26	58	21	32
Lindane	4.1	32	10	16	81
Malathion	18	25	6.6	17	66
Mecarbam	34	50	57	18	74
Metalaxyl	39	22	13	26	35
Metconazole	4.3	9.5	29	3.6	59
Methamidophos	44	55	63	70	64
Methidathion	88	19	24	72	81
Methiocarb	36	9.3	36	6.5	37
Monocrotophos	161	56	60	35	61
Myclobutanil	56	15	18	19	31
o,p'-DDT	15	21	23	6.2	43
Ofurace	27	37	67	13	71
Omethoate	41	30	42	20	45
o-phenylphenol	52	16	10	19	31
Oxadixyl	15	38	30	32	24
p,p'-DDD	57	33	12	31	45
p,p'-DDE	4.5	35	5.7	17	15
p,p'-DDT	14	49	28	33	56
Paclobutrazol	9.3	14	42	14	68
Parathion	16	36	15	9.7	70
Parathion-methyl	5.1	36	8.8	15	76
Penconazole	35	47	25	5.0	54
Pendimethalin	8.6	13	12	20	46
Permethrin	10	12	17	7.1	37
Phosalone	87	13	19	34	88
Piperonyl butoxide	18	13	7.7	5.3	19

Substances	Grape LOQ ($\mu g.kg^{-1}$)	Lemon LOQ ($\mu g.kg^{-1}$)	Onion LOQ ($\mu g.kg^{-1}$)	Tomato LOQ ($\mu g.kg^{-1}$)	Leaf LOQ ($\mu g.kg^{-1}$)
Pirimicarb	29	14	6.7	24	17
Pirimiphos-methyl	29	11	26	27	19
Prochloraz	41	53	27	35	41
Procymidone	24	18	18	17	27
Propamocarb	39	38	67	28	45
Propargite	36	49	66	49	85
Propiconazole	11	27	31	7.1	52
Propyzamide	28	34	18	15	20
Pyridaben	44	47	12	40	22
Pyrimethanil	28	13	8.5	32	21
Pyriproxyfen	80	4.9	38	14	25
Quinalphos	49	22	29	30	70
Quinoxyfen	28	11	29	12	17
Quintozene	34	23	39	13	24
Tebuconazole	35	38	29	30	89
Tecnazene	19	25	20	28	70
Terbutryne	16	5.4	6.0	24	29
Tetradifon	58	59	28	26	71
Tetramethrin	16	6.1	19	9.5	48
Thiabendazole	74	42	48	22	35
Tolclofos-methyl	16	28	14	21	68
Tolylfluanid	46	46	50	37	82
Triadimefon	13	8.9	11	13	64
Triadimenol	32	8.5	24	15	56
Triazophos	11	12	19	6.6	58
Trifloxystrobin	15	33	41	22	46
Trifluralin	8.6	45	8.0	13	45
Vinclozoline	47	15	10	14	20

Table 3 pinpoints that the LODs and LOQs with GC-MS in SIM mode for foodstuff were between 0.4 and 48.2 $\mu g.kg^{-1}$ and 1.2 and 161 $\mu g.kg^{-1}$, respectively. Less than 20 % of the substances presented LOQs lower than the organic farming pesticide residue threshold of 10 $\mu g.kg^{-1}$ whereas between 50 and 85 % of the LODs, depending on the matrix, were below

this limit [LESUEUR et al, 2007c] (**Appendix 2**). Concerning the leaf samples, the LODs and LOQs for GC-amenable substances varied between 2.3 and 29.9 $\mu g.kg^{-1}$, and between 7.6 and 99.5 $\mu g.kg^{-1}$, repectively. As already mentioned for leaf samples, it is important to state whether a pesticide has been applied during the florescence, which is defined as a residue concentration lower than 100 $\mu g.kg^{-1}$. Although the detection and quantification limits for leaf samples were much higher than for foodstuff, partly explainable by a lower concentration factor, all the substances were satisfying the 100 $\mu g.kg^{-1}$ criterium for leaf samples.

Concerning the HPLC-amenable substances, the first commitment was the reduction of the method from 49 to 46 substances as detailed elsewhere [LESUEUR et al, 2007b] (**Appendix 3**). Chlorfluazuron could hardly be detected at a 10 $\mu g.ml^{-1}$ (20 000 $\mu g.kg^{-1}$) level (LOD and LOQ in full scan 6 180 $\mu g.kg^{-1}$ and 20 600 $\mu g.kg^{-1}$, respectively), which was far off the authorised MRLs, benomyl was rapidly converted to carbendazim [BERNAL et al., 1997; MALLAT et al., 1997] and acephate, which is thermally labile, pH-labile and very polar, was not retained on the column (R.T.: 0.7 min) and rapidly undetectable in the standard solution. Indeed, these three substances were removed from the present method that finally resulted in a multiresidue method for 46 pesticides.

Table 4 presents the achieved LOQs in food and leaf samples for the selected 46 HPLC-amenable substances in full scan.

Table 4: LOQs for HPLC-amenable analytes in full scan mode

Substances	Grape LOQ ($\mu g.kg^{-1}$)	Lemon LOQ ($\mu g.kg^{-1}$)	Onion LOQ ($\mu g.kg^{-1}$)	Tomato LOQ ($\mu g.kg^{-1}$)	Leaf LOQ ($\mu g.kg^{-1}$)
Acetamiprid	31	31	31	37	55
Aldicarb	14	25	14	0	55
Avermectin B1a	119	172	175	171	395
Bendiocarb	13	51	22	21	66
Butocarboxim	12	24	12	18	48
Carbaryl	18	58	27	33	92
Carbendazim	17	40	20	24	43
Carbofuran	16	26	20	15	24
Clomazone	15	30	31	16	39
Clothianidin	44	30	41	51	45
Cyromazine	22	14	22	19	28

Substances	Grape LOQ ($\mu g.kg^{-1}$)	Lemon LOQ ($\mu g.kg^{-1}$)	Onion LOQ ($\mu g.kg^{-1}$)	Tomato LOQ ($\mu g.kg^{-1}$)	Leaf LOQ ($\mu g.kg^{-1}$)
Demeton-S	9.7	28	30	19	41
Dictrotophos	39	49	36	39	63
Diflubenzuron	21	37	35	30	73
Dimethoate	40	34	35	45	93
Diuron	29	31	27	24	59
Ethiofencarb	22	46	39	37	60
Ethirimol	21	18	21	15	17
Fenamiphos	13	22	23	12	31
Fenpyroximate	10	14	11	14	31
Fenthion	42	36	52	22	128
Flufenoxuron	63	39	24	53	165
Furathiocarb	3.3	7.8	8.5	4.6	26
Hexythiazox	39	37	24	33	105
Imazalil	5.8	8.9	10	5.0	9.2
Imidacloprid	51	20	36	41	103
Indoxacarb	52	30	49	52	244
Linuron	36	28	40	23.	85
Metamitron	18	31	19	22	36
Methabenzthiazuron	9.7	20	19	11	31
Metobromuron	31	30	39	25	64
Metoxuron	13	33	14	16	36
Monocrotophos	57	134	24	74	312
Monolinuron	22	33	32	16	41
Omethoate	140	382	141	195	390
Oxycarboxim	16	30	17	19	42
Paclobutrazol	5.9	12	10	5.8	15
Pencycuron	13	22	18	17	43
Propamocarb	19	25	29	27	58
Pyraclostrobin	8.2	16	18	15	22
Rotenone	32	35.	48	22	56
Tebufenozide	8.2	22	15	16	26
Thiabendazole	27	33	28	31	47
Thiamethoxam	116	222	108	152	262

Substances	Grape LOQ ($\mu g.kg^{-1}$)	Lemon LOQ ($\mu g.kg^{-1}$)	Onion LOQ ($\mu g.kg^{-1}$)	Tomato LOQ ($\mu g.kg^{-1}$)	Leaf LOQ ($\mu g.kg^{-1}$)
Thiofanox	12	39	17	25	59
Triflumuron	22	30	20	38	4.8

As presented in **Table 4** with HPLC-MS in full scan mode, LODs and LOQs in foodstuff between 1.0 and 115 $\mu g.kg^{-1}$ and 3.3 and 382 $\mu g.kg^{-1}$ were achieved. Furathiocarb showed the lowest LOD/LOQ independent of the matrix and omethoate and avermectin B1a the highest ones, respectively. The 10 $\mu g.kg^{-1}$ threshold was in most cases exceeded for the LOQs whereas it was respected for the LODs for 67 to 79 % of the substances, depending on the matrix. The lowest LOD and LOQ achieved with leaf samples were as low as 1.4 $\mu g.kg^{-1}$ and 4.8 $\mu g.kg^{-1}$, respectively in the full scan mode. The LODs and LOQs for leaf samples were higher than for foodstuff for the same reason as for GC-amenable substances i.e. lower concentration factor. In full scan mode, 44 of the 46 substances showed LODs lower than 100 $\mu g.kg^{-1}$ i.e. satisfying the required 100 $\mu g.kg^{-1}$ fresh leaves for organic farming. Only two substances (avermectin B1a and omethoate) gave LODs higher than 100 $\mu g.kg^{-1}$. On the same way, 37 substances presented LOQ below 100 $\mu g.kg^{-1}$ and nine substances between 100 and 400 $\mu g.kg^{-1}$.

For leaf samples, LODs and LOQs were estimated in the SRM mode as presented in Lesueur et al. (2007b) and in **Appendix 3**. In this mode, LODs and LOQs were lower and 45 of the 46 substances presented LODs lower than 30 $\mu g.kg^{-1}$. Seemingly, the LOQs in the SRM mode were below 100 $\mu g.kg^{-1}$ for these same substances. Only avermectin B1a was illustrated with higher values (LOD: 63 $\mu g.kg^{-1}$; LOQ: 210 $\mu g.kg^{-1}$).

Concerning the nine substances which were analysed with GC-MS and HPLC-MS, it was pinpointed that carbaryl, cyromazine, dimethoate and monocrotophos presented similar LODs/LOQs on both devices, omethoate presented definitely far better LODs/LOQs with GC-MS and the remaining imazalil, paclobutrazol, propamocarb and thiabendazole were more sensitively analysed with HPLC-MS.

Štajnbaher et al. (2003) worked with GC-SQ/MS and reported LOQs between 10 and 20 $\mu g.kg^{-1}$ for 90 GC-amenable pesticides after extraction with ethyl acetate with SPE and clean-up with diethylaminopropyl. After a simple extraction with ethyl acetate and analysis with GC-TQ/MS, Martínez Vidal et al. (2006) reported LODs between 0.01 and 3.21 $\mu g.kg^{-1}$ and LOQs between 0.04 and 9.64 $\mu g.kg^{-1}$ in cucumber for 130 pesticides. Leandro et al.

(2005) carried out experiments with baby food extracted with the QuECheRS method and analysed with large injection volume GC-TQ/MS and could achieve, with this apparatus and an injection volume of 8 µl, LOQs down to 1-3 $\mu g.kg^{-1}$. Seemingly, with the QuECheRS method followed by analysis with GC-TOF/MS, Díez et al. (2006) achieved LODs between 1.1 and 2.3 $\mu g.kg^{-1}$ in barley.

The reported LODs and LOQs in the literature for HPLC-IT/MS detectors are also varying a lot depending on the tediousness of the preparation method. Blasco's as well as Soler's research groups have been intensively working on the detection of pesticides in foodstuff with HPLC-IT/MS and reported LOQs from a few $\mu g.kg^{-1}$ to the higher $\mu g.kg^{-1}$ range: after a quite simple extraction with ethyl acetate, Soler et al. (2004a) achieved LODs of 0.5-20 $\mu g.kg^{-1}$ for six pesticides in oranges with HPLC-IT/MS, comparable to those they obtained with HPLC-TQ/MS; with the same extraction, they presented LOQs in the range 10-400 $\mu g.kg^{-1}$ for nine pesticides in oranges and strawberries [SOLER et al., 2004b]; Blasco et al. (2004) studied the quantification of six pesticides in oranges in the MS, MS^2 and MS^3 modes and reported LOQs between 0.5 and 10 $\mu g.kg^{-1}$ in MS mode, between 1 and 200 $\mu g.kg^{-1}$ in MS^2 mode and between 1 and 300 $\mu g.kg^{-1}$ in MS^3 mode. The extraction of pesticides from foodstuff with PLE combined to their analysis with HPLC-IT/MS resulted in LOQs from 10 to 90 $\mu g.kg^{-1}$ for 10 pesticides in peaches and oranges [BLASCO et al., 2005] and from 10 to 70 $\mu g.kg^{-1}$ for carbosulfan and its metabolites in oranges [SOLER et al., 2006]. Zrostlíková et al. (2003) analysed 17 pesticides with HPLC-IT(MS) from apples and apricots after extraction with acetonitrile and SPE and reported LODs between 0.1 and 10 $\mu g.kg^{-1}$ and LOQs from 2 to 24 $\mu g.kg^{-1}$.

The obtained LODs and LOQs with both GC-MS and HPLC-MS were, for some substances, so far not fulfilling the 10 $\mu g.kg^{-1}$ threshold required for organic farming foodstuff. This could be overcome by the introduction of an additional concentration and/or clean-up step in the extraction method. For HPLC-amenable analytes, it could be possible to preconcentrate the extracts as it is already done for the GC analyses. The drawback in such a step is a simultaneous preconcentration of the matrix that can possibly affect the LODs/LOQs and accelerate the damaging of the MS detector. For GC-MS, a thermodesorption system has been studied as explained in Chapter 5.4 and **Appendix 5**.

5.2.3 Recovery

The recovery of the method was tested at a 10*LOQ fortification level in the SIM mode for the GC-amenable substances and at LOQ and 10*LOQ fortification levels in full

scan mode for HPLC-amenable substances. For leaf samples, ten substances were tested at LOQ and 10*LOQ fortification levels in SRM mode. Different blank matrices were used for the 2 fortification levels. Detailed results concerning the recovery and repeatability of the QuECheRS method for the representative foodstuff and leaf matrices shown in LESUEUR et al. (2007b; 2007c) (**Appendix 2** and **Appendix 3**) are given in **Table 5** to **Table 8**.

Table 5 presents the recovery and repeatability expressed as relative standard deviation (RSD) obtained for the grape, lemon, onion, tomato and leaf matrices at a 500 $\mu g.kg^{-1}$ fresh weight and 1,000 $\mu g.kg^{-1}$ fresh weight for the foodstuff matrices and the leaf samples, repectively.

Table 5: GC-MS fortification experiments (recovery and repeatability) at 10*LOQ fortification level in full scan mode (i.e. ca. 500 µg.kg^{-1} fresh weight for foodstuff and 1,000 µg.kg^{-1} fresh weight for leaf samples)

Substances	Fortification concentration (µg.l^{-1})	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Atrazin desethyl	500	98.7	5.8	88.3	9.9	78.6	16.8	99.5	7.2	92.5	8.1
Azoxystrobin	503	119.3	1.2	113.6	7.8	96.8	10.2	113.9	8.2	111.2	4.5
Benalaxyl	503	105.9	3.5	85.9	5.6	83.2	9.7	86.7	10.9	88.2	8.7
Bifenthrin	503	99.6	3.5	75.5	6.3	78.7	8.1	77.2	10.8	75.2	10.1
Biphenyl	573	61.9	10.9	32.4	10.6	50.4	10.5	57.5	4.0	48.7	10.4
Bitertanol	530	109.8	3.7	115.0	4.7	106.2	11.5	108.4	8.1	109.7	9.4
Bromopropylate	508	112.2	3.2	116.9	8.2	79.4	9.8	93.3	10.0	102.4	9.4
Bupirimate	525	98.6	4.7	82.8	6.8	65.8	10.2	88.0	10.4	79.4	7.9
Buprofezin	505	107.6	5.2	73.4	7.1	83.3	10.6	83.0	10.7	81.4	8.8
Carbaryl	550	91.7	5.1	76.7	5.4	49.1	11.3	92.0	10.3	72.4	8.7
Carboxin	503	85.4	4.4	65.1	10.7	47.6	11.9	73.5	9.3	69.9	6.9
Chlorfenvinphos	685	98.3	2.9	106.0	9.9	95.9	11.4	110.0	5.5	108.9	6.7
Chlorothalonil	510	102.1	14.5	76.4	9.2	119.4	6.6	82.3	4.2	100.1	5.5
Chlorpropham	505	94.1	2.9	93.5	7.4	75.1	9.6	93.8	9.9	91.4	9.7
Chlorpyrifos	515	104.5	3.7	86.2	7.1	76.0	9.9	90.5	9.1	92.1	7.8
Chlorpyrifos-methyl	543	102.7	3.1	107.0	9.4	70.3	8.6	95.8	10.5	96.4	5.7
Chlorthaldimethyl	520	101.8	2.5	81.9	6.5	73.8	9.5	90.0	9.3	96.2	5.6
Cyfluthrin	505	111.8	4.0	118.3	4.2	88.7	11.0	103.1	10.2	108.1	9.6

Substances	Fortification concentration ($\mu g.l^{-1}$)	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Cypermethrin	543	112.6	2.1	118.7	5.3	89.3	10.5	113.5	9.3	113.2	11.4
Cyproconazole	500	110.1	3.3	85.3	5.3	86.4	11.2	88.9	10.4	90.2	10.1
Cyprodinil	505	108.3	2.3	78.0	5.8	73.7	9.7	88.2	10.0	79.4	6.6
Cyromazine	500	45.5	11.2	31.6	9.0	29.2	3.9	42.9	10.1	41.1	6.7
Deltamethrin	530	116.5	8.7	119.3	8.3	84.9	9.8	110.8	5.0	108.7	5.9
Diazinon	640	92.6	3.7	76.5	10.8	78.2	9.9	86.6	10.1	81.5	8.8
Dichlorvos	655	80.9	9.5	76.2	9.0	72.8	11.2	70.3	15.2	73.1	12.7
Dicloran	515	93.8	5.2	92.8	10.3	91.3	9.6	99.4	8.6	94.2	7.5
Dicofol	498	117.5	7.1	90.1	7.9	74.4	12.3	92.2	9.3	93.1	10.1
Dieldrin	508	101.3	2.9	86.4	11.7	82.8	11.2	92.7	6.2	89.4	9.7
Difenoconazole	490	114.4	1.4	118.0	6.1	95.9	11.5	119.0	11.2	112.4	13.0
Dimethoate	515	116.2	4.0	117.3	9.0	106.0	12.3	131.7	7.4	121.1	9.7
Dimethomorph	510	119.1	1.8	119.9	6.3	103.9	13.1	117.7	10.8	115.4	10.7
Diphenylamine	495	86.3	2.1	71.5	6.3	64.0	13.1	80.6	10.8	72.4	6.7
Ethion	675	111.5	3.1	97.1	10.8	89.9	11.5	100.9	5.9	106.7	3.7
Ethoprophos	723	94.4	4.9	76.3	8.3	80.7	9.4	88.8	11.7	83.4	9.6
Fenarimol	525	113.5	2.8	104.7	5.3	82.6	11.9	94.3	10.7	98.1	5.8
Fenbuconazole	503	111.6	3.3	118.5	10.1	105.4	12.6	106.6	8.3	112.8	6.9
Fenhexamid	505	119.0	1.6	119.1	9.2	82.9	10.2	115.2	11.0	113.1	7.8
Fenithrothion	755	113.7	3.4	120.0	6.7	74.6	8.5	111.3	9.9	115.7	10.0

Substances	Fortification concentration ($\mu g.l^{-1}$)	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Fenoxycarb	505	111.6	6.5	119.4	5.7	96.2	13.3	115.4	9.2	118.2	11.6
Fenpropathrin	520	102.3	2.1	97.7	11.2	91.0	11.3	97.4	7.7	91.7	8.3
Fenvalerate	555	120.2	1.8	118.7	10.2	110.0	12.4	110.7	8.4	119.4	13.1
Fludioxonil	510	77.7	8.8	104.7	6.4	86.4	10.6	101.3	9.2	85.7	9.8
Fluquinconazole	535	99.8	3.5	92.1	10.8	96.2	12.0	97.5	8.9	93.4	11.5
Flusilazole	555	107.2	3.7	84.6	5.8	82.1	11.1	89.3	11.0	86.4	9.7
Fluvalinate-tau	468	118.5	2.6	118.9	5.5	119.7	10.8	112.2	10.5	116.7	10.4
Heptachlor-exo-epoxid	543	100.5	3.7	83.0	10.9	82.9	10.3	89.9	7.6	86.7	8.9
Heptaclor-endo-epoxid	535	103.1	3.6	75.1	10.5	77.0	12.2	90.3	3.8	79.5	6.9
Heptenophos	680	95.9	7.4	84.2	6.7	89.1	9.3	99.2	10.7	93.4	8.7
Imazalil	510	33.4	10.7	92.0	8.5	77.6	10.9	88.2	11.1	86.4	5.7
Iprodione	508	119.3	4.1	92.8	11.2	57.7	11.3	98.8	11.1	72.4	10.4
Kresoxim-methyl	518	107.0	5.6	88.1	4.8	86.8	10.9	91.3	11.3	90.1	8.9
Lindane	515	119.1	4.4	116.7	7.7	117.3	6.1	119.1	4.3	121.4	6.7
Malathion	603	111.1	2.4	101.7	10.1	75.8	9.3	93.8	6.8	89.1	3.9
Mecarbam	643	111.4	4.0	97.0	9.8	93.9	11.5	105.6	6.2	102.7	5.9
Metalaxyl	510	103.9	3.6	85.6	6.0	77.3	10.6	90.4	9.3	89.4	6.7
Metconazole	588	95.9	3.3	104.9	10.2	87.0	8.0	92.3	9.2	94.7	7.5

Substances	Fortification concentration ($\mu g.l^{-1}$)	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Methamidophos	483	81.1	10.4	44.4	17.3	118.9	6.6	128.3	9.8	76.4	8.3
Methidathion	533	117.5	3.4	110.8	11.7	75.0	11.1	108.2	9.0	96.4	8.9
Methiocarb	520	82.4	10.2	115.7	8.5	40.2	8.0	93.9	9.8	76.4	7.6
Monocrotophos	503	116.0	5.9	118.5	7.6	83.1	11.6	72.7	5.8	95.4	6.9
Myclobutanil	513	107.6	4.6	85.4	5.6	83.2	10.7	89.8	10.1	86.4	8.9
o,p'-DDT	505	94.7	4.0	77.3	11.2	71.0	8.7	77.6	5.7	73.4	4.9
Ofurace	495	113.2	5.8	117.9	5.9	77.7	5.3	109.5	11.3	116.8	6.7
Omethoate	513	104.7	11.9	111.9	11.3	115.7	11.5	119.6	5.0	116.8	6.2
o-phenylphenol	535	85.1	3.2	77.3	5.2	71.1	9.0	86.3	9.8	84.3	9.8
Oxadixyl	528	105.8	4.4	105.6	11.3	83.0	11.0	98.0	6.6	94.3	7.9
p,p'-DDD	520	110.8	3.2	92.5	10.5	92.9	11.4	102.0	4.8	98.8	6.7
p,p'-DDE	523	94.9	3.3	76.1	10.9	78.1	10.4	82.9	3.8	83.4	8.7
p,p'-DDT	530	89.9	7.3	94.7	13.3	79.7	10.5	90.2	9.6	93.1	6.7
Paclobutrazol	510	110.3	4.2	96.9	10.7	95.3	11.7	99.2	6.1	103.4	3.8
Parathion	653	107.7	4.6	95.4	10.9	94.8	11.7	100.8	6.5	93.7	7.3
Parathion-methyl	500	119.9	6.1	112.3	10.4	100.0	11.8	114.4	7.2	116.2	8.2
Penconazole	508	111.6	3.1	85.8	5.9	79.5	9.9	93.4	10.9	89.7	11.5
Pendimethalin	513	99.9	3.8	83.9	8.6	83.7	10.5	92.9	5.9	87.7	6.4
Permethrin	478	95.4	10.1	103.1	11.2	92.8	11.4	98.2	6.9	96.7	6.9
Phosalone	510	108.8	4.5	116.1	6.9	87.3	11.3	98.5	9.2	103.7	10.8

Substances	Fortification concentration ($\mu g.l^{-1}$)	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Piperonyl butoxide	175	99.2	8.0	85.1	6.1	77.2	11.3	86.0	11.6	79.8	5.7
Pirimicarb	503	71.9	4.7	82.9	5.7	63.7	12.1	90.3	11.4	84.3	6.7
Pirimiphos-methyl	658	102.6	1.9	89.5	6.5	71.8	10.1	93.0	9.7	84.7	7.5
Prochloraz	495	110.9	8.8	114.9	3.9	83.5	7.3	115.6	7.1	113.8	6.7
Procymidone	500	113.1	4.1	83.5	5.6	83.1	10.1	90.9	11.4	87.9	8.0
Propamocarb	715	40.3	7.6	71.9	11.2	58.3	5.4	73.3	10.7	66.6	7.9
Propargite	538	110.3	3.3	116.4	3.7	55.4	8.4	107.4	8.8	97.8	8.9
Propiconazole	540	114.7	4.5	110.6	11.4	97.7	13.1	113.2	6.1	108.9	6.6
Propyzamide	495	97.9	3.0	90.9	6.7	73.8	9.3	92.5	11.0	98.3	10.5
Pyridaben	510	110.9	5.1	104.6	10.1	82.3	9.7	91.3	10.2	89.5	4.4
Pyrimethanil	543	90.8	3.6	81.1	6.7	69.4	8.9	87.5	11.1	86.4	9.9
Pyriproxyfen	503	110.1	3.3	117.5	10.5	80.7	10.4	90.0	10.6	111.6	6.3
Quinalphos	733	113.1	2.9	95.2	6.5	81.9	10.8	97.8	10.3	89.5	3.6
Quinoxyfen	515	115.1	7.2	102.4	4.4	55.4	7.0	102.2	10.6	107.6	5.5
Quintozene	533	79.3	2.2	70.6	8.1	63.2	7.7	76.5	9.4	72.4	8.8
Tebuconazole	498	108.7	3.4	93.4	5.8	82.8	9.7	92.2	10.5	89.7	6.6
Tecnazene	503	80.9	10.3	57.5	6.1	69.1	8.3	77.0	9.2	59.7	8.9
Terbutryne	503	101.6	4.1	85.7	11.6	84.4	10.6	94.2	6.6	78.4	11.0
Tetradifon	505	117.7	6.1	119.7	8.1	78.1	10.4	119.9	7.1	116.4	6.3
Tetramethrin	500	100.9	3.3	99.1	11.4	91.7	11.8	99.6	7.4	97.6	6.6

Substances	Fortification concentration ($\mu g.l^{-1}$)	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Thiabendazole	508	109.1	7.3	119.4	11.5	101.6	12.9	105.5	7.5	110.6	6.9
Tolclofos-methyl	728	102.2	2.2	89.1	7.3	69.7	8.8	91.2	9.7	88.6	7.8
Tolylfluanid	500	101.8	5.0	94.8	6.7	73.9	7.9	61.9	11.0	64.5	9.7
Triadimefon	528	106.1	4.5	87.7	8.1	78.2	10.2	90.6	11.4	79.8	7.8
Triadimenol	503	112.8	4.2	86.6	5.4	89.8	11.5	90.1	10.1	85.6	9.7
Triazophos	463	112.5	8.8	119.7	9.3	109.5	12.2	119.1	8.5	116.5	7.8
Trifloxystrobin	523	106.8	1.4	88.7	4.7	85.7	9.9	92.5	11.2	88.8	6.7
Trifluralin	520	92.1	4.2	74.8	10.7	81.6	9.4	88.0	8.5	73.2	7.8
Vinclozoline	510	103.1	2.7	85.2	7.6	68.1	8.6	93.8	10.1	73.5	8.7

The method was found to be precise and accurate for GC analyses with recoveries between 70 and 110 % and RSD lower than 15 % for almost all the substances. The recovery of the pesticides was depending on the substance and the matrix with 61 to 82 % of the substances presenting a recovery in the range of 70-110 % as recommended by the SANCO Guideline [COMMISSION OF THE EUROPEAN COMMUNITIES, 2006]. Between 6 and 30 % of the substances presented a recovery higher than 110 %. Less than 5 % of the substances showed recoveries lower than 70 % except onion that tended to low recoveries with 17 % of the substances with a recovery lower than 70 % and 82 % in the range of 70-110 %, respectively. The repeatability was satisfying the SANCO Guidelines' recommendation with a RSD lower than 20 % for almost all the substances (between 1.1 % for dicofol in grape and 16.8 % for atrazin-desethyl in onion) apart for methamidophos in lemon (27.3 %) and dichlorvos in tomato (20.2 %). A trend to high recovery across the matrix selection was recognised for some pyrethroid insecticides (cyfluthrin, cypermethrin, deltamethrin, fenvalerate and fluvalinate-tau), some conazole fungicides (difenoconazole, fenbuconazole, prochloraz and propiconazole), some organothiophosphate insecticides (dimethoate, fenitrothion, omethoate, parathion-methyl and triazophos), some anilide fungicides (fenoxycarb and ofurace) and the other pesticides azoxystrobin, dimetomorph, fenoxycarb, lindane, propargite, pyriproxyfen and tetradifon. On the other hand, some substances like biphenyl, cyromazine, propamocarb, and, tecnazene and in less extend carboxin and dichlorvos tended to low recoveries in all matrices. The recoveries obtained for the GC-amenable substances in this study were in the same range than reported in the literature [ANASTASSIADES et al, 2003a; DÍEZ et al., 2006; LEANDRO et al., 2005] after extraction with the QuECheRS method except for carbaryl, cyprodinil, dichorvos and o-phenylphenol where lower recoveries than reported were obtained and for methamidophos and omethoate that presented inhere higher recoveries. High recoveries for some of the selected substances may be explained by the simple clean-up step leading to a limited removal of the impurities from the samples responsible for an enhancement of the signal. Concerning the substances with low recovery, cyromazine is for instance a small polar basic molecule (pKa = 5.22), easily hydrolysed at extreme pH, owing an ionic behaviour, which makes its analysis quite tedious [LESUEUR et al., 2007b] (**Appendix 3**). Biphenyl is a high lipophilic substance that can easily bind to the solid sample matrix. Based on our experience, propamocarb was rapidly degradated not only in samples but also in standards. Finally, dichlorvos was already reported with recoveries of max. 78 % at 500 $\mu g.kg^{-1}$ [ŠTAJNBAHER et al.,2003].

Table 6 and **Table 7** present in details the results showed in Lesueur et al. (2007b; 2007c) (**Appendix 2** and **Appendix 3**) about the recovery and repeatability of the HPLC-amenable substances at LOQ (i.e. ca 50 $\mu g.kg^{-1}$ fresh weight and 100 $\mu g.kg^{-1}$ fresh weight for foodstuff

and leaf samples, respectively) and 10*LOQ (i.e. ca 500 µg.kg^{-1} fresh weight and 1,000 µg.kg^{-1} fresh weight for foodstuff and leaf samples, respectively) fortification levels in the full scan mode for food and leaf samples.

Table 6: HPLC-MS fortification experiments (recovery and repeatability) at LOQ fortification level in full scan mode (i.e. ca. 50 µg.kg^{-1} fresh weight for foodstuff and 100 µg.kg^{-1} fresh weight for leaf samples)

Substances	Fortification concentration foodstuff/leaf (µg.l^{-1})	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Acetamiprid	55 / 111	100.5	7.1	68.0	2.7	87.9	6.0	81.4	6.3	88.8	13.1
Aldicarb	50 / 100	67.5	5.6	50.1	6.6	87.2	2.8	81.2	3.4	109.7	11.3
Avermectin B1a	51 / 102	< LOQ	-	< LOQ	-	< LOQ	-	< LOQ	-	< LOQ	-
Bendiocarb	54 / 109	101.6	3.9	59.4	7.3	118.2	6.7	116.4	2.8	79.3	6.4
Butocarboxim	49 / 98	67.2	5.1	50.3	6.5	87.0	2.8	80.6	3.5	101.1	14.4
Carbaryl	50 / 100	97.1	3.4	78.5	4.8	118.9	9.1	119.5	3.5	85.7	6.4
Carbendazim	51 /101	74.7	3.2	85.7	6.5	80.1	4.8	78.7	5.8	76.6	4.6
Carbofuran	51 / 102	67.6	1.0	48.8	1.8	89.4	4.5	69.3	3.6	95.4	2.9
Clomazone	47 / 95	94.0	4.5	64.4	2.4	74.7	5.0	78.6	4.2	101.8	6.4
Clothianidin	51 / 102	85.3	7.1	55.5	6.3	95.3	2.9	102.9	6.6	174.9	9.9
Cyromazine	50 / 101	40.2	1.9	22.9	1.9	41.6	3.2	36.0	10.8	48.2	9.6
Demeton-S	46 / 91	79.0	3.6	66.8	3.8	71.5	3.1	83.1	7.4	139.2	20.7
Dicrotophos	75 / 150	85.6	6.9	64.8	4.2	88.8	5.4	79.8	6.6	96.8	8.9
Diflubenzuron	51 / 103	89.1	7.2	69.7	6.1	86.6	5.6	95.8	10.2	106.8	6.7
Dimethoate	53 / 106	91.6	5.0	61.6	5.1	94.3	4.9	89.1	4.5	76.3	14.7
Diuron	52 / 104	89.4	5.8	58.0	5.6	78.7	9.0	83.9	4.6	89.1	7.1
Ethiofencarb	54 / 108	70.8	1.8	90.9	5.6	96.7	4.0	115.8	6.0	108.4	14.9

Substances	Fortification concentration foodstuff/leaf ($\mu g.l^{-1}$)	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Ethirimol	50 / 99	58.0	9.3	51.1	1.5	58.3	3.6	66.1	1.5	80.7	14.0
Fenamiphos	54 / 108	104.1	1.8	96.5	4.7	88.5	2.5	93.6	3.9	99.6	4.5
Fenpyroximate	49 / 98	97.4	6.0	56.0	3.5	84.9	11.5	87.5	9.8	85.1	7.1
Fenthion	84 / 167	93.4	5.6	77.1	4.6	71.1	5.2	82.9	4.8	98.3	9.8
Flufenoxuron	51 / 102	< LOQ	-	72.7	6.7	44.2	5.3	99.0	9.9	109.3	8.9
Furathiocarb	49 / 97	80.2	5.4	64.9	8.3	80.2	9.3	94.3	10.5	89.7	14.4
Hexythiazox	53 / 106	73.0	2.4	69.8	2.1	< LOQ	-	82.6	3.0	< LOQ	-
Imazalil	50 / 99	91.9	2.0	118.4	6.4	83.5	3.2	69.9	2.8	77.4	3.1
Imidacloprid	53 / 105	92.1	2.4	47.7	11.1	78.0	6.3	85.3	5.4	86.5	5.9
Indoxacarb	57 / 113	103.5	10.0	60.2	11.0	85.9	7.5	92.9	10.0	97.2	2.6
Linuron	50 / 101	92.5	6.6	61.6	4.3	91.6	7.0	83.7	6.8	81.1	5.4
Metamitron	51 / 102	95.9	6.0	67.9	3.7	92.5	2.4	85.5	4.4	74.0	5.1
Methabenzthiazuron	51 / 102	91.7	4.6	54.4	3.9	78.8	4.9	80.5	2.8	88.4	2.5
Metobromuron	53 / 106	93.4	3.8	59.9	9.6	76.4	4.5	83.1	3.2	95.8	11.4
Metoxuron	50 /100	94.1	7.8	66.9	3.0	90.9	4.7	83.2	5.0	79.3	8.1
Monocrotophos	50 / 99	< LOQ	-	< LOQ	-	51.7	2.0	< LOQ	-	< LOQ	-
Monolinuron	50 /101	94.9	3.3	64.6	5.8	88.3	6.0	85.0	7.6	91.6	4.9
Omethoate	50 / 99	< LOQ	-	< LOQ	-	< LOQ	-	< LOQ	-	< LOQ	-
Oxycarboxim	51 / 102	49.6	4.3	56.4	5.3	78.5	7.7	116.9	7.9	70.6	8.8

Substances	Fortification concentration foodstuff/leaf ($\mu g.l^{-1}$)	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Paclobutrazol	51 / 101	96.0	1.0	56.6	4.8	92.3	4.7	90.1	2.1	96.2	3.8
Pencycuron	49 / 99	85.6	2.8	54.1	4.5	104.1	10.0	85.0	5.5	92.0	3.3
Propamocarb	50 / 101	39.2	4.1	45.2	5.1	56.5	7.7	60.8	7.0	70.1	10.7
Pyraclostrobin	51 / 102	92.0	6.5	78.8	4.9	85.5	3.6	91.1	4.3	109.2	8.2
Rotenone	49 / 98	107.2	5.5	71.2	8.0	113.9	5.8	104.3	6.7	104.1	4.8
Tebufenozide	55 / 102	90.7	2.9	66.2	2.5	117.0	10.4	94.3	4.1	108.6	9.8
Thiabendazole	51 / 102	80.4	2.4	57.7	2.2	78.7	2.0	81.3	3.6	71.4	7.9
Thiamethoxam	52 / 104	< LOQ	-	< LOQ	-	< LOQ	-	< LOQ	-	< LOQ	-
Thiofanox	53 / 106	70.5	4.3	69.4	5.8	93.1	4.6	90.4	5.2	< LOQ	-
Triflumuron	54 / 106	90.6	11.0	55.3	12.1	53.8	7.5	93.2	9.3	82.1	13.9

Table 7: HPLC-MS fortification experiments (recovery and repeatability) at a 10*LOQ fortification level in full scan mode (i.e. ca. 500 µg.kg^{-1} fresh weight for foodstuff and 1,000 µg.kg^{-1} fresh weight for leaf samples)

Substances	Fortification concentration foodstuff/leaf (µg.l^{-1})	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Acetamiprid	554 / 1,110	97.1	0.7	91.0	2.3	97.4	2.6	106.7	2.3	98.0	3.1
Aldicarb	500 / 1000	95.2	2.8	74.6	1.9	91.2	2.0	98.7	2.5	76.0	1.6
Avermectin B1a	510 / 1,020	107.1	4.6	87.2	9.8	103.1	3.9	91.4	3.2	85.5	9.7
Bendiocarb	544 / 1,090	99.0	2.9	83.5	3.2	93.0	3.3	103.2	3.6	98.1	1.9
Butocarboxim	491 / 980	94.0	2.7	74.8	2.3	92.8	2.6	98.9	2.0	73.4	1.4
Carbaryl	499 / 997	98.0	6.1	96.9	4.1	97.1	3.8	102.6	3.8	107.8	4.7
Carbendazim	505 / 1,010	92.8	3.7	91.7	4.4	95.1	3.4	99.3	3.5	80.7	5.7
Carbofuran	510 / 1,020	94.5	1.8	93.1	2.7	117.2	5.3	102.3	1.3	99.7	2.5
Clomazone	473 / 945	98.3	2.1	90.1	3.2	78.6	2.3	106.7	3.4	98.5	1.6
Clothianidin	512 / 1,025	96.2	2.2	87.3	4.0	98.4	3.2	106.5	3.1	100.3	4.1
Cyromazine	505 / 1,010	45.9	4.3	27.8	1.1	47.9	6.7	98.0	2.6	26.1	2.0
Demeton-S	456 / 910	94.7	3.2	92.8	2.4	50.8	3.2	98.7	3.1	92.0	2.3
Dicrotophos	750 / 1,500	94.9	2.2	94.9	1.8	96.4	2.0	100.4	1.5	95.9	1.1
Diflubenzuron	515 / 1,030	100.0	3.5	82.2	3.7	83.9	6.3	110.2	3.0	102.6	4.3
Dimethoate	529 / 1,060	97.2	3.1	93.2	1.4	95.9	1.8	102.9	2.3	102.2	1.3
Diuron	519 / 1,040	98.2	2.2	92.1	2.8	89.4	3.8	101.8	2.5	93.0	4.4
Ethiofencarb	541 / 1,080	80.1	2.6	81.1	1.7	80.3	1.5	87.2	2.6	81.1	2.8

Substances	Fortification concentration foodstuff/leaf ($\mu g.l^{-1}$)	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Ethirimol	495 / 990	67.0	1.8	75.3	4.0	71.9	2.0	81.4	2.8	60.5	6.1
Fenamiphos	541 / 1,080	95.9	2.7	112.8	4.0	94.9	2.1	105.7	3.1	96.5	2.3
Fenpyroximate	490 / 980	97.4	3.7	92.2	7.4	79.3	7.1	102.9	2.5	154.9	30.9
Fenthion	835 / 1,670	95.6	3.1	112.4	3.7	74.4	1.9	104.0	2.4	90.7	2.9
Flufenoxuron	511 / 1,020	106.9	6.5	96.8	11.4	80.9	6.8	119.5	3.5	90.3	13.0
Furathiocarb	485 / 970	103.5	1.9	106.4	3.9	74.0	7.1	113.2	2.5	74.0	8.4
Hexythiazox	535 / 1,070	99.7	4.1	72.4	10.2	61.5	5.2	84.5	3.9	174.3	30.5
Imazalil	495 / 990	94.8	2.2	119.6	3.2	99.1	3.3	103.7	2.2	89.9	2.8
Imidacloprid	527 / 1,055	101.0	2.1	92.6	2.8	92.6	4.0	102.9	2.5	99.0	2.7
Indoxacarb	567 / 1,135	99.7	4.2	74.8	6.1	69.5	4.9	118.4	2.0	73.6	4.7
Linuron	504 / 1,010	101.6	3.9	93.3	4.4	74.1	4.1	102.7	2.7	102.0	2.0
Metamitron	510 / 1,020	95.9	2.4	85.5	1.5	97.9	2.1	103.2	2.2	89.4	5.7
Methabenzthiazuron	510 / 1,020	98.7	1.9	75.7	2.7	92.8	2.5	103.5	1.8	96.1	3.3
Metobromuron	530 / 1,060	97.4	3.8	85.5	6.5	94.4	3.8	99.8	2.0	99.7	2.8
Metoxuron	500 / 1000	98.4	1.8	92.7	4.1	100.7	2.5	105.8	1.7	96.0	4.0
Monocrotophos	495 / 990	95.5	2.8	85.7	5.7	95.5	2.2	97.0	4.3	105.4	2.3
Monolinuron	505 / 1,010	102.2	1.9	86.4	1.9	95.4	2.7	106.5	2.6	99.9	1.9
Omethoate	497 / 990	84.6	5.9	86.3	4.4	87.1	3.4	84.3	5.8	94.0	7.7
Oxycarboxim	510 /1,020	71.8	3.4	52.7	1.8	79.9	5.2	108.5	3.3	71.1	3.9

Substances	Fortification concentration foodstuff/leaf ($\mu g.l^{-1}$)	Grape		Lemon		Onion		Tomato		Leaf	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Paclobutrazol	508 / 1,015	99.1	3.5	85.3	4.8	94.0	1.9	105.9	2.8	98.9	3.2
Pencycuron	494 / 990	91.1	3.9	91.7	4.3	77.8	8.9	83.6	3.9	89.5	16.0
Propamocarb	503 / 1,010	49.7	3.2	67.9	2.7	78.1	3.4	71.6	5.1	91.0	8.5
Pyraclostrobin	510 / 1,020	95.1	3.0	84.7	3.1	62.0	3.4	116.9	3.0	77.3	7.5
Rotenone	492 / 985	104.0	2.5	103.1	4.2	96.4	3.5	106.0	3.6	93.6	4.0
Tebufenozide	554 / 1,110	100.5	2.7	97.4	2.1	99.2	3.3	111.1	1.8	92.6	2.6
Thiabendazole	508 / 1,020	93.4	2.5	78.2	2.2	96.2	2.1	106.6	1.3	70.4	5.8
Thiamethoxam	520 / 1,040	99.6	5.5	100.7	6.0	94.3	2.9	102.3	3.0	96.6	3.0
Thiofanox	529 / 1,060	89.9	1.0	92.6	3.3	81.0	2.3	98.3	1.6	95.3	3.2
Triflumuron	540 / 1,080	78.1	7.6	71.8	5.8	70.7	3.8	94.0	5.2	90.7	4.7

The method was judged as precise and accurate. As far as the HPLC-amenable pesticides are concerned, 87 to 93 % of the substances, depending on the matrix, at 10*LOQ fortification level and 78 to 85 % at LOQ fortification level, respectively, presented recoveries of 70 to 110 % except for lemon at 50 $\mu g.kg^{-1}$ that tended to low recoveries with 26 % of the substances in the range of 70-110 % and 72 % lower than 70 % with a minimum at 22.9 % recovery for cyromazine. The experiments were repeatable with a RSD lower than 20 % (from 0.7 % for acetamiprid in grape to 11.4 % for flufenoxuron in lemon) at 500 $\mu g.kg^{-1}$ and lower than 15 % (between 1.0 % for paclobutrazoland 12.1 % for triflumuron in lemon) at 50 $\mu g.kg^{-1}$ for the food matrices. The repeatability was less satisfying for leaf samples with RSD values ranging from 1.1 % (dicrotophos) to 16.0 % (pencycuron) for all the substances except for fenpyroximate (30.9 %) and hexythiazox (30.5 %) at the 1,000 $\mu g.kg^{-1}$ fortification level and lower than 20 % at 100 $\mu g.kg^{-1}$ apart for demeton-S (20.7 %). No pesticide in grape showed recovery higher than 110 % at both fortification level. At the lower fortification level, carbaryl was the only substance with a trend to higher recovery whereas carbofuran, cyromazine, ethirimol and propamocarb presented low recoveries. For the HPLC-amenable substances reported in the literature [BLASCO et al., 2004; BLASCO et al., 2005; ZROSTLÍKOVÁ et al., 2003] (carbaryl, carbendazim, carbofuran, diflubenzuron, flufenoxuron, imidacloprid, imazalil, linuron, thiabendazole, triflumuron,) at comparable fortification level (10 to 1 000 $\mu g.kg^{-1}$) in similar matrices (apple, apricot and orange) but different extraction methods, the recoveries obtained with HPLC-MS were comparable. Noticeable is the very low recovery for carbofuran (2 to 5 % in apple and apricot) obtained by Zrostlíková et al. (2003) due to its lost during the SPE clean-up of the extracts in comparison to the results obtained by Soler et al. (2006) reporting the analysis of carbofuran as main metabolite of carbosulfan with recoveries of 90.2 % and 95.4 % at the 10 and 100 $\mu g.kg^{-1}$ fortification levels, respectively. Cyromazine showed, once again, at both fortification levels poor recoveries (26.1 % at LOQ and 48.2 % at 10 times the LOQ) for the same reasons as explainable in the former paragraph.

Different leaf blank matrices were used for the two fortification levels resulting in different interference peaks of the same m/z ratio and R.T. for some analytes. This leaded to recoveries for the concerned analytes (like hexythiazox) higher than 110 % in the full scan mode as shown in Lesueur et al. (2007b) and in **Appendix 3**. It could be certified in the SRM mode that these interfering substances in the blank matrices were not our analytes as detailed in **Table 8** presenting the recovery and repeatability of eleven analytes in SRM mode.

Table 8: HPLC-MS fortification experiments (recovery and repeatability) at LOQ and 10*LOQ fortification levels in SRM mode for leaf samples (i.e. ca. 100 µg.kg^{-1} and 1,000 µg.kg^{-1} fresh weight)

Substance	LOQ			10*LOQ		
	Fortification concentration (µg.l^{-1})	Recovery (%)	RSD (%)	Fortification concentration (µg.l^{-1})	Recovery (%)	RSD (%)
Carbendazim	101.0	73.8	7.4	1,010	79.0	3.4
Diflubenzuron	102.8	84.8	5.2	1,030	87.1	6.1
Fenamiphos	108.2	92.1	4.0	1,082	98.0	6.7
Fenpyroximate	98.0	71.9	15.8	980	88.2	14.9
Flufenoxuron	102.2	96.1	17.9	1,022	80.2	6.1
Hexythiazox	106.8	95.5	16.0	1,070	88.4	5.2
Imidacloprid	105.4	79.9	3.7	1,054	89.7	2.1
Indoxacarb	113.4	96.0	12.5	1,134	92.9	4.8
Propamocarb	100.6	72.4	6.1	1,006	95.0	8.3
Pyraclostrobin	102.0	98.1	10.4	1,020	92.3	4.9
Tebufenozide	110.8	108.4	1.5	1,108	100.0	3.0

Matrix interferences were confirmed when analysing the fortification samples in SRM mode. In this mode at the LOQ fortification level, recoveries between 71.9 % (fenpyroximate) and 108.4 % (tebufenozide) were achieved with repeatabilities ranging from 1.5 % (tebufenozide) to 17.9 % (flufenoxuron). At 10*LOQ fortification level, recoveries between 79 % (carbendazim) and 100 % (tebufenozide) were obtained coupled with repeatabilities between 2.1 % (imidacloprid) and 14.9 % (fenpyroximate). The matrix intereference were especially noticeable for fenpyroximate and hexythiazox, whose recovery decreased from 154.9 % to 88.2 % and from 174.3 to 88.4 %, respectively, from full scan to SRM mode.

5.2.4 Proficiency tests

The detailed proficiency test results are presented in Lesueur et al. (2007c) and in **Appendix 2**. It was shown that almost all z-scores were between –2 and 2, defining the good compliance of a proficiency test. For the three substances azoxystrobin, bromopropylate and bupirimate in melon at low residue concentration, z-scores between 2.4 and 4.5 were obtained, signifying an overestimation of the assigned value, which is justified by assigned values between our LODs and LOQs.

Iprodione and azoxystrobin were as well overestimated in tomato with z-scores of 3.0 and 2.7, respectively. Since iprodione is quickly degraded, its concentration in the standard decreased, which leaded to the overestimation of the concentration in the samples. An iprodione standard should be frequently prepared to reduce the risk of a substance loss.
Azoxystrobin presents a high recovery (114 % at 500 µg.kg^{-1}) in tomato, which can explain its overestimation in this product. Deltamethrin in flour presented a z-score of –2.2. Since flour is a high fat content matrix and deltamethrin shows a log K_{OW} of 4.6 [FOOTPRINT] meaning a higher attraction to the apolar and fat containing phase, a consequently low recovery of the substance and the underestimation of the assigned value are possible.

The single proficiency test realised with HPLC-MS for a citrus fruit (lemon) presented 4 pesticides which were all detected and quantified (calibration in solvent). The 3 substances carbofuran, dimethoate and omethoate showed satisfing z-score of 1.5, -0.6 and –1.8, respectively. Only aldicarb showed a z-score slightly higher than the accepted limit of 2 with a value of –2.3. The low recovery of aldicarb in lemon at 50 µg.kg^{-1} of 50.1 % can explain the underestimation of the proficiency test value in this commodity. For dimethoate and omethate, the suppression of the signal from the matrix implies a higher concentration when working with matrix-matched standard and thus a z-score closer to 0 i.e. a better estimation of the concentration.

5.2.5 Comparison samples

In the around 600 samples extracted with the European Guideline DIN EN 12393 and the QuECheRS method 438 residues of 59 pesticides validated with both methods were found as shown in **Table 9**. The “Yes” and “No” results in **Table 9** correspond to a succeded z-score test (–2 < z-score < 2) and a failed z-score test (z-score < -2 or z-score > 2), respectively.

Table 9: Comparison of samples extracted with the European Guideline DIN EN 12393 and the QuECheRS method

Substances	Number of residues	high sugar content	high acidic content	high sulphur content	high water content
Azoxystrobin	5				Yes (5)
Benalaxyl	1				Yes (1)
Bifenthrin	7				Yes (7)
Bupirimate	4				Yes (4)
Buprofezin	2				Yes (2)
Carbaryl	7				Yes (5); **No (2)**
Chlorothalonil	2				Yes (2)
Chlorpropham	2				Yes (2)
Chlorpyrifos	27	**No (1)**	Yes (7)		Yes (19)
Chlorpyrifos-methyl	13				Yes (13)
Chlorthaldimethyl	1				Yes (1)
Cypermethrin	2				Yes (2)
Cyprodinil	37				Yes (37)
Deltamethrin	13				Yes (12); **No (1)**
Dichlorvos	1		Yes (1)		
Dicloran	2				Yes (2)
Dicofol	2				Yes (2)
Difenoconazole	6				Yes (6)
Dimethoate	2				Yes (2)
Dimethomorph	11				Yes (10); **No (1)**
Diphenylamine	3				Yes (3)
Fenarimol	6				Yes (6)
Fenhexamid	12				Yes (10); **No (2)**
Fenoxycarb	12				Yes (9); **No (3)**
Fenpropathrin	1				Yes (1)
Fludioxonil	18				Yes (18)
Imazalil	15		Yes (7)		Yes (8)
Iprodione	47		Yes (1); **No (1)**		Yes (40); **No (5)**
Kresoxim-methyl	7				Yes (7)
Malathion	1				Yes (1)
Metalaxyl	13				Yes (13)

Substances	Number of residues	high sugar content	high acidic content	high sulphur content	high water content
Methidathion	1		Yes (1)		**No (1)**
Methiocarb	4				Yes (4)
Myclobutanil	2	Yes (1)			Yes (1)
o-phenylphenol	2		Yes (2)		
p,p'-DDE	2				Yes (2)
Parathion-methyl	1				Yes (1)
Penconazole	3				Yes (3)
Phosalone	2				Yes (2)
Piperonyl butoxide	2				Yes (2)
Pirimicarb	4				Yes (4)
Pirimiphos-methyl	4				Yes (4)
Prochloraz	3		Yes (1)		Yes (2)
Procymidone	20				Yes (19); **No (1)**
Propamocarb	32				Yes (32)
Propyzamide	7	Yes (1)			Yes (5); **No (1)**
Pyridaben	1				Yes (1)
Pyrimethanil	9		Yes (1)		Yes (8)
Pyriproxyfen	1				Yes (1)
Quinoxyfen	7				Yes (6); **No (1)**
Tebuconazole	14				Yes (13); **No (1)**
Thiabendazole	12				Yes (12)
Tolclofos-methyl	2				Yes (2)
Tolylfluanid	7				Yes (6); **No (1)**
Triadimefon	2				Yes (2)
Triadimenol	8				Yes (8)
Trifloxystrobin	2				Yes (2)
Trifluralin	1				Yes (1)
Vinclozoline	1				Yes (1)

As can be seen in **Table 9**, only 5 % of the results obtained with the QuECheRS method were significantly different from those obtained with the European Guideline DIN EN 12393. Furthermore, these were most of time substances for which many more results with satisfying z-scores in the same matrix class were at disposital like carbaryl, deltamethrin,

dimetomorph, fenhexamid, fenoxycarb, iprodione, procymidone, propyzamide, quinoxyfen, tebuconazole and tolylfluanid. Two substances presented noticeable because single results: chlorpyriphos in a high sugar content matrix (apple) and methidathion in a high water content matrix (sweet pepper). In each failed z-score test, z-scores were higher than two, meaning an overestimation of the concentration by the QuECheRS method. As already mentioned, due to the limited clean-up of the procedure, the matrix influence is more perceptible than with the European Guideline DIN EN 12393 and most of time results are (slightly) enhanced.

5.3 Analysis of pesticides in soil samples after extraction with the QuECheRS method and comparison with other extraction methods

The results of this study have been published in Lesueur et al. (2007d) presented in **Appendix 4**. It was shown that the 24 selected pesticides for the analysis of soil samples presented a linear behaviour with GC-MS and HPLC-MS/MS analysis in the standard concentration range of 0.010-2 $\mu g.ml^{-1}$ corresponding to a soil concentration range between 4-800 $ng.g^{-1}$ and 20-2000 $ng.g^{-1}$, depending on the extraction method. The lowest LODs/LOQs were achieved with the European Guideline DIN EN 12393 and the PLE method and the highest with the QueCheRS method. **Table 10** presents the achieved LODs/LOQs for the different extraction methods with GC-MS and HPLC-MS/MS, respectively.

Table 10: Linearity, LODs and LOQs of the selected pesticides with GC-MS and HPLC-IT/MS in soil samples

Substances	QuECheRS		DFG S19		PLE		USE	
	LOD $ng.g^{-1}$	LOQ $ng.g^{-1}$	LOD $ng.g^{-1}$	LOQ $ng.g^{-1}$	LOD $ng.g^{-1}$	LOQ $ng.g^{-1}$	LOD $ng.g^{-1}$	LOQ $ng.g^{-1}$
Atrazine	13	43	5	17	4	12	7	23
Desethylatrazine	13	43	5	17	4	12	7	23
Desisopropylatrazine	11	38	5	15	3	10	6	20
Carbendazim	0.02	0.08	0.01	0.03	0.006	0.02	0.01	0.04
Chlorfenvinphos	22	73	8.7	29	6	19	12	39
Chloroxuron	0.10	0.33	0.04	0.13	0.03	0.09	0.05	0.17
Chlorpyrifos	24	79	10	32	6	21	13	42
Chlorpyrifos-methyl	37	125	15	50	10	33	20	67
Deltamethrin	14	47	6	20	3.8	13	8	25
Dieldrin	88	292	35	117	23	78	47	156
Diuron	12	39	5	16	3	11	6	21

Substances	QuECheRS		DFG S19		PLE		USE	
	LOD $ng.g^{-1}$	LOQ $ng.g^{-1}$	LOD $ng.g^{-1}$	LOQ $ng.g^{-1}$	LOD $ng.g^{-1}$	LOQ $ng.g^{-1}$	LOD $ng.g^{-1}$	LOQ $ng.g^{-1}$
Flufenoxuron	0.23	0.77	0.09	0.30	0.06	0.21	0.12	0.41
Isoproturon	0.17	0.56	0.07	0.20	0.04	0.15	0.09	0.30
Lindane	13	42	5	17	3	11	7	22
Linuron	0.09	0.30	0.04	0.12	0.02	0.08	0.05	0.16
Metamitron	0.05	0.16	0.02	0.07	0.01	0.04	0.03	0.09
Methabenzthiazuron	0.17	0.58	0.07	0.23	0.05	0.16	0.09	0.31
Metobromuron	0.12	0.42	0.05	0.17	0.03	0.11	0.07	0.22
Metoxuron	0.08	0.26	0.03	0.10	0.02	0.07	0.04	0.14
Monolinuron	0.10	0.35	0.04	0.14	0.03	0.09	0.06	0.19
Pencycuron	0.09	0.30	0.04	0.12	0.02	0.08	0.05	0.16
Simazine	14	48	6	19	4	13	8	25
Trifluraline	20	65	8	26	5	17	10	35
Vinclozoline	20	68	8	27	5	18	11	36

Table 10 shows that with the present methods the HPLC-amenable substances presented LODs and LOQs in SRM mode in the low $ng.g^{-1}$ range from 0.006 $ng.g^{-1}$ (carbendazim) to 0.23 $ng.g^{-1}$ (flufenoxuron) and from 0.022 to 0.77 $ng.g^{-1}$, respectively, except for diuron with LOD from 3.1 to 11.8 $ng.g^{-1}$ and LOQ from 10.5 to 39.2 $ng.g^{-1}$. Unlikely, GC-amenable analytes and in particular chlorpyrifos-methyl and dieldrin presented higher LODs and LOQs in SIM mode in the range from 3.0 $ng.g^{-1}$ (desisopropylatrazine) to 87.5 $ng.g^{-1}$ (dieldrin) and from 10 to 292 $ng.g^{-1}$, respectively.

It was noticed that only the QuECheRS and the USE methods could recover all the selected substances. Carbendazim and metamitron were recovered neither with the European Guideline DIN EN 12393 nor with PLE as well as additionally monolinuron with PLE. Carbendazim, metamitron and monolinuron own the lowest octanol-water partition coefficient (K_{OW}) of all the selected substances (between 0.8 and 2.2) implying a possibly high repartition in the water phase and as a consequence low concentration in the analysed organic phase.

Figure 1 to **Figure 3** present the recoveries obtained for the three soil reference materials with the four extraction methods.

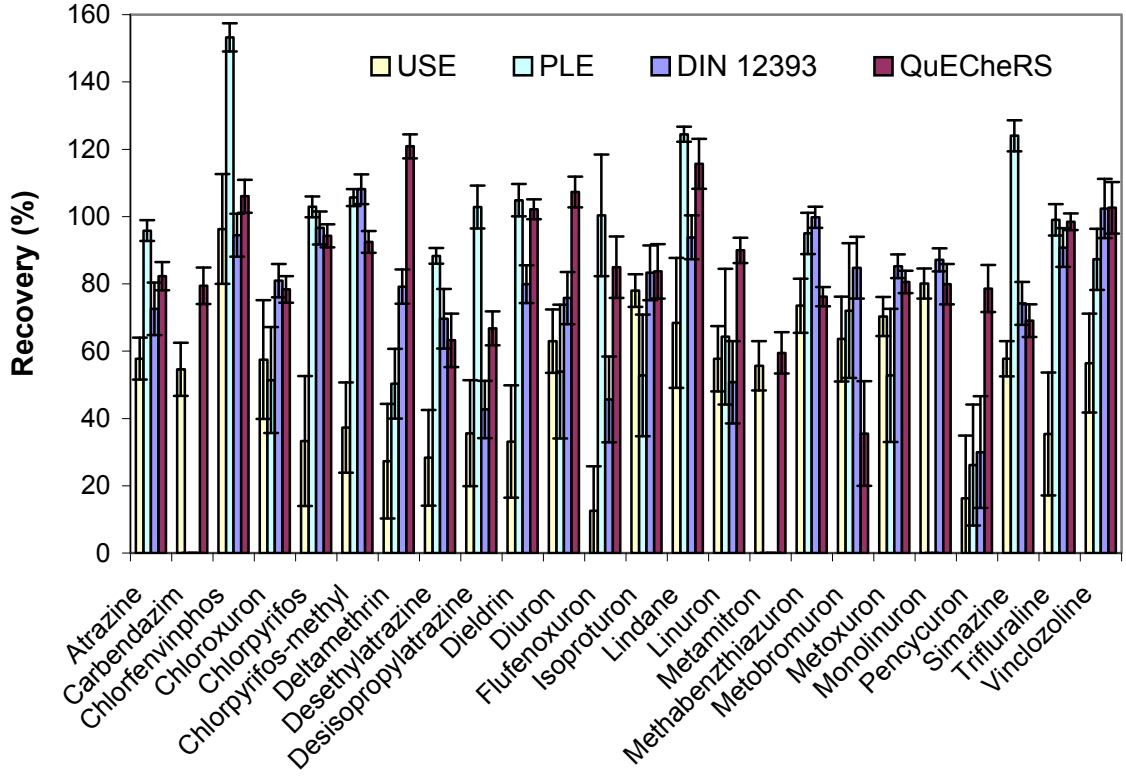

Figure 1: Recovery (%) and RSD (%) of the pesticides at 500 ng.g^{-1} from EUROSOIL 7

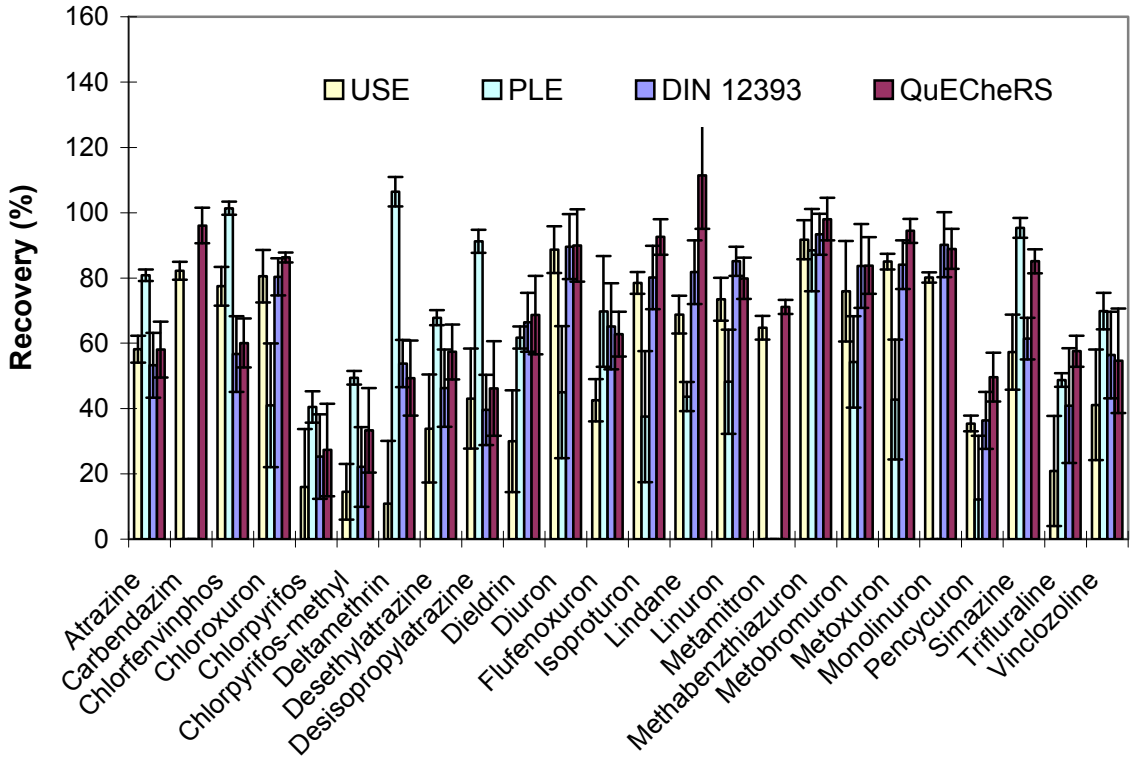

Figure 2: Recovery (%)and RSD (%) of the pesticides at 500 ng.g^{-1} from SO26

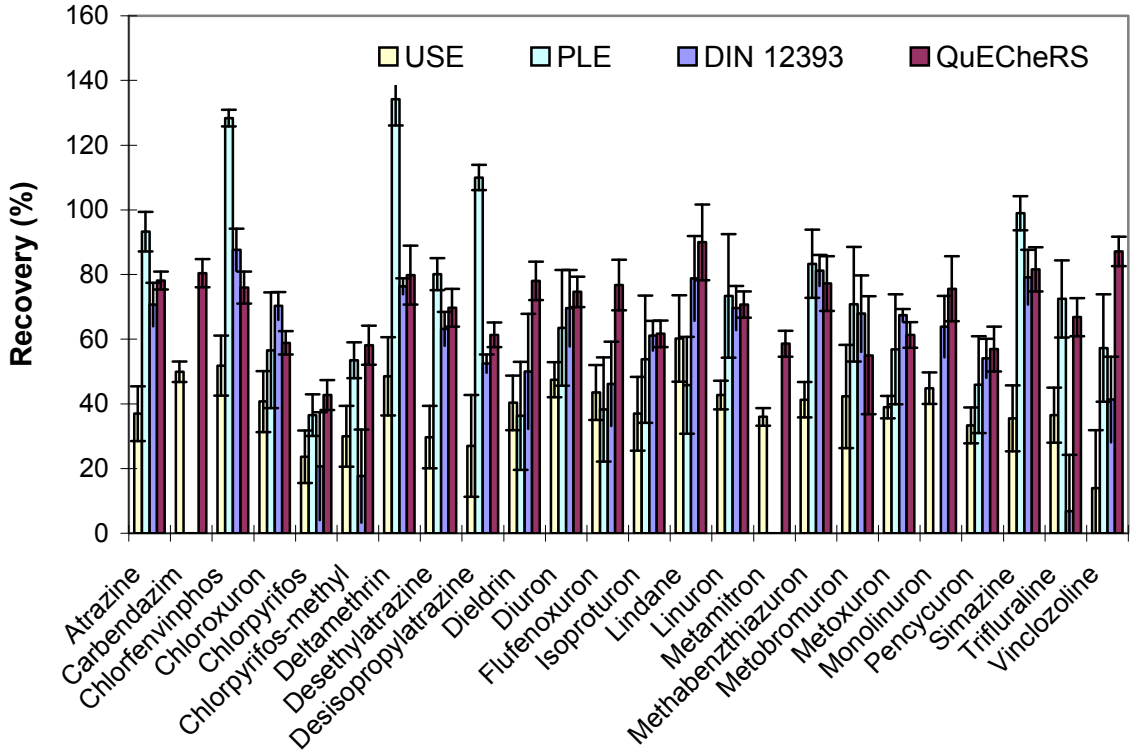

Figure 3: Recovery (%)and RSD (%) of the pesticides at 500 ng.g^{-1} from sea sand

As detailed in **Figure 1** to **Figure 3**, when considering only the substances that were recovered from the materials, the QuECheRS method showed the highest recoveries (between 27.3 and 120.9 % with a median recovery of 72.7 % for the 3 materials). It was followed by the European Guideline DIN EN 12393 (between 6.8 and 108.1 % with a median recovery of 65.7 % for 3 materials) and the PLE (between 12.2 and 153.2 % with a median recovery of 63.5 % for 3 materials) that presented comparable results. Finally, the lowest recoveries were obtained with the USE (between 10.9 and 96.3 % with a median recovery of 57.0 % for the 3 materials). The QuECheRS method was the method with the highest rate of substances (around 50 %) in the three matrices satisfying the recovery range of 70-110 %.

The repeatability was similar and acceptable below 20 % for all the methods: QuECheRS method (RSD between 1.5 and 18.3 % with a median repeatability of 5.4 %); European Guideline DIN EN 12393 (RSD between 1.9 and 17.8 % with a median repeatability of 7.7 %); USE (RSD between 1.5 and 19.3 % with a median repeatability of 8.5 %) and PLE (RSD between 1.8 and 20.2 % with a median repeatability of 6.4 %). Nevertheless, it was on the average slightly higher for the USE and the PLE.

Organochlorine pesticides like chlorpyriphos, chlorpyriphos-methyl, deltamethrin and dieldrin have a high affinity to the organic humic substances of soil matrices (high K_{OW}) with which they develop chemical bonds [ANDREU et al., 2004; BLUME, 1992; RIPPEN, 1987]. These bonds are stronger than the Van der Wals bonds related to surface processes and can not broken down through the energy produced by the ultrasonic dispersion since it is too weak [MAYER et al., 2002]. Lindane owns the highest water solubility and the lowest soil sorption coefficient, which can explain the better recovery than with the other organochlorine pesticides [SCHEFFER et al., 2002a]. The secondary and tertiary amine pesticides (phenylureas, triazines and their metabolites) tend to adsorb on the soils' inter-crystalline layers of clay minerals [SCHEFFER et al., 2002b] that can not be reached with ultrasonic vibration and makes this extraction less efficient with these substances [MAYER et al., 2002].

Higher recoveries were achieved with the EUROSOIL 7 than with the SO 26 and in any case than with the sea sand whatever with all the extraction methods applied (especially for chlorpyrifos and chlorpyrifos-methyl). Since recoveries as high as 150 % (chlorfenvinphos, deltamethrin, lindane and simazine) were obtained with PLE, a clean-up step is strongly recommended. Trifluralin, chlorpyriphos and chlorpyriphos-methyl presented extreme low recoveries from sea sand with the European Guideline DIN EN 12393 although this method has been demonstrated to be effective for these analytes but for other matrices. The adsorption of ionisable substances like atrazine and its metabolites is reported to increase with decreasing pH [ANDREU et al., 2004; BLUME, 1992; RIPPEN, 1987], which explains the rather low recoveries of atrazine, desethylatrazine and desisopropylatrazine with less acidic extraction methods.

The European Guideline DIN EN 12393 has been developed for the analysis of GC-amenable substances and is known to be low efficient in the recovery of polar pesticides like phenylureas or carbamates. Some researchers favored the use of acetone as organic solvent for the extraction of pesticides. Lambropoulou et al. (2004) noticed that water, acetone, acetonitrile and methanol showed similar results for the extraction of vinclozoline but that acetone was significantly more efficient in the case of dicloran. Tor et al. (2006) expressed the importance of getting inside the aggregates in the case of organochlorine pesticides (lindane, dieldrin), especially with "aged soils" since these analytes are adsorbed on and in soil aggregates. They recognized acetone, more polar, possibly assisted with mechanical forces, as the best solvent for the break-up and disintegration of aggregates leading to a better contact between particles and solvent and thus a better extraction. By studying the USE of tetramethrin and chlorpropham, Babíc et al. (1998) noticed that acetonitrile was the only solvent out of seven (acetone, dichlormethane, chloroform, hexane,

benzene, acetonitrile and diethyl ether) that was not able to recover these substances. On the other hand, Gonçalves et al. (2005) recommend acetonitrile for the extraction of organochlorine pesticides from soil samples. Nevertheless most of the studies approve the use of water/solvent mixture since better recoveries are reported in the case of water-assisted extraction than with pure solvent extraction (Zhu et al., 2000). The use of acetone might favor the extraction of analytes from soil matrices but together with that of co-eluents inherent to the materials. This resulted in less clean extracts leading to the drawbacks of higher LODs/LOQs and the need for a clean-up step, which is always critical in the case of multiresidue methods.

5.4 Development of a TDAS system for the analysis of apolar and middle polar pesticides (GC-MS)

These results have been presented in Lesueur et al. (2006). More detailed results can be found in **Appendix 5**.

With the injection of single and mix standards with the thermodesorption system set with a purge flow to split vent of 5 $ml.min^{-1}$ at 5 min, it was quickly noticed that the less volatile the substance i.e. the higher the R.T., the higher the carry-over of the substance in the following runs. Consequently 3 groups were observed:

- ✓ Substances with no or slight carry-over (less than 10 % of the total signal). Roughly from dichlorvos to buprofezin apart from chlorthaldimethyl and imazalil.
- ✓ Substances with a “medium” carry-over (20 % to 75 % of the total signal). From bupirimate to endosulfan sulfate with the addtion of chlorthaldimethyl and imazalil and the exception of fludioxonil.
- ✓ Substances with an extreme carry over (more than 90 % of the total signal). From kresoxim-methyl up to fluvalinate-tau and the additon of fludioxonil.

Increasing the purge flow to split vent from 5 to 70 $ml.min^{-1}$ and decreasing the starting time of the purge flow from 5 to 0 min had the advantage of preventing the carry-over of the substance in the next run but also the disadvantage of decreasing the signal by different way as shown for benalaxyl, ethion and kresoxim-methyl. The total response for kresoxim-methyl decreased with increasing purge flow to split vent from 5 to 70 $ml.min^{-1}$ and reached a maximum at 50 $ml.min^{-1}$ for ethion and at 25 $ml.min^{-1}$ for benalaxyl.

With this design of the thermodesorption system, it seemed improbable to find an optimum for almost 50 substances. Nevertheless and as expected, high purge flow to split vent (70 $ml.min^{-1}$ at 0 min) presented the drawback of drastically burke the response of the detector.

A set of experiments determined the location of the contamination in or on the injection needle at the bottom of the oven introducing the sample from the thermodesorption system into the liner of the GC. A new design of the thermodesorption system has now been developed and furher experiments have to be carried out to assess the feasibility of this system for the monitoring and in the best case the quantification of pesticide multiresdues in foodstuff.

5.5 Application: pesticide residues in fruit and vegetable samples: analytical results of two year´s pesticide investigations

These results have been published in Lesueur et al. (2007a) and are shown in **Appendix 6**. All the samples in this study were extracted according to the European Guideline DIN EN 12393 and analysed with GC-MS since the QuECheRS method and the HPLC-MS results were neither validated nor available until 2006.

Table 11 and **Figure 4** present the pesticide residue findings for the years 2004 and 2005.

Table 11: Pesticide contamination in foodstuff on the Austrian market in 2004 and 2005 (in number of samples)

Year	Type of product	Number of samples	Total number of residues	Number of pesticide residues per sample													
	Org. / Conv.			0	1	2	3	4	5	6	7	8	9	10	11	12	13
2004	Organic	604	65	553	41	8	2	0	0	0	0	0	0	0	0	0	0
	Conventional	889	1411	343	213	140	71	51	25	17	7	8	5	7	0	2	0
	Sum	1493	1476	896	254	148	73	51	25	17	7	8	5	7	0	2	0
2005	Organic	440	90	391	32	8	3	3	1	0	0	2	0	0	0	0	0
	Conventional	1336	2018	610	294	156	87	80	28	19	13	10	13	10	5	6	5
	Sum	1776	2108	1001	326	164	90	83	29	19	13	12	13	10	5	6	5

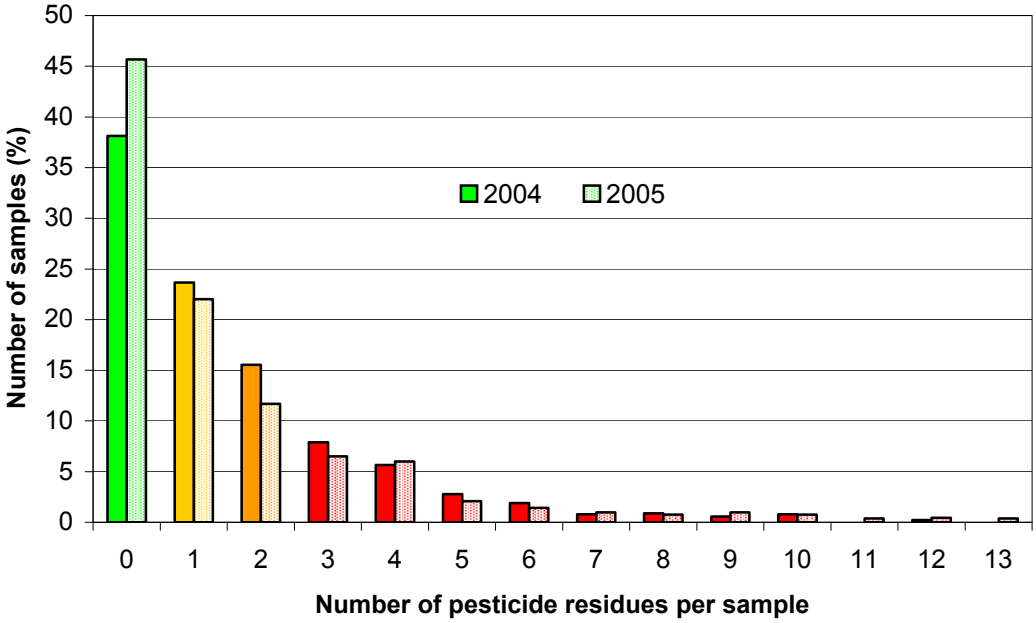

Figure 4: Percentage (%) of conventional farming products contaminated with pesticides in 2004 and 2005

As represented in **Table 11**, it was demonstrated that 90 % of the organic farming products were below LOD, which means for organic farming samples, for almost all the analysed substances, below the authorised maximum value of 10 $\mu g.kg^{-1}$ fresh product. It also showed that the total number of samples with detectable pesticide residues decreased from 62 to 54 % from 2004 to 2005. It was stated as presented in **Figure 4** that the number of conventional farming samples with one pesticide residue decreased from 24 to 22 % and the numbers of conventional farming samples with two and more pesticide residues was reduced from 37 to 32 % between 2004 and 2005.

These data also show that some samples were still very contaminated not only in the concentration but also in the number of different pesticide residues. Still 14 % of conventional farming samples contained from 4 to 13 pesticides in 2004 and in 2005. Less than 2 % of the samples (i.e. 25 samples per year) contained between 10 and even 13 pesticide residues.

This monitoring over 2 years allowed us to build a database in which each foodstuff could be associated to typical pesticide residues and reciprocally as well as to establish a list of

pesticide hits in 2004 and 2005. More details are available in Lesueur et al. [2007a] and in **Appendix 6**.

There was an increase of the number of organic farming samples exceeding MRLs from 3.9 to 8.2 %, but a decrease of the contaminated conventional farming products from 8.4 to 7.7 %. 38 organic farming products presented single residue exceeding the 10 $\mu g.kg^{-1}$ threshold (22 in 2004 and 16 in 2005), twelve organic farming products presented two multiresidues exceeding 10 $\mu g.kg^{-1}$ (two in 2004 and ten in 2005), six organic farming products presented three multiresidues exceeding 10 $\mu g.kg^{-1}$ in 2005 and finally four organic farming product presented four multiresidues exceeding 10 $\mu g.kg^{-1}$ in 2005. We declared 145 conventional farming products with single residue exceeding the MRLs (69 in 2004 and 76 in 2005), 24 conventional farming products with two multiresidues exceeding the MRLs (four in 2004 and 20 in 2005), six conventional farming products with three multiresidues exceeding the MRLs (three in 2004 and three in 2005) and finally four conventional farming product with four multiresidues exceeding the MRLs in 2005. More details concerning the pesticides exceeding MRLs for organic and conventional farming products are obtainable in Lesueur et al. [2007a] and in **Appendix 6**.

6 Conclusions

At first we showed that it is possible to operate a simultaneous screening and quantification of pesticide multiresidues in foodstuff for routine determination with GC-MS.

The development of a simultaneous full scan/SIM mode acquisition GC-MS method following the QuECheRS extraction method for the analysis of 105 GC-amenable pesticides was reliable, repeatable and accurate for the analysis of foodstuff (grape, lemon, onion and tomato) and leaf samples. All the selected substances showed a linear range with correlation factors R^2 higher than 0.99.

A HPLC-IT/MS method following the QuECheRS extraction method for the analysis of 46 HPLC-amenable pesticides was also reliable, repeatable and accurate for the qualitative and quantitative analysis of foodstuff (grape, lemon, onion and tomato) and leaf samples. Three substances, benomyl, acephate and chlorfluazuron, could not be analysed with HPLC-MS because they were rapidely converted to carbendazim (benomyl), thermally labile and pH-labile (acephate) or producing a low signal (chlorfluazuron). Although most of the substances presented a linear range ($R^2 > 0.99$), four substances (furathiocarb, indoxacarb, oxycarboxim and pyraclostrobin) showed quadratic functions with correlation factors R^2 higher than 0.99.

The LODs and LOQs achieved with GC-MS and HPLC-MS were in the same range: from 1 to 400 $\mu g.kg^{-1}$ fresh weight. The LODs were for two thirds of the selected pesticides meeting the authorised values of 10 $\mu g.kg^{-1}$ fresh weight required for organic farming samples but were still too high for the remaining of them. The recovery, repeatability and accuracy of the QuECheRS method with both GC-MS and HPLC-MS were meeting the SANCO Guidelines' requirements for almost all the substances and the matrices (grape, lemon, onion, tomato and leaf) with lower recoveries for the two difficult matrices onion and lemon.

The analysis of proficiency tests with the QuECheRS method showed for more than 80 % of the analytes z-score results in the accepted range. For some substances like azoxystrobin, bromopropylate, bupirimate and iprodione, the results were either over- or underestimated, mainly because of the low concentration in the test or the degradation of the substances in the standards.

For the analysis of 24 herbicides and insecticides from soil samples with GC-MS and HPLC-IT/MS after extraction with four different methods, the QuECheRS method presented the

highest recoveries (median recovery of 72.7 %) followed by the European Guideline DIN EN 12393 (median recovery of 65.7 %) and the PLE (median recovery of 63.5 %) whereas the USE showed the lowest recovery (median recovery of 57.0 %) at a 500 $ng.g^{-1}$ fortification level. Especially, it was shown that only the QuECheRS method and the USE could recover all the selected pesticides whereas the European Guideline DIN EN 12393 could recover neither carbendazim nor metamitron and the PLE could not recover carbendazim, metamitron and monolinuron at 500 $ng.g^{-1}$. The QuECheRS method was the most adapted method with around 50 % of the substances with recoveries in the recommended range of 70-110 %. Some substances presented recoveries as high as 150 % with PLE implying the need for a cleaning step. The ultrasonic energy seemed to be too low to extract the substances that either create bonds with humic substances or adsorb on the inter-crystalline layers of clay minerals. The European Guideline DIN EN 12393 and the PLE were in this study not adapted for the extraction of polar pesticides.

The first findings of the adaptation of a direct thermodesorption injection, increasing the injection volume by a factor 20 compared to liquid injection and reducing the fouling of the device, for the analysis of pesticide residues with GC-MS showed a good repeatability and a quantitative response of the signal for volatile substances. For less volatile substances low signal responses and contamination of the standards in the next runs were reported. An extended design of the thermal desorption system should achieve a quantitative response of the GC-MS for all the substances and avoid their carry-over in the next runs.

Finally, the evaluation of the foodstuff database showed a decrease of the number of conventional farming samples contaminated with pesticides and of conventional farming samples with residues exceeding MRLs between 2004 and 2005. During this period, the number of organic farming samples with residues exceeding the assigned value of 10 $\mu g.kg^{-1}$ increased by a factor 2 from 2004 to 2005. A significant amount of conventional farming samples were found with multiresidue contaminations up to 13 pesticides. It was alarming that some samples presented very high contamination exceeding the authorized MRLs several times.

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Appendix

APPENDIX 1.

Lesueur, C., Gartner, M., 2005. Routine identification and quantification of pesticide multiresidues in fruit and vegetable samples with full scan, SIM and deconvolution reporting software. *Die Ernährung*, 29 (11), pp. 466-471

APPENDIX 2.

Lesueur, C., Knittl, P., Gartner, M., Fuerhacker, M., 2007. Analysis of 140 apolar, middle polar and polar pesticides from conventional farming foodstuff samples after extraction with the modified QuECheRS method and analysis with GC/SQ-MS and HPLC/IT-MS. *Food control*, **submitted**

APPENDIX 3.

Lesueur, C., Gartner, M., Mentler, A., Fuerhacker, M., 2007. Qualitative and quantitative analysis of polar pesticide multiresidues in leaf samples with liquid chromatography – ion trap mass selective detector: validation of the method. *Int. J. Environ. Anal. Chem.*, **in Press** (*Available online since 19 November 2007*)

APPENDIX 4.

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Lesueur, C., Gartner, M., Fuerhacker, M., 2006. First findings of an innovative direct thermal desorption-gas chromatography-mass spectrometry applied to pesticide multiresidue methods In: 1st European Chemistry Congress, 27-31 August 2006, Budapest, Hungary, P48

APPENDIX 6.

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Routine Identification and Quantification of Pesticide Multiresidues in Fruit and Vegetable Samples With Full Scan, SIM and Deconvolution Reporting Software.

Céline Lesueur and Michael Gartner

Die Ernährung, **29** (11), pp. 466-471, 2005

APPENDIX 2.

Analysis of 140 Apolar, Middle Polar and Polar Pesticides From Conventional Farming Foodstuff Samples After Extraction with The Modified QuECheRS Method and Analysis with GC/SQ-MS and HPLC-IT/MS.

Céline Lesueur, Patrik Knittl, Michale Gartner and Maria Fuerhacker

Food control, **submitted, 2007**

APPENDIX 3.

Qualitative and Quantitative Analysis of Polar Pesticide Multiresidues in Leaf Samples with Liquid Chromatography – Ion Trap Mass Selective Detector: Validation of the Method.

Céline Lesueur, Michael Gartner, Axel Mentler and Maria Fuerhacker

Int. J. Environ. Anal. Chem., **in Press** (*Available online since 19 November 2007*), 2007

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Comparison of Four Extraction Methods for the Analysis of 24 Pesticides in Soil Samples with Gas Chromatography – Mass Spectrometry and Liquid Chromatography – Ion Trap – Mass Spectrometry.

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Talanta, **in Press, 2007**

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First Findings of an Innovative Direct Thermal Desorption - Gas Chromatography - Mass Spectrometry Applied to Pesticide Multiresidue Methods

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In: 1st European Chemistry Congress, 27-31 August 2006, Budapest, Hungary, P48

APPENDIX 6.

Pesticide Residues in Fruit and Vegetable Samples: Analytical Results of 2 Year´s Pesticide Investigations

Céline Lesueur, Michael Gartner, Patrik Knittl, Peter List, Stefan Wimmer, Verena Sieler and Maria Fuerhacker

Die Ernährung, 31 (6), pp. 247-259, 2007

Résumé

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Routine Identification and Quantification of Pesticide Multiresidues in Fruit and Vegetable Samples with Full Scan, SIM and Deconvolution Reporting Software

C. LESUEUR, M. GARTNER

Abtract

In our study we developed a gas chromatography/mass spectrometry (GC/MS) system for the monitoring of the currently most common pesticides in fruits and vegetables detectable with gas chromatography. This method allows the simultaneous screening of 113 pesticides and quantification of the 68 most relevant among them as well as the collecting of data for library search which, combined to a deconvolution reporting software, achieves a screening of the samples in a databank of over 560 pesticides. We achieved limits of detection and limits of quantification as low as 2 and 6 µg/kg produce respectively in a wide range of samples and in each case under the required 10 µg/kg limit for biological food after extraction with aceton, partitioning with ethylacetate/cyclohexane (1:1) and clean-up with gel permeation chromatography (GPC).
This Scan/SIM measurement coupled with a Systematic Deconvolution Reporting Software was applied to the pesticide analysis of real food samples and represents a successful and very satisfying tool for the efficient, quick and routine pesticide multiresidue analysis.

Key words:
GC/MS, multiresidue method, pesticides, SIM, full scan

1. Introduction

Since the introduction of DDT as a pesticide in 1939 the worldwide utilisation of synthetic pesticides of diverse structures has incredibly increased, reaching 50.000 tons a year around 1945 and arisen by approximately a 50-fold in 1999. The pesticide use can even be seen higher since their toxicity and biological activity has been increased by a factor 10 in the last 50 years. For these reasons the systematic control of our food has become a high priority.

Pesticides are commonly analysed either by gas chromatography (GC) or liquid chromatography (LC). Nowadays multiresidue methods are more frequently achieved through GC-MS [1, 2, 3, 4, 5, 6] after a customarily sample preparation, which generally entails several steps and serves to the clean-up and the enrichment of the samples.

The sample preparation of pesticides from food matrixes is typically achieved with a tedious, laborious and time-consuming liquid-liquid extraction with typically acetone [7, 8, 9, 5, 6], acetonitrile [3, 10, 4] or ethylacetate [1, 9, 11, 12].

Couples of MS detectors from simple quadropole [3, 5, 6] to triple quadropole [1, 10] through ion trap [11] are employed for the detection of pesticides with GC-MS. Up to now when working with simple quadropole MS systems it was common practice [5, 6] i) to screen the samples without any target list by acquiring a total ion chromatogram (TIC) in full scan, ii) to repeat the measurement in the SIM mode to quantify the samples. For positive samples this meant a minimum of 2 measurements, which was twice more time- and money-consuming.

In the last months Agilent Technologies has developed a software capable of acquiring a full scan parallel and simultaneous to SIM data. This allows the simultaneous screening and quantification of a sample as well as its confirmation through a coupled systematic deconvolution reporting software.

The goal of our study was to apply this newly available possibility for the routine and daily detection of pesticide multiresidues in real fruit and vegetable samples in concentration ranges as low as the so-called biological quality of 10 µg/kg.

2. Experimental

2.1. Materials

Table 1 lists the retention time (min), the target ion and the qualifier ions of the 113 pesticides selected for the full scan screening and the 68 pesticides for the SIM mode quantification. The listed pesticides

have been selected on the ground of their occurrence and relevance as residue in foodstuff as well as according to the their analytical feasibility with GC. Each analyte was provided either from Sigma-Aldrich or from Ehrenstorfer with the highest available purity. The standard salts were at first dissolved in acetone to reach concentrations around 1.000 ng/µl and the single standard solutions were then mixed up altogether to reach final concentrations of 10 ng/µl, 5 ng/µl, 2 ng/µl, 1 ng/µl and 0.2 ng/µl.

Tab. 1: Retention time (min), target and qualifier ions of the substances analysed in full scan and in SIM mode

Analyte	R.T. [min]	Target ion (m/z)	Qualifiers (m/z)	Scan	SIM
Dichlorvos	5.7	185	109, 220	X	
Methamidophos	5.7	94	95, 141, 64	X	
Biphenyl	7.0	154	153, 152	X	X
Propamocarb	7.1	58	188, 143	X	X
o-Phenyphenol	8.8	170	141, 115	X	X
Carbaryl	8.8	144	115, 116, 145	X	X
Methiocarb	9.3	168	153, 109	X	X
Heptenophos	9.7	124	200, 215	X	
Omethoate	10.0	156	110, 109, 79	X	X
Diphenylamine	10.1	169	168, 167, 170	X	X
Tecnazene	10.2	261	215, 203	X	
Ethoprophos	10.7	158	200, 242	X	X
Chlorpropham	11.1	127	213, 171, 153	X	X
Atrazin-desethyl	11.4	172	187, 145	X	
Trifluralin	11.6	306	264, 290, 307	X	
Monocrotophos	11.8	127	67, 192, 97	X	
Dicloran	12.7	206	176, 124, 208	X	X
Dimethoate	12.7	87	93, 125, 143	X	X
Lindan	13.5	219	254, 181	X	
Cyromazin	13.5	151	165, 166	X	
Quintozene	13.7	295	265, 249	X	X
Propyzamide	14.0	173	175, 240, 255	X	X
Pyrimethanil	14.3	198	199, 200	X	X
Diazinon	14.4	304	276, 199	X	
Flufenoxuron	14.5	331	268, 227, 233	X	
Chlorothalonil	15.1	266	264, 268	X	X
Pirimicarb	15.7	166	72, 238, 167	X	X
Chlorpyriphos-methyl	16.6	286	288, 323, 290	X	X
Parathion-methyl	16.6	263	125, 109, 79	X	
Vinclozoline	16.7	285	198, 189, 241	X	X
Tolclofos-methyl	16.8	265	267, 125, 266	X	X
Metalaxyl	17.3	206	160, 132, 249	X	X
Fenitrothion	18.1	277	260	X	X
Terbutryne	18.1	226	185, 241, 170	X	
Primiphos-methyl	18.3	290	276, 305, 125	X	X
Aldrin	18.5	263	261, 265, 293	X	
Malathion	18.8	173	158, 125	X	X
Chlorpyriphos	19.3	314	197, 97, 258	X	X
Parathion	19.4	291	109, 97, 235	X	
Triadimefon	19.5	208	181, 128	X	X
Chlorethal-dimethyl	19.5	301	332, 223	X	X
Dicofol	19.8	139	111, 141, 75	X	X
Heptachlor-exo-epoxid (cis)	20.8	353	355, 351, 357	X	
Cyprodinil	20.8	224	225	X	X
Heptachlor-endo-epoxid (trans)	21.0	183	253, 289, 353	X	
Pendimethanil	21.1	252	281	X	
Penconazole	21.2	248	159, 161, 250	X	X
Tolylfluanid	21.3	181	238, 137	X	X
Captan	21.4	79	149, 80, 77	X	
Thiabendazole	21.4	201	174	X	X
Chlorfenvinphos	21.6	267	269, 323, 325	X	
Folpet	21.8	260	295, 262	X	
Mecarbam	21.8	131	97, 159, 329	X	
Quinalphos	21.8	146	157, 241, 298	X	
Triadimenol	21.9	112	128, 168	X	X
Tetramethrin	21.9	164	123, 165, 81	X	
Paclobutrazole	22.0	236	125, 238, 167	X	
Procymidone	22.1	96	283, 285, 67	X	X
Methidathion	22.4	145	85, 93, 302	X	X
Alpha Endosulfan	22.6	241	207, 195	X	X
Dieldrin	23.9	279	277, 237, 345	X	
Imazalil	23.9	215	173, 217, 175	X	X
Azaconazole	23.9	217	173	X	
p,p'-DDE	24.1	246	318, 248, 316	X	
Fludioxonil	24.4	248	127, 154, 182	X	X
Carboxin	24.5	143	235	X	
Myclobutanil	24.6	179	150, 206, 288	X	X
Buprofezin	24.6	105	172, 106, 249	X	X
Flusilazole	24.7	233	315, 206	X	X
Bupirimate	24.9	273	316, 208	X	X
Kresoxim-methyl	25.0	206	282, 131	X	X
Beta Endosulfan	25.1	195	237, 241, 207	X	X
Cyproconazole	25.1	222	224	X	X
p,p'-DDD	25.8	235	237, 165, 320	X	
Iprodione	26.0	187	189, 244, 246	X	X
Oxadixyl	26.0	163	105, 132, 278	X	
Ethion	26.0	231	153, 97, 233	X	
Triazophos	26.6	161	162, 172, 285	X	
Ofurace	26.7	132	160, 232, 281	X	
Benalaxyl	26.8	148	91, 206, 234	X	X
Quinoxyfen	27.0	237	272, 307, 238	X	X
Fenhexamid	27.1	97	177, 266, 301	X	X
Propiconazole	27.2	259	191, 173	X	
Trifloxystrobin	27.3	116	132, 186, 222	X	X
Endosulfan sulfat	27.4	387	272, 237, 261	X	X
Tebuconazole	27.6	125	250, 70, 207	X	X

Analyte	R.T. [min]	Target ion (m/z)	Qualifiers (m/z)	Scan	SIM
Propargite	27.8	135	173, 335, 350	X	
Piperonyl butoxide	28.0	176	177, 178	X	X
Fenoxycarb	28.8	116	186, 88, 207	X	X
Bromopropylate	28.8	341	339, 343, 183	X	X
Bifenthrin	28.9	181	165, 166, 182	X	X
Fenpropathrin	29.1	181	209, 265	X	
Metconazole	29.3	125	70, 250, 319	X	
Tetradifon	29.6	159	111, 356, 229	X	X
Phosalone	29.8	182	121, 184, 367	X	X
Pyriproxyfen	30.0	136	226, 96	X	X
Lambda-Cyhalothrin	30.2	181	197, 208, 209	X	X
Fenarimol	30.6	139	219, 330, 251	X	X
Bitertanol	31.5	170	152, 141, 112	X	
Permethrin	31.6	183	163, 165, 184	X	
Pyridaben	31.7	147	364, 309	X	X
Fluquinconazole	31.8	340	313, 286, 108	X	
Prochloraz	31.9	180	70, 308, 310	X	X
Fenbuconazole	32.4	129	198	X	
Cyfluthrin	32.5	163	165, 206, 226	X	X
Cypermethrin	33.0	181	209, 163	X	X
Etofenprox	33.1	163	181, 147, 165	X	
Fenvalerate	34.5	167	125, 181, 225	X	
Fluvalinate-tau	34.9	250	252, 251, 209	X	
Difenoconazole	35.3	323	265, 325, 267	X	X
Deltamethrin	36.1	181	253, 251, 255	X	X
Azoxystrobin	36.8	344	388, 345, 75	X	X
Dimethomorph	36.9	301	303, 387, 165	X	X

2.2. Sample preparation

The samples were extracted according to the DFG S19 method [9]. This conventional method consists of an extraction step with acetone followed by partitioning with ethylacetate/cyclohexane (1:1), clean-up by Gel Permeation Chromatography (GPC) and analysis with GC-MS. Aldrin, forbidden for use since over 20 years, was used as internal standard spiked at the partitioning step.

This extraction presents the advantage of providing clean samples concentrated by a factor 20 through the different extraction and cleaning steps.

2.3. Analyses

The analyses were performed on two Hewlett-Packard (Agilent Technologies) GC/MS Model 6890N Series gas chromatography coupled to a 5973N mass selective detector and on a Hewlett-Packard (Agilent Technologies) GC/MS Model 6890N Series gas chromatography coupled to a 5975 mass selective detector. The details concerning our devices are presented in *Table 2*. The Agilent Chemstation

Tab. 2: Gas Chromatograph and mass spectrometer parameters

GC	Agilent technologies 6890N – 5973N	Agilent technologies 6890N – 5975
Autosampler	CTC CombiPal	Agilent Technologies 7683B autoinjector
Autosampler control software	CTC CombiPal Cycle Composer version 1.5.2	
Inlet	EPC Split /Splitless	
Mode	Splitless, 1.0 µl injected	
Inlet Temperature	280 °C	
Pressure	22 to 27 psi (Chlorpyriphos-methyl RT relocked to 16.596 min)	
Purge flow	50.0 ml/min	
Purge time	2 min	
Total flow	55. 9 ml/min	
Gas saver	15 ml/min	
Gas type	Helium	
Inlet liner	Agilent Technologies liner splitless, single-taper, glass woll, deactivated, p/n 5062-3587	
Oven		
Initial temperature	70 °C for 2 min	
Ramp 1	25 °C/min to 150 °C not hold	
Ramp 2	3 °C/min to 200 °C not hold	
Ramp 3	8 °C/min to 280 °C hold for 10 min	
Ramp 4	15 °C/min to 320 °C hold for 2.47 min	
Total run time	47 min (last standard elutes around 37 min)	
Equilibration time	0.5 min	
Column	Agilent Technologies HP 5 MS	
Length	30.0 m	
Diameter	0.25 mm i.d.	
Film thickness	0.25 µm	
Mode	Constant pressure	
Nominal initial flow	2.5 ml/min	
MSD	Agilent Technologies 5973N inert MSD	Agilent Technologies 5975 inert MSD
Tune file	Atune.U	
Mode	Simultaneous full Scan – SIM mode	
Electron energy	- 70 eV	
Solvent delay	3.2 min	
EM voltage	Atune Voltage	
Low mass (m/z)	50	
High mass (m/z)	550	
Threshold		
Sampling	2	
A/D samples		
Scan/s		
Quad temperature	150 °C	
Source temperature	250 °C	

Software G1701DA version D.02.00.237 was used for data analysis.

3. Results and discussion

Figure 1 shows an overlay of the simultaneously acquired full scan and SIM data of a 10 ng/µl (10 ppm) standard. This confirmed that one can truly and trustily acquire scan simultaneous to SIM data without decreasing the selectivity in the SIM mode.

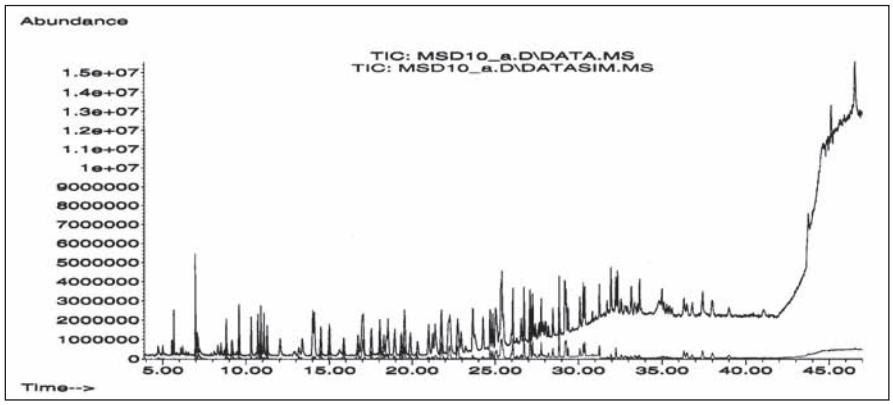

Fig. 1: Scan and SIM data of a 10 ng/µl injection volume (10 ppm) standard

This was confirmed when calibrating the SIM method for the selected 68 analytes as shown in *Table 3* since we obtained a linearity of the calibration curves over two orders of magnitude. Furthermore, with the present SIM method we achieved limits of detection (LOD) as low as 0,3 to 40 pg/µl injection volume and limits of quantification (LOQ) from 1 to 130 pg/µl injection volume for pyrimethanil and cyfluthrin, respectively, as pinpointed in *Table 3*. This corresponds for real samples to a maximal LOD of 2 µg/kg produce and a maximal LOQ of 6 µg/kg produce, far under the biological limit value of 10 µg/kg.

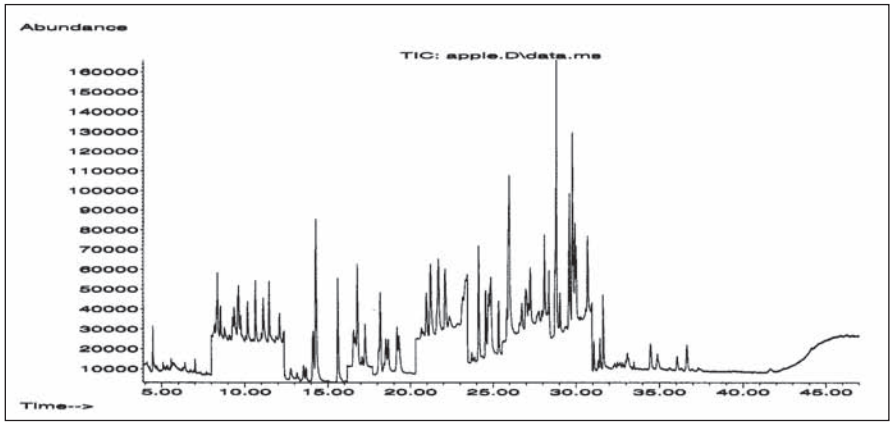

Fig. 2: SIM data of an apple spiked at 200 µg/kg

Figure 2 selects the SIM acquisition of an apple sample spiked at 200 µg/kg attesting a satisfying chromatogram even with matrix effects and interferences.

Finally each simultaneous full scan and SIM acquisition was coupled with a Deconvolution Reporting Software (DRS) as related in *Figure 3* and *Table 4* for a potato sample. Practically the Total Ion Chromatogram (TIC) is used by the DRS for library search, the full scan is required for the qualification of the sample for the selected 113 pesticides and the SIM data are necessary for the quantification of the analytes in the sample. As depicted in *Table 4* the DRS found several pesticides in the TIC of the potato. The proposed pesticides in the potato sample were aldrin, azobenzene and chlorpropham. As already mentioned we use aldrin as internal standard for the extraction and injection in the GC. Azobenzene was not confirmed by both AMDIS and NIST, implying that it was most likely false positive as confirmed in full scan but chlorpropham was confirmed in full scan and quantified at 10 µg/kg with the SIM data.

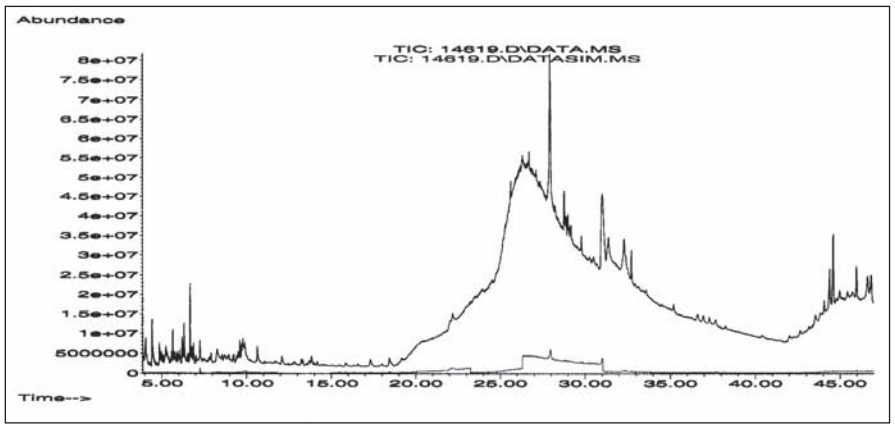

Fig. 3: Full scan and SIM data of a potato sample containing 10 µg/kg chlorpropham

4. Conclusions

Our study shows the new possibility of a simultaneous screening and quantifying of real samples for the routine determination of pesticide multiresidues in produce at level down to biological quality, making possible a quicker and cheaper analysis.

Tab. 3: Calibration in SIM mode, LOQ and LOD in (pg/µl injection volume) for the selected 68 substances

Analyte	SIM mode	LOQ [pg/µl]	LOD [pg/µl]
Biphenyl	10433308*x - 70992, R2=1	1.3	0.4
Propamocarb	11396308*x - 3165671, R2=0.9991	4.5	1.4
o-Phenyphenol	5153053*x - 312910, R2=0.9999	1.6	0.5
Carbaryl	2142727*x + 458631, R2=0.9987	2.4	0.7
Methiocarb	1190963*x + 423901, R2=0.9971	3.7	1.1
Omethoate	1499292*x - 678466, R2=0.9955	15.0	4.5
Diphenylamine	7607888*x - 347546, R2=1	12.9	3.9
Ethoprophos	1843197*x - 299493, R2=0.9997	3.5	1.0
Chlorpropham	2419119*x - 179546, R2=0.9999	2.9	0.9
Dicloran	958174*x - 357177, R2=0.9971	12.9	3.9
Dimethoate	2308907*x - 466824, R2=0.9992	20.2	6.1
Quintozene	445094*x - 117329, R2=0.9987	8.0	2.4
Propyzamide	3435781*x - 463956, R2=0.9996	6.6	2.0
Pyrimethanil	9838309*x - 577297, R2=0.9999	1.0	0.3
Chlorothalonil	799986*x - 73158, R2=0.9998	20.3	6.1
Pirimicarb	4711914*x - 244632, R2=0.9997	1.7	0.5
Chlorpyriphos-methyl	2578156*x - 485384, R2=0.9993	1.5	0.5
Vinclozoline	679641*x - 76340, R2=0.9997	6.5	1.9
Tolclofos-methyl	5071707*x - 622976, R2=0.9998	22.5	6.8
Matalaxyl	1468831*x - 122468, R2=0.9999	11.7	3.5
Fenitrothion	1180029*x - 758619, R2=0.9960	45.3	13.6
Primiphos-methyl	1944252*x - 332985, R2=0.9996	1.9	0.6
Malathion	1627767*x - 563692, R2=0.9983	21.9	6.6
Chlorpyriphos	781199*x - 67639, R2=0.9999	10.2	3.1
Triadimefon	1628374*x - 247393, R2=0.9996	21.4	6.4
Chlorethaldimethyl	2637626*x -83239, R2=1	1.9	0.6
Dicofol	3804692*x + 12608, R2=0.9999	17.8	5.4
Cyprodinil	6795946*x - 379135, R2=0.9999	4.7	1.4
Penconazole	3167033*x - 513886, R2=0.9994	18.1	5.4
Tolylfluanid	492730*x - 127864, R2=0.9986	81.6	24.5
Thiabendazole	4068545*x - 656915, R2=0.9995	11.7	3.5
Triadimenol	2821639*x - 786439, R2=0.9983	18.8	5.6
Procymidone	3280634*x + 86278, R2=1	9.1	2.7
Methidathion	2621667*x - 197116, R2=0.9997	27.1	8.1
Alpha endosulfan	144975*x, R2=1	10.9	3.3
Imazalil	825800*x - 334457, R2=0.9968	16.0	4.8
Fludioxonil	3384455*x - 62556, R2=1	13.4	4.0
Myclobutanil	2366256*x - 601997, R2=0.9987	10.4	3.1
Buprofezin	2840955*x + 108213, R2=0.9998	20.8	6.2
Flusilazole	5175270*x - 1087859, R2=0.9993	11.2	3.4
Bupirimate	1869961*x - 36506, R2=1	9.6	2.9
Kresoxim-methyl	2182717*x - 494157, R2=0.9982	10.6	3.2
Beta endosulfan	118317*x, R2=1	12.0	3.6
Cyproconazole	3141776*x - 785019, R2=0.9987	16.0	4.8
Iprodione	533530*x + 14119, R2=0.9995	20.6	6.2
Benalaxyl	5458211*x - 332316, R2=0.9999	4.7	1.4
Quinoxyfen	4104621*x - 234281, R2=0.9999	5.7	1.7
Fenhexamid	2044195*x - 783391, R2=0.9970	76.8	23.0
Trifloxystrobin	3162219*x - 235538, R2=0.9999	15.2	4.6
Endosulfan sulfat	263612*x, R2=1	11.8	3.6
Tebuconazole	2282812*x + 736842, R2=0.9978	29.6	8.9
Piperonyl butoxide	4919988*x - 260056, R2=0.9994	6.2	1.9
Fenoxycarb	3144298*x - 1323051, R2=0.9955	11.0	3.3
Bromopropylate	2675401*x - 587237, R2=0.9990	21.8	6.5
Bifenthrin	10489183*x - 676854, R2=0.9999	3.1	0.9
Tetradifon	1627766*x - 43866 , R2=1	35.3	10.6
Phosalone	1643579*x - 106545, R2=0.9988	29.4	8.8
Pyriproxyfen	9608755*x - 811342, R2=0.9999	5.0	1.5
Lambda-Cyhalothrin	1315031*x, R2=1	66.1	19.8
Fenarimol	1988455*x - 318382, R2=0.9995	12.8	3.8
Pyridaben	9873779*x - 1583458, R2=0.9994	7.2	2.2
Prochloraz	1312650*x - 930584, R2=0.9871	13.8	4.1
Cyfluthrin	1115717*x - 324293, R2=0.9929	129.5	38.8
Cypermethrin	1228954*x - 622876, R2=0.9949	49.8	14.9
Difenoconazole	2208808*x - 1427243, R2=0.9890	11.6	3.5
Deltamethrin	758517*x - 642012, R2=0.9840	39.0	11.7
Azoxystrobin	2798414*x - 974141, R2=0.9975	8.6	2.6
Dimethomorph	3840589*x - 1294430, R2=0.9974	26.5	7.9

MSD Deconvolution Report
Sample Name: 400 µl
Data File: O:\msdchem\1\DATA\PE05605\14619.D
Date/Time: 12:00:03 PM Tuesday, Oct 4 2005

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
4.0249	108394	m-Cresol		42	-22.8		
4.0249	95487	2-Methylphenol		47	-12.2		
4.0249	20739586	2-Octyn-1-ol				68	1
4.0249	29474111	1-Hydroxymethyl-2-methyl-1-cyclohexene				70	1
9.4716	25013165	Butylated hydroxyanisole		62	74.1		
9.4716	121006	3-tert-Butyl-4-hydroxyanisole				68	1
9.8539	84662	Diethyl phthalate		70	-6.1		
9.8539	112068016	S-(-)-1,1-Diphenylprolinol				70	1
10.6672	103333	AZOBENZENE IUPAC NAME : diphenyldiazeneE : diphenyldiazeneE : diphenyldiaze		44	4.7		
10.6672	22319295	4-Hepten-3-one, 5-ethyl-2,4-dimethyl-				72	1
11.1695	101213	CHLORPROPHAM IUPAC NAME : isopropyl 3-chlorocarbanilate TRADE NAMES: CIPC, CH		41	7.1	52	26
18.4624	309002	Aldrin		90	-4.7	80	1
29.8401	117817	Bis(2-ethylhexyl)phthalate		92	12.0	84	3
13.748		Phenanthrene-d10	10				

Tab. 4: Report for potato sample containing 10 µg/kg chlorpropham generated from the deconvolution reporting software

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Analysis of 140 pesticides from conventional farming foodstuff samples after extraction with the modified QuECheRS method

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Abstract

Pesticides in foodstuff are becoming a major issue due to their intensive use in agriculture. Thus an appropriate control of their residues in food samples has to be operated. In this study we analysed 105 pesticides with GC/SQ-MS and 46 pesticides with HPLC/IT-MS after extraction with the QuECheRS method in four matrices (grape, lemon, onion and tomatoes). For GC-amenable substances, the LOD and LOQ ranged from 0.4 to 48.2 μg/kg and from 1.2 to161 μg/kg, respectively. For HPLC-amenable substances, they varied from 1.0 to 115 μg/kg and from 3.3 to 382 μg/kg, respectively. With GC/MS, 61–82% of the substances showed a recovery in the range of 70–110% and 6–30 % presented a recovery higher than 110% at the 500 μg/kg fortification level. With HPLC/MS, 87–93% of the substances presented recoveries in the range of 70–110% at the 500 μg/kg fortification level compared to 78–85% at the 50 μg/kg fortification level. Lemon and onion showed poor recoveries but are known to be difficult matrices (high acidic and high sulfur content, respectively). The method was proved to be repeatable with RSD lower than 20% at 500 μg/kg and lower than 15% at 50 μg/kg with both devices.

Keywords: Gas chromatography; Liquid chromatography; Mass spectrometry; Pesticide residues; Foodstuff

1. Introduction

More and more different pesticides are used nowadays in agriculture. Since pesticides are potentially harmful to the environment and consequently to human beings through the consumption of pesticide contaminated food and water, the European Community established maximum residue levels (MRLs), based on the assumption that good agricultural practice is applied at the use of pesticides in farming, for pesticide residues in water (Commission of the European Communities, 2000) and foodstuff (Commission of the European Communities, 1990).

As a consequence food commodities have to be controlled to assure the non-violation of the MRLs. For apolar and middle polar pesticides, the detection of pesticide residues is commonly achieved through analysis with gas chromatography (GC) coupled to single quadropole (SQ) and, less frequently, triple quadropole (QQQ) mass spectrometers (MS) after extraction according to the DIN Norm 12393 parts 1–3 (DIN EN 12393, 1998) adapted from the German Norm DFG S19 (DFG-Methode S19, 1999) or according to the Luke Method (Luke, Froberg, Doose, & Masumato, 1981) and more recently according to the (modified) QuECheRS (standing for "Quick, Easy, Rugged and Safe") method (Anastassiades & Lehotay, 2003) drafted as European Norm (CEN/TC 275 & Draft, 2006). The analysis of foodstuff with GC/MS has already been successfully implemented in numerous laboratories for the determination of pesticide multiresidues in food samples (Díez, Traag, Zommer, Marinero, & Atienza,

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2006; Leandro, Fussell, & Keely, 2005; Lesueur & Gartner, 2005; Lesueur et al., 2007; Martínez Vidal, Arrebola Liébanas, González Rodríguez, Garrido Frenich, & Fernández Moreno, 2006; Stan, 2000; Štajnbaher & Zupančič-Kralj, 2003).

As far as polar pesticides are concerned and since these pesticides have been emerging in the recent years on the market, there is a crucial need for new extraction and detection methods. The pesticide detection is generally achieved with liquid chromatography (HPLC) mass spectrometry after extraction with methanol known as the Alder method (Klein & Alder, 2003) and drafted as European Norm (DFG-Methode 704) or with acetonitrile according to the modified QuECheRS method as already mentioned (Anastassiades & Lehotay, 2003; CEN/TC 275, N 236, 2006). The detection, i.e. qualification and quantification of polar pesticides from food samples was until recently achieved with HPLC/QQQ-MS, because QQQ are stable, sensitive, selective and have a wide linear range (Garrido Frenich, Martínez Vidal, López López, Cortés Aguado, & Martínez Salvador, 2004; Hernández et al., 2006; Jansson, Pihlström, Österdahl, & Markides, 2004; Leandro, Hancock, Fussell, & Keely, 2007; Mol, van Dam, & Steijger, 2003; Pizzutti et al., 2007; Sannino, Bolzoni, & Bandini, 2004) in MRM (multiple reaction monitoring) mode and unlike SQ can discard false positive results. Other detectors like time-of-flight (TOF) and ion trap (IT) mass detectors were up to now principally used as confirmation and identification systems based on their exact mass measurement capacity (TOF) or operation in MS^n mode (IT) but lower sensitivity and linearity. Nevertheless, recent publications report the development of HPLC/IT-MS methods for the detection of pesticide multiresidues in foodstuff (Blasco, Font, & Picó, 2004, 2005; Lesueur, Gartner, Mentler, & Fuerhacker, in press; Soler, Manes, & Picó, 2004a, 2004b, 2006; Zrostlíková, Hajšlová, Kovalczuk, Štepán, & Poustka, 2003).

The goal of our work was to validate the modified QuECheRS method for food samples according to the SANCO guidelines (Commission of the European Communities, 2002, 2006) in combination with GC/SQ-MS and HPLC/IT-MS for the multiresidue analysis of a selection of 140 apolar, middle polar and polar pesticides.

2. Material and methods

2.1. Reagents and chemicals

Pesticide standards were purchased either from Dr. Ehrenstorfer or from Sigma–Aldrich with the highest available purity. Magnesium sulfate anhydrous, sodium chloride and sodium citrate dihydrate were purchased from J.T. Baker, di-sodium hydrogen citrate sesquihydrate was provided from Fluka and Bondesil-PSA 40 μm was from Varian. Ultra-residue reagent acetone, ultra-residue reagent ethylacetate, ultra-residue reagent acetonitrile, HPLC/MS grade methanol, ultra-HPLC/MS grade water and HPLC/MS grade formic acid were purchased from J.T. Baker.

Single standard stock solutions were prepared dissolving 10 mg of standard in 10 ml acetonitrile and further diluted with acetonitrile to 10 μg/ml. GC/MS and HPLC/MS multicompound standard stock solutions were prepared dissolving 10 mg of each standard in 1000 ml acetonitrile (10 μg/ml) and further diluted with acetonitrile to achieve concentrations of 5 μg/ml, 2 μg/ml, 0.5 μg/ml, 0.2 μg/ml and 0.05 μg/ml. 10 μg/ml matrix-matched multicompound standards were obtained by evaporating 10 ml of a 10 μg/ml acetonitrile standard in a gentle stream of nitrogen until almost dryness and dissolving it with a blank extract of lemon, grape, onion or tomato up to 10 ml. Each 10 μg/ml standard was then further diluted with the corresponding matrix blank extract to 5 μg/ml, 2 μg/ml, 0.5 μg/ml, 0.2 μg/ml and 0.05 μg/ml. The single and multicompound standards were stored at 4 °C in the dark.

Blank samples used for the fortification experiments and the matrix-matched standards were fruit and vegetable samples extracted with the QuECheRS method and presenting no residues with GC/MS and HPLC/MS.

2.2. Sample preparation

The fruit and vegetable samples were prepared with the modified QuECheRS method (Anastassiades & Lehotay, 2003; CEN/TC 275, N 236, 2006). Roughly summarised it consisted of (1) homogenise around 500 g food samples; (2) weigh 10 g previously chopped fresh sample into a 50 ml teflon centrifuge tube; (3) add 10 ml acetonitrile (ACN) and shake the sample vigorously for 1 min using a vortex mixer; (4) add 4 g magnesium sulfate anhydrous ($MgSO_4$), 1 g sodium chloride (NaCl), 1 g sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and 0.5 g di-sodium hydrogen citrate sesquihydrate ($C_6H_6Na_2O_7 \cdot 1.5H_2O$) and vortex immediately for 1 min; (5) for acidic sample add 600 μl of a 6 N NaOH solution to reach a pH value between 5 and 5.5; (6) centrifuge the extracts for 3 min at 5000 rpm; (7) transfer a 6 ml aliquot of the upper layer into a 15 ml teflon centrifuge tube containing 150 mg PSA and 950 mg $MgSO_4$; (8) centrifuge the extracts for 3 min at 5000 rpm; (9) filter through 0.45 μm filter; (10) transfer 1.5 ml of the extract into an autosampler vial containing 15 μl of a 5% formic acid solution (for the stabilisation of the extracts) for GC/MS and HPLC/MS analysis; (11) for the analysis with GC/MS, evaporate the extract to dryness before dilution in 150 μl acetone/ethylacetate (1:1, v/v).

Triphenylphosphate (TPP) was used as internal standard and spiked at the initial step to reach a 1 μg/ml concentration in the HPLC/MS extract and a 10 μg/ml concentration in the GC/MS extract.

2.3. Analyses

The GC/MS analyses were performed on 3 Hewlett-Packard (Agilent Technologies) GC/MS Model 6890 N

Series gas chromatography coupled to 5973 N and 5975 mass selective detectors. A HP 5 MS (30 m × 0.25 mm i.d.) (Agilent Technologies, Waldbronn, Germany) fused silica capillary column with a 0.25 μm film thickness was used with helium as carrier gas at a constant pressure daily adjusted (chlorpyriphos-methyl RT relocked to 16.596 min). 1.0 μl of the sample was injected in the splitless mode at 280 °C. The GC oven was operated with the following temperature program: initial temperature 70 °C held for 2 min, ramped at 25 °C/min to 150 °C not held, followed by a ramp of 3 °C/min to 200 °C not hold, followed by another ramp of 8 °C/min to 280 °C held for 10 min and finally ramped to 320 °C at 15 °C/min held for 2.47 min. The total run time was 47 min. The interface was kept at 250 °C, the quadropole at 150 °C and the mass spectra were obtained at an electron energy of 70 eV. The Agilent Chemstation Software G1701DA version D.02.00.237 was used for data analysis and the analyses were operated on the principle of a simultaneous full scan/SIM mode method presented elsewhere (Lesueur & Gartner, 2005; Lesueur et al., 2007). The GC/MS analyses were carried out in 2 runs with 2 full scan/SIM mode methods containing each around 55 substances.

The high-performance liquid chromatography system was an Agilent Technologies HP-1100 Series (Agilent Technologies, Waldbronn) controlled with the Agilent Technologies Chemstation for LC 3D System Software. Chromatographic separation was achieved using a Zorbax SB-C18 analytical column 2.1 × 150 mm, 3.5 μm particle size from Agilent Technologies at a flow rate of 300 μl/min. The mobile phases consisted of (A) H_2O–MeOH, 90–9.95% (v/v) with 0.05% HCOOH and (B) H_2O–MeOH, 9.95–90% (v/v) with 0.05% HCOOH. The gradient was 100% A at 0 min, 100% A at 1 min, 0% A at 10 min, 0% A at 17 min, 100% A at 20 min. The post time was 2 min with 100% A and the stop time 22 min. The HPLC system was interfaced to an Agilent Technologies mass spectrometer LC/MSD trap XCT Plus (Agilent Technologies, Waldbronn) equipped with an electrospray ionisation (ESI) interface operated in positive mode and controlled with the Agilent Technologies LC/MSD trap software 5.3. The nebulizer gaz (nitrogen) pressure was 40 psi, the drying gas flow rate was 8 ml/min and the drying gas temperature was 325 °C. The capillary voltage was −4500 V, the endplate offset was fixed at −500 V. The ion trap was operated in the ion charge control (ICC) mode with a target ion count of 150,000 and a maximum accumulation time of 50 ms. The IT mass detector operated in full scan and selected ion monitoring (SRM) modes for confirmation with a 0.6 V fragmentation amplitude as already presented elsewhere (Lesueur et al., in press).

The identification and confirmation of the pesticides were realised like recommended by the European SANCO Guidelines (Commission of the European Communities, 2002, 2006). In this point, the basic premise is that a correct identification requires 3-ion criteria for permitted substances and 4-ion criteria for banned substances. This implies thereby with SQ mass detectors 1 target ion and 2 qualifiers for permitted substances and 1 target ion and 3 qualifiers for banned substances whereas this is reduced to 1 precursor ion and 2 product ions with an IT mass detector, which was achieved when possible.

2.4. Pesticide selection and validation study

We selected 105 pesticides for the GC/MS analysis and 46 for the HPLC analysis, based on their relevance in food samples and chromatographic properties, for the validation of the QuECheRS method according to the European SANCO Guideline (Commission of the European Communities, 2006) in 4 reference matrices: tomato (high water content), lemon (high acid content), grape (high sugar content) and onion (high sulfur content). Nine pesticides (carbaryl, cyromazine, dimethoate, imazalil, monocrotophos, omethoate, paclobutrazol, propamocarb and thiabendazole) were analysed with both GC/MS and HPLC/MS.

LOD and LOQ were estimated in the full scan mode for the GC/MS and HPLC/MS analyses for the target ion and the parent ion, respectively, as the lowest concentration injected that yielded to a S/N ratio of 3 and 10, respectively. Accuracy and precision (i.e. repeatability expressed in term of relative standard deviation (RSD, %)) of the method were tested with recovery experiments, performed with seven replicates of blank samples spiked at about 10 times the LOQ, i.e. 500 μg/kg for GC/MS analysis and at about the LOQ (50 μg/kg) and 10 times the LOQ, i.e. 500 μg/kg for HPLC/MS analyses. The spiked samples were allowed to stand for 30 min before extraction to allow the pesticides to penetrate into the matrix.

3. Results and discussion

3.1. Validation of the method

3.1.1. Linearity of the standard in matrix-matched standards

The 105 GC-amenable substances showed linearity in the SIM mode over a concentration range of 0.050–10 μg/ml for the standards in acetonitrile. For all the substances we obtained correlation factors R^2 higher than 0.99 as already reported elsewhere (Lesueur & Gartner, 2005). Most of the 46 HPLC-amenable substances were linear over a concentration range between 0.05 and 5 μg/ml or 0.05 and 2 μg/ml as already presented elsewhere (Lesueur et al., in press) with correlation factors R^2 higher than 0.99. Avermectin B1a, which had a higher LOQ, was linear between 0.2 and 10 μg/ml with R^2 higher than 0.99. Four substances (furathiocarb, indoxacarb, oxycarboxim and pyraclostrobin) did not show linear ranges but quadratic functions with correlation factor R^2 higher than 0.99.

Linear ranges over two orders of magnitude have also been reported in the MS, MS^2 and MS^3 modes with IT mass detectors (Blasco et al., 2004, 2005; Soler et al., 2004a, 2004b, 2006). Linear ranges of three orders of magnitude have even been reported in MS^3 mode for pesticides

Table 1
GC/MS fortification experiments (recovery and repeatability) at a 500 μg/kg fresh weight fortification level

Substance	Fortification concentration (μg/kg)	Grape		Lemon		Onion		Tomato	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Atrazin-desethyl	500	99	5.8	88	9.9	79	16.8	100	7.2
Azoxystrobin	503	119	1.2	114	7.8	97	10.2	114	8.2
Benalaxyl	503	106	3.5	86	5.6	83	9.7	87	10.9
Bifenthrin	503	100	3.5	76	6.3	79	8.1	77	10.8
Biphenyl	573	62	10.9	32	10.6	50	10.5	58	4.0
Bitertanol	530	110	3.7	115	4.7	106	11.5	108	8.1
Bromopropylate	508	112	3.2	117	8.2	80	9.8	93	10.0
Bupirimate	525	99	4.7	83	6.8	66	10.2	88	10.4
Buprofezin	505	108	5.2	73	7.1	83	10.6	83	10.7
Carbaryl	550	92	5.1	77	5.4	49	11.3	92	10.3
Carboxin	503	85	4.4	65	10.7	48	11.9	74	9.3
Chlorfenvinphos	685	98	2.9	106	9.9	96	11.4	110	5.5
Chlorothalonil	510	102	14.5	76	9.2	119	6.6	82	4.2
Chlorpropham	505	94	2.9	94	7.4	75	9.6	94	9.9
Chlorpyrifos	515	105	3.7	86	7.1	76	9.9	91	9.1
Chlorpyrifos-methyl	543	103	3.1	107	9.4	70	8.6	96	10.5
Chlorthaldimethyl	520	102	2.5	82	6.5	74	9.5	90	9.3
Cyfluthrin	505	112	4.0	118	4.2	89	11.0	103	10.2
Cypermethrin	543	113	2.1	119	5.3	89	10.5	114	9.3
Cyproconazole	500	110	3.3	85	5.3	86	11.2	89	10.4
Cyprodinil	505	108	2.3	78	5.8	74	9.7	88	10.0
Cyromazine	500	46	11.2	32	9.0	29	3.9	43	10.1
Deltamethrin	530	117	8.7	119	8.3	85	9.8	111	5.0
Diazinon	640	93	3.7	77	10.8	78	9.9	87	10.1
Dichlorvos	655	81	9.5	76	9.0	73	11.2	70	15.2
Dicloran	515	94	5.2	93	10.3	91	9.6	99	8.6
Dicofol	498	118	7.1	90	7.9	74	12.3	92	9.3
Dieldrin	508	101	2.9	86	11.7	83	11.2	93	6.2
Difenoconazole	490	114	1.4	118	6.1	96	11.5	119	11.2
Dimethoate	515	116	4.0	117	9.0	106	12.3	132	7.4
Dimethomorph	510	119	1.8	120	6.3	104	13.1	118	10.8
Diphenylamine	495	86	2.1	72	6.3	64	13.1	81	10.8
Ethion	675	112	3.1	97	10.8	90	11.5	101	5.9
Ethoprophos	723	94	4.9	76	8.3	81	9.4	89	11.7
Fenarimol	525	114	2.8	105	5.3	83	11.9	94	10.7
Fenbuconazole	503	112	3.3	119	10.1	105	12.6	107	8.3
Fenhexamid	505	119	1.6	119	9.2	83	10.2	115	11.0
Fenithrothion	755	114	3.4	120	6.7	75	8.5	111	9.9
Fenoxycarb	505	112	6.5	119	5.7	96	13.3	115	9.2
Fenpropathrin	520	102	2.1	98	11.2	91	11.3	97	7.7
Fenvalerate	555	120	1.8	119	10.2	110	12.4	111	8.4
Fludioxonil	510	78	8.8	105	6.4	86	10.6	101	9.2
Fluquinconazole	535	100	3.5	92	10.8	96	12.0	98	8.9
Flusilazole	555	107	3.7	85	5.8	82	11.1	89	11.0
Fluvalinate-tau	468	119	2.6	119	5.5	120	10.8	112	10.5
Heptachlor-exo-epoxid	543	101	3.7	83	10.9	83	10.3	90	7.6
Heptaclor-endo-epoxid	535	103	3.6	75	10.5	77	12.2	90	3.8
Heptenophos	680	96	7.4	84	6.7	89	9.3	99	10.7
Imazalil	510	33	10.7	92	8.5	78	10.9	88	11.1
Iprodione	508	119	4.1	93	11.2	58	11.3	99	11.1
Kresoxim-methyl	518	107	5.6	88	4.8	87	10.9	91	11.3
Lindane	515	119	4.4	117	7.7	117	6.1	119	4.3
Malathion	603	111	2.4	102	10.1	76	9.3	94	6.8
Mecarbam	643	111	4.0	97	9.8	94	11.5	106	6.2
Metalaxyl	510	104	3.6	86	6.0	77	10.6	90	9.3
Metconazole	588	96	3.3	105	10.2	87	8.0	92	9.2
Methamidophos	483	81	10.4	44	17.3	119	6.6	128	9.8
Methidathion	533	118	3.4	111	11.7	75	11.1	108	9.0
Methiocarb	520	82	10.2	116	8.5	40	8.0	94	9.8
Monocrotophos	503	116	5.9	119	7.6	83	11.6	73	5.8
Myclobutanil	513	108	4.6	85	5.6	83	10.7	90	10.1

(*continued on next page*)

Table 1 (*continued*)

Substance	Fortification concentration (μg/kg)	Grape		Lemon		Onion		Tomato	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
o,p′-DDT	505	95	4.0	77	11.2	71	8.7	78	5.7
Ofurace	495	113	5.8	118	5.9	78	5.3	110	11.3
Omethoate	513	105	11.9	112	11.3	116	11.5	120	5.0
o-Phenylphenol	535	85	3.2	77	5.2	71	9.0	86	9.8
Oxadixyl	528	106	4.4	106	11.3	83	11.0	98	6.6
p,p′-DDD	520	111	3.2	93	10.5	93	11.4	102	4.8
p,p′-DDE	523	95	3.3	76	10.9	78	10.4	83	3.8
p,p′-DDT	530	90	7.3	95	13.3	80	10.5	90	9.6
Paclobutrazol	510	110	4.2	97	10.7	95	11.7	99	6.1
Parathion	653	108	4.6	95	10.9	95	11.7	101	6.5
Parathion-methyl	500	120	6.1	112	10.4	100	11.8	114	7.2
Penconazole	508	112	3.1	86	5.9	80	9.9	93	10.9
Pendimethalin	513	100	3.8	84	8.6	84	10.5	93	5.9
Permethrin	478	95	10.1	103	11.2	93	11.4	98	6.9
Phosalone	510	109	4.5	116	6.9	87	11.3	99	9.2
Piperonyl butoxide	175	99	8.0	85	6.1	77	11.3	86	11.6
Pirimicarb	503	72	4.7	83	5.7	64	12.1	90	11.4
Pirimiphos-methyl	658	103	1.9	90	6.5	72	10.1	93	9.7
Prochloraz	495	111	8.8	115	3.9	84	7.3	116	7.1
Procymidone	500	113	4.1	84	5.6	83	10.1	91	11.4
Propamocarb	715	40	7.6	72	11.2	58	5.4	73	10.7
Propargite	538	110	3.3	116	3.7	55	8.4	107	8.8
Propiconazole	540	115	4.5	111	11.4	98	13.1	113	6.1
Propyzamide	495	98	3.0	91	6.7	74	9.3	93	11.0
Pyridaben	510	111	5.1	105	10.1	82	9.7	91	10.2
Pyrimethanil	543	91	3.6	81	6.7	69	8.9	88	11.1
Pyriproxyfen	503	110	3.3	118	10.5	81	10.4	90	10.6
Quinalphos	733	113	2.9	95	6.5	82	10.8	98	10.3
Quinoxyfen	515	115	7.2	102	4.4	55	7.0	102	10.6
Quintozene	533	79	2.2	71	8.1	63	7.7	77	9.4
Tebuconazole	498	109	3.4	93	5.8	83	9.7	92	10.5
Tecnazene	503	81	10.3	58	6.1	69	8.3	77	9.2
Terbutryne	503	102	4.1	86	11.6	84	10.6	94	6.6
Tetradifon	505	118	6.1	120	8.1	78	10.4	120	7.1
Tetramethrin	500	101	3.3	99	11.4	92	11.8	100	7.4
Thiabendazole	508	109	7.3	119	11.5	102	12.9	106	7.5
Tolclofos-methyl	728	102	2.2	89	7.3	70	8.8	91	9.7
Tolylfluanid	500	102	5.0	95	6.7	74	7.9	62	11.0
Triadimefon	528	106	4.5	88	8.1	78	10.2	91	11.4
Triadimenol	503	113	4.2	87	5.4	90	11.5	90	10.1
Triazophos	463	113	8.8	120	9.3	110	12.2	119	8.5
Trifloxystrobin	523	107	1.4	89	4.7	86	9.9	93	11.2
Trifluralin	520	92	4.2	75	10.7	82	9.4	88	8.5
Vinclozoline	510	103	2.7	85	7.6	68	8.6	94	10.1

in orange matrix-matched standards (Soler et al., 2004a). Nevertheless it seems that QQQ detectors are more linear (Sannino et al., 2004) although many studies report linear ranges of two orders of magnitude too (Hernández et al., 2006; Mol et al., 2003).

3.1.2. LOD and LOQ

With GC/MS the LOD and LOQ were between 0.4 and 48.2 μg/kg and 1.2 and 161 μg/kg, respectively. Less than 20% of the substances presented LOQ lower than 10 μg/kg whereas between 50 and 85% of the LOD, depending on the matrix, were below this limit. With HPLC/MS we achieved LOD and LOQ between 1.0 and 115 μg/kg and 3.3 and 382 μg/kg. Furathiocarb showed independent from the matrix the lowest LOD and LOQ and omethoate and avermectin B1a the highest ones, respectively. The 10 μg/kg threshold was in most cases exceeded for the LOQ whereas it was achieved for the LOD for 67–79% of the substances.

The reported LOD and LOQ in the literature for IT detectors are varying a lot depending on the tediousness of the preparation method. Blasco et al. (2004, 2005) as

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well as Soler et al. (2004a, 2004b, 2006) have been intensively working on the detection of pesticides in foodstuff with HPLC/IT-MS and reported LOQ from a few μg/kg to the higher μg/kg range. After a quite simple extraction with ethyl acetate, Soler et al. (2004a) achieved LOD of 0.5–20 μg/kg for six pesticides in oranges and LOQ in the range of 10–400 μg/kg for nine pesticides in oranges and strawberries (Soler et al., 2004b) with HPLC/IT-MS. Blasco et al. (2004) studied the quantification of six pesticides in oranges in the MS, MS^2 and MS^3 modes and reported LOQ between 0.5 and 10 μg/kg in MS mode, between 1 and 200 μg/kg in MS^2 mode and between 1 and 300 μg/kg in MS^3 mode. Zrostlíková et al. (2003) analysed 17 pesticides in apples and apricots with HPLC/IT-MS after extraction with acetonitrile and SPE and reported LODs between 0.1 and 10 μg/kg and LOQ from 2 to 24 μg/kg.

The obtained LOD and LOQ are so far not for all substances fulfilling the 10 μg/kg threshold required for organic farming foodstuff. This can be overcome on the one hand by an optimisation of the mass detectors and on the other hand by the introduction of an additional concentration and/or clean-up step in the extraction method. Both solutions are being now considered in our laboratory.

Table 2
HPLC/MS fortification experiments (recovery and repeatability) at a 50 μg/kg fresh weight fortification level

Substance	Fortification concentration (μg/kg)	Grape		Lemon		Onion		Tomato	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Acetamiprid	55.4	101	7.1	68	2.7	88	6.0	81	6.3
Aldicarb	50.0	68	5.6	50	6.6	87	2.8	81	3.4
Avermectin B1a	51.0	<LOQ	–	<LOQ	–	<LOQ	–	<LOQ	–
Bendiocarb	54.4	102	3.9	59	7.3	118	6.7	116	2.8
Butocarboxim	49.1	67	5.1	50	6.5	87	2.8	81	3.5
Carbaryl	49.9	97	3.4	79	4.8	119	9.1	120	3.5
Carbendazim	50.5	75	3.2	86	6.5	80	4.8	79	5.8
Carbofuran	50.9	68	1.0	49	1.8	89	4.5	69	3.6
Clomazone	47.3	94	4.5	64	2.4	75	5.0	79	4.2
Clothianidin	51.2	85	7.1	56	6.3	95	2.9	103	6.6
Cyromazine	50.4	40	1.9	23	1.9	42	3.2	36	10.8
Demeton-S	45.6	79	3.6	67	3.8	72	3.1	83	7.4
Dicrotophos	75.0	86	6.9	65	4.2	89	5.4	80	6.6
Diflubenzuron	51.4	89	7.2	70	6.1	87	5.6	96	10.2
Dimethoate	52.9	92	5.0	62	5.1	94	4.9	89	4.5
Diuron	51.9	89	5.8	58	5.6	79	9.0	84	4.6
Ethiofencarb	54.1	71	1.8	91	5.6	97	4.0	116	6.0
Ethirimol	49.5	58	9.3	51	1.5	58	3.6	66	1.5
Fenamiphos	54.1	104	1.8	97	4.7	89	2.5	94	3.9
Fenpyroximate	49.0	97	6.0	56	3.5	85	11.5	88	9.8
Fenthion	83.5	93	5.6	77	4.6	71	5.2	83	4.8
Flufenoxuron	51.0	<LOQ	–	73	6.7	44	5.3	99	9.9
Furathiocarb	48.5	80	5.4	65	8.3	80	9.3	94	10.5
Hexythiazox	53.4	73	2.4	70	2.1	<LOQ	–	83	3.0
Imazalil	49.5	92	2.0	118	6.4	84	3.2	70	2.8
Imidacloprid	52.7	92	2.4	48	11.1	78	6.3	85	5.4
Indoxacarb	56.7	104	10.0	60	11.0	86	7.5	93	10.0
Linuron	50.3	93	6.6	62	4.3	92	7.0	84	6.8
Metamitron	50.9	96	6.0	68	3.7	93	2.4	86	4.4
Methabenzthiazuron	50.9	92	4.6	54	3.9	79	4.9	81	2.8
Metobromuron	52.9	93	3.8	60	9.6	76	4.5	83	3.2
Metoxuron	50.0	94	7.8	67	3.0	91	4.7	83	5.0
Monocrotophos	49.5	<LOQ	–	<LOQ	–	52	2.0	<LOQ	–
Monolinuron	50.4	95	3.3	65	5.8	88	6.0	85	7.6
Omethoate	49.7	<LOQ	–	<LOQ	–	<LOQ	–	<LOQ	–
Oxycarboxim	50.9	50	4.3	56	5.3	79	7.7	117	7.9
Paclobutrazol	50.7	96	1.0	57	4.8	92	4.7	90	2.1
Pencycuron	49.4	86	2.8	54	4.5	104	10.0	85	5.5
Propamocarb	50.3	39	4.1	45	5.1	57	7.7	61	7.0
Pyraclostrobin	50.9	92	6.5	79	4.9	86	3.6	91	4.3
Rotenone	49.2	107	5.5	71	8.0	114	5.8	104	6.7
Tebufenozide	55.4	91	2.9	66	2.5	117	10.4	94	4.1
Thiabendazole	50.8	80	2.4	58	2.2	79	2.0	81	3.6
Thiamethoxam	51.9	<LOQ	–	<LOQ	–	<LOQ	–	<LOQ	–
Thiofanox	52.9	71	4.3	69	5.8	93	4.6	90	5.2
Triflumuron	53.9	91	11.0	55	12.1	54	7.5	93	9.3

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3.1.3. Recovery

Table 1 presents the recovery and repeatability of the fortification experiments at 500 μg/kg for the four selected matrices with GC/MS whereas Tables 2 and 3 show the recovery and repeatability of the fortification experiments at 50 and 500 μg/kg, respectively for the same matrices with HPLC/MS.

The recovery of the pesticides (calculated with multi-compound standards in acetonitrile) was depending on the substance and the matrix, less on the fortification level. The recovery of the substances analysed with GC/MS tended to be high: 61–82% of the GC-amenable pesticides presented a recovery in the range of 70–110% as recommended by the SANCO Guideline (Commission of the European Communities, 2006) whereas 6–30% of the substances presented a recovery higher than 110%. Less than 5% of the substances showed recoveries lower than 70% except onion that tended to low recoveries with 17% of the substances with a recovery lower than 70% and 82% in the range of 70–110%, respectively. The repeatability was satisfying with a RSD lower than 20% (between 1.1% for dicofol in grape and 16.8% for atrazin-desethyl

Table 3
HPLC/MS fortification experiments (recovery and repeatability) at a 500 μg/kg fresh weight fortification level

Substance	Fortification concentration (μg/kg)	Grape		Lemon		Onion		Tomato	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Acetamiprid	554.4	97	0.7	91	2.3	97	2.6	107	2.3
Aldicarb	499.5	95	2.8	75	1.9	91	2.0	99	2.5
Avermectin B1a	509.8	107	4.6	87	9.8	103	3.9	91	3.2
Bendiocarb	543.9	99	2.9	84	3.2	93	3.3	103	3.6
Butocarboxim	491.0	94	2.7	75	2.3	93	2.6	99	2.0
Carbaryl	498.5	98	6.1	97	4.1	97	3.8	103	3.8
Carbendazim	504.9	93	3.7	92	4.4	95	3.4	99	3.5
Carbofuran	509.5	95	1.8	93	2.7	117	5.3	102	1.3
Clomazone	472.5	98	2.1	90	3.2	79	2.3	107	3.4
Clothianidin	512.4	96	2.2	87	4.0	98	3.2	107	3.1
Cyromazine	504.5	46	4.3	28	1.1	48	6.7	98	2.6
Demeton-S	455.8	95	3.2	93	2.4	51	3.2	99	3.1
Dicrotophos	749.7	95	2.2	95	1.8	96	2.0	100	1.5
Diflubenzuron	514.5	100	3.5	82	3.7	84	6.3	110	3.0
Dimethoate	528.9	97	3.1	93	1.4	96	1.8	103	2.3
Diuron	519.4	98	2.2	92	2.8	89	3.8	102	2.5
Ethiofencarb	540.6	80	2.6	81	1.7	80	1.5	87	2.6
Ethirimol	495.0	67	1.8	75	4.0	72	2.0	81	2.8
Fenamiphos	540.6	96	2.7	113	4.0	95	2.1	106	3.1
Fenpyroximate	489.9	97	3.7	92	7.4	79	7.1	103	2.5
Fenthion	835.3	96	3.1	112	3.7	74	1.9	104	2.4
Flufenoxuron	510.6	107	6.5	97	11.4	81	6.8	120	3.5
Furathiocarb	484.6	104	1.9	106	3.9	74	7.1	113	2.5
Hexythiazox	534.5	100	4.1	72	10.2	62	5.2	85	3.9
Imazalil	494.5	95	2.2	120	3.2	99	3.3	104	2.2
Imidacloprid	527.4	101	2.1	93	2.8	93	4.0	103	2.5
Indoxacarb	567.2	100	4.2	75	6.1	70	4.9	118	2.0
Linuron	503.5	102	3.9	93	4.4	74	4.1	103	2.7
Metamitron	509.5	96	2.4	86	1.5	98	2.1	103	2.2
Methabenzthiazuron	509.5	99	1.9	76	2.7	93	2.5	104	1.8
Metobromuron	529.5	97	3.8	86	6.5	94	3.8	100	2.0
Metoxuron	499.5	98	1.8	93	4.1	101	2.5	106	1.7
Monocrotophos	495.0	96	2.8	86	5.7	96	2.2	97	4.3
Monolinuron	504.5	102	1.9	86	1.9	95	2.7	107	2.6
Omethoate	496.7	85	5.9	86	4.4	87	3.4	84	5.8
Oxycarboxim	509.5	72	3.4	53	1.8	80	5.2	109	3.3
Paclobutrazol	507.5	99	3.5	85	4.8	94	1.9	106	2.8
Pencycuron	494.0	91	3.9	92	4.3	78	8.9	84	3.9
Propamocarb	503.0	50	3.2	68	2.7	78	3.4	72	5.1
Pyraclostrobin	509.5	95	3.0	85	3.1	62	3.4	117	3.0
Rotenone	491.9	104	2.5	103	4.2	96	3.5	106	3.6
Tebufenozide	554.4	101	2.7	97	2.1	99	3.3	111	1.8
Thiabendazole	508.0	93	2.5	78	2.2	96	2.1	107	1.3
Thiamethoxam	519.5	100	5.5	101	6.0	94	2.9	102	3.0
Thiofanox	529.2	90	1.0	93	3.3	81	2.3	98	1.6
Triflumuron	539.5	78	7.6	72	5.8	71	3.8	94	5.2

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in onion) apart for methamidophos in lemon (27.3%) and dichlorvos in tomato (20.2%). A trend to high recovery across the matrix selection was recognised for some pyrethroid insecticides (cyfluthrin, cypermethrin, deltamethrin, fenvalerate and fluvalinate-tau), some conazole fungicides (difenoconazole, fenbuconazole, prochloraz and propiconazole), some organothiophosphate insecticides (dimethoate, fenitrothion, omethoate, parathion-methyl and triazophos), some anilide fungicides (fenoxycarb and ofurace) and the other pesticides azoxystrobin, dimetomorph, fenoxycarb, lindane, propargite, pyriproxyfen and tetradifon. On the other hand some substances like biphenyl, cyromazine, propamocarb and tecnazene and in less extend carboxin and dichlorvos tended to low recoveries in all matrices. The recoveries obtained for the GC-amenable substances in this study are in the same range than reported in the literature (Anastassiades & Lehotay, 2003; Díez et al., 2006; Leandro et al., 2005) after extraction with the QuECheRS method except for carbaryl, cyprodinil, dichorvos and *o*-phenylphenol where we obtained lower recoveries than reported and for methamidophos and omethoate that presented inhere higher recoveries. High recoveries for some of the selected substances may be explained by the simple clean-up step leading to a limited removal of the impurities from the samples responsible for an enhancement of the MS signal. Concerning the substances with low recovery, cyromazine is for instance a small polar basic molecule ($pK_a = 5.22$), easily hydrolysed at extreme pH, owing an ionic behaviour, which makes its analysis quite tedious (Lesueur et al., in press). Biphenyl is a high lipophilic substance that can easily bind to the solid sample matrix. Based on our experience propamocarb is rapidly degradated not only in samples but also in standards. Finally, dichlorvos was already reported with recoveries of max. 78% at 500 μg/kg (Štajnbaher & Zupančič-Kralj, 2003).

As far as the LC-amenable pesticides are concerned, 87–93% of the substances at the 500 μg/kg fortification level and 78–85% at 50 μg/kg, respectively, presented recoveries of 70–110% except for lemon at 50 μg/kg that tended to low recoveries with 26% of the substances in the range of 70–110% and 72% lower than 70% with a minimum at 22.9% recovery for cyromazine. The experiments were repeatable with a RSD lower than 20% (from 0.7% for acetamiprid in grape to 11.4% for flufenoxuron in lemon) at 500 μg/kg and lower than 15% (between 1.0% for paclobutrazol and 12.1% for triflumuron in lemon) at 50 μg/kg. No pesticide in grape showed recovery higher than 110% at both fortification level. At the lower fortification level carbaryl was the only substance with a trend to higher recovery (up to 119.5%) whereas carbofuran, cyromazine, ethirimol and propamocarb presented low recoveries (from 22.6% for cyromazine in lemon to 69.3% for carbofuran in tomato). For the LC-amenable substances reported in the literature (Blasco et al., 2004, 2005; Zrostlíková et al., 2003) (carbaryl, carbendazim, carbofuran, diflubenzuron, flufenoxuron, imidacloprid, imazalil, linuron, thiabendazole, triflumuron,) at comparable fortification level (10–1000 μg/kg) in similar matrices (apple, apricot and orange) but different extraction method, the recoveries obtained with HPLC/MS were comparable. Noticeable is the very low recovery for carbofuran (2–5% in apple and apricot) obtained by Zrostlíková et al. (2003) due to its loss during the SPE clean-up of the extracts in comparison to the results obtained by Soler et al. (2006) that report the analysis of carbofuran as main metabolite of carbosulfan with recoveries of 90.2% and 95.4% at the 10 and 100 μg/kg fortification levels, respectively.

For the nine substances analysed with GC/MS and HPLC/MS, the recoveries fitted together; cyromazine and propamocarb showed low recoveries with both devices, paclobutrazol recoveries between 70% and 110%. Nevertheless dimethoate, omethoate and thiabendazole presented higher recoveries with GC/MS than with HPLC/MS, carbaryl and imazalil lower recoveries. For monocrotophos it was depending on the matrix. Dimethoate, monocrotophos and omethoate were preferably analysed with GC/MS, imazalil, propamocarb and thiabendazole with HPLC/MS due to their peak form, which was in accordance with the better LOD/LOQ obtained either with GC/MS or with HPLC/MS. Because of its very low retention on the HPLC/MS column, cyromazine was more easily analysed with GC/MS. The quantification of carbaryl and paclobutrazol was equally achieved with GC/MS and HPLC/MS.

4. Conclusions

The QuECheRS method applied to the analysis of apolar, middle polar and polar pesticides from fruit and vegetable matrices with GC/MS and HPLC/MS was proved to be accurate with the analysis of 140 substances in reference matrices. For some substances, the results were either over- or underestimated, mainly because of the low concentration in the test or the degradation of the substances in the standards. The LODs and LOQs achieved with GC/MS and HPLC/MS were in the same range from 1 to 400 μg/kg fresh weight.

The recovery, the repeatability and the accuracy of the method were satisfying for almost all the substances and the matrices (grape, lemon, onion and tomato) at the LOQ and ten times the LOQ fortification levels with lower recoveries for the two difficult matrices onion and lemon.

Recoveries from the matrices were depending on the matrix and the substance. With GC/MS, 61–82% of the substances showed a recovery in the range 70–110% and 6–30% presented a recovery higher than 110%. The LC-amenable substances presented recoveries in the range 70–110% for 87–93% of the substances at the 500 μg/kg fortification level and 78–85% at the 50 μg/kg fortification level, respectively. Onion and lemon were presented lower recoveries. The method was proved to be repeatable with RSD lower than 20% at 500 μg/kg and lower than 15 % at 50 μg/kg as recommended by the European SANCO

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Guideline (Commission of the European Communities, 2006). The QuECheRS method, contrary to other traditional extraction methods, was not only proved to be quick, but also applicable to GC/MS and HPLC/MS analysis with a good accuracy.

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First results of an innovative thermodesorption (TDAS) system for pesticide multiresidue GC-MS methods

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Abstract

For the analysis of pesticides the time has come for quicker but also dirtier and less enriched extraction methods. To oversome these effects a thermodesorption system appears as a solution for the determination of apolar till middle pesticides in GC-MS.
By comparing a thermodesorption injection system to the liquid injection of a standardised GC-MS pesticide multiresidue method, we established encouraging results for volatile substances but stated a carry-over of the standards in the next runs for less volatile pesticides. This was solved with high purge flows to split vent but reduced the GC-MS responses of 1/8 leading to responses as low as with liquid injection, making the thermodesorption system and its concentration factor effect senseless. As working progress, we are working on anew design of the thermodesoption unit.

Introduction

Nowadays pesticides are commonly analysed by GC-MS methods following a customarily sample preparation after liquid extraction, which generally entails several steps for the clean-up and the enrichment of the samples [DFG S19, 1999]. Since these methods are still highly tedious and time-consuming new methods like QuECheRS [Anastassiades, 2003] have been developed. The Quechers method is already successfully for the determination of hardly detectable pesticides in produce with GC-MS. It is highly reducing the preparation time and costs of the food samples but implies thereby a far reduced clean-up step and no enrichment of the samples.
The fouling of the device through the limited clean-up of the food matrixes is one problem that still remains and the high LOD/LOQ in the samples due to the reduced enrichment of the samples is another problem. Both effects can be partially overcome with a thermodesorption system (TDAS) [Chromtech, 2004] by increasing the injected volume from 1 to 20 µl and multiplying the enrichement of the samples by a factor 20.

In this study we investigated the use of a thermodesorption system as alternative injection system and compared it with a standard liquid injection in a standardised multiresidue methods.

Material and methods

Each analyte was provided either from Sigma-Aldrich or from Ehrenstorfer with the highest available purity. We selected 46 relevant pesticides recommended by the FAPAS scheme institution and compared the liquid injection and the thermodesorption of 1 µl of 1 ppm single standard solutions in acetonitrile
All analyses were performed on a HP Model 6890N Series GC coupled to a 5973N MS detector (GC/MS), equipped with a split-splitless injection inlet, electronic pressure control and a CTC CombiPal autosampler as well as with a thermodesorption unit PAL TDAS 2000 from Chromtech (Fig. 1 and 2). The capillary column was a HP 5 MS (Agilent, 30 m*0.25 mm i.d.*0.25 µm), with He as mobile phase at 3.3 ml/min for the liquid injection and at 4.5 ml/min for 5 min followed by 3.3 ml/min with thermodesorption. The analyses were carried out in the selected ion mode (SIM) with an electron energy of -70 eV . The thermodesorption system was set at 320°C for 4 min.
In the liquid injection of the standardised multiresidue method we used a purge flow to split vent of 50 ml/min at 2 min. Thermodesorption samples were prepared by injecting 20 µl of standard on glass wool in thermodesorption vials and let it evaporate till dryness. In the 1st thermodesortion experiment set-up we successively injected the standards with a purge flow to split vent of 5 ml/min at 5 min. In the 2nd thermodesorption experiment set-up, we injected the standards followed by series of blanks with a purge flow to split vent ranging from 5 to 70 ml/min at 0 min.

Fig.1 and 2: TDAS system and vials

Results

During the 1st thermodesorption experiment set-up, we noticed a carry-over of the less volatile substances to the following runs. As the order of the GC-MS responses are quite different for the 46 standards, we drawn the results in 2 figures where the substances are classified per increasing retention time (Fig. 3 and 4). Fig. 3 points out that the 26 less volatile pesticides are not contaminating the next runs. On the contrary, Fig. 3 and 4 show, from Imazalil on, that the higher the retention time i.e. the less volatile the pesticide, the higher the carry-over.

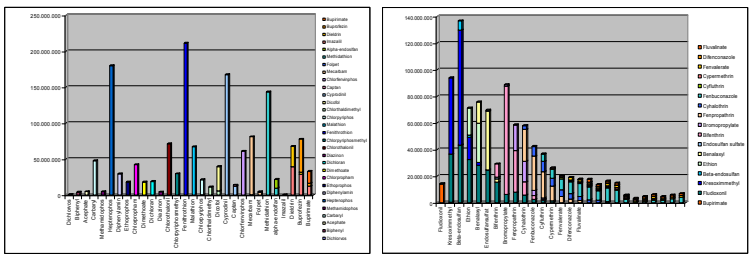
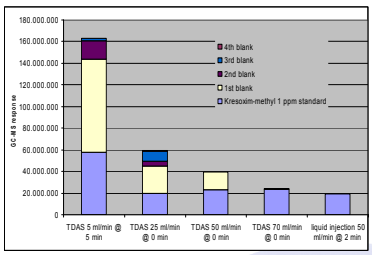


Fig.3 and 4: GC/MS response of the thermodesorption injection of single standards (1 ppm) ordered per retention time (min) in the 1st thermodesorption experiment set-up.

In an attempt to decrease this contamination, we increased the purge flow to split vent from 5 to 70 ml/min at 0 min in the 2nd thermodesorption experiment set-up. Injecting a 1 ppm standard and successive blanks established a reproducible response of the signal in the desired run but unfortunately also of the contamination in the next blank as seen for kresoxim-methyl in Fig. 5.



With a high purge flow to split vent we prevented the contamination of the standard in the next runs but significantly reduced the GC-MS response. Comparing the thermodesorption of 20 µl of 1 ppm standard to the liquid injection of 1 µl of 1 ppm standard we expected a increase of the response by a factor 20.
Fig.5: Influence of the purge flow on the thermodesorption of Kresoxim-methyl

Whereas the total response (standard + carry-overs) of 20 µl of 1 ppm kresoxim-methyl with a low purge flow to split vent (5 ml/min at 5 min) was 8 fold higher than the liquid injection of 1 µl of 1 ppm kresoxim-methyl, the response of 20 µl of 1 ppm kresoxim-methyl without carry-over i.e. with high purge flow to split vent (70 ml/min at 0 min) was as low as the reponse of the liquid injection of 1 µl of 1 ppm kresoxim-methyl making useless the thermodesorption system.

Sets of experiments(not shown) localised the contamination in the needle introducing the sample from the TDAS in the liner of the GC.

Conclusion

In this study we compared an innovative thermodesorption injection system with a conventional liquid injection for the GC-MS analysis of pesticide multiresidue methods. For easily volatile substances we could achieve promising results with standards. For less volatile pesticides, we noticed a carry-over of standards in the next runs, which could be avoided by increasing the purge flow to split vent but had the drawback of reducing the GC-MS response of 1/8 achieving responses as high as with liquid injection, inhering the thermodesorption concentration factor effect. As the time we are optimising the system to prevent this carry-over and obtain higher GC-MS responses as with the liquid injection.

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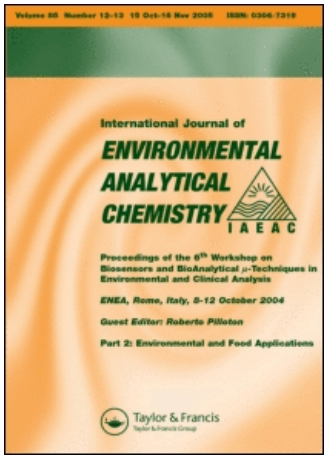

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Qualitative and quantitative analysis of polar pesticide multiresidues in leaf samples with a liquid chromatography-ion-trap mass-selective detector

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Qualitative and quantitative analysis of polar pesticide multiresidues in leaf samples with a liquid chromatography–ion-trap mass-selective detector

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Since 1995, in Austria, an agricultural programme (ÖPUL) has promoted an environmentally friendly and extensive production with restricted pesticide use. To observe the achievement of this goal, the pesticides in leaf samples are monitored. This study aimed to develop a multiresidue method for the qualitative and quantitative analysis of 46 pesticides in leaf samples with HPLC-IT-MS equipped with an electrospray ionization in positive mode after extraction with the QuECheRS method. The method has been validated for leaf samples based on the SANCO European Guideline at two fortification levels (LOQ and 10 times LOQ). The recoveries of the pesticides, with a few exceptions, were between 70 and 110% at both fortification levels and modes (full scan and selected reaction monitoring, SRM) with acceptable precision (RSD < 16%). For most pesticides, the method was linear over two orders of magnitude, repeatable, and accurate. Although the matrix effect was relevant for only a few pesticides, matrix-matched standards were used. The quantification of real samples in both modes fitted well, but a confirmation in the SRM mode was always necessary to avoid false-positive samples. Unfortunately, the method is not yet sensitive enough for organic farming foodstuff, since the limits of detection and quantification are still too high (between 1.5 and 218 $\mu g\,kg^{-1}$ and between 4.8 and 725 $\mu g\,kg^{-1}$ in full scan, respectively) compared with the Austrian authorized value of 100 $\mu g\,kg^{-1}$ fresh leaf sample defined in the ÖPUL programme.

Keywords: Polar pesticides; Liquid chromatography; Ion-trap mass spectrometry; Multiresidue method

1. Introduction

As pesticides are potentially harmful to humans, the European Community established directives and maximum residue levels (MRLs) in water (Directive 2000/60/EC [1]) and

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foodstuff (Directive 90/642/EEC [2]). Nevertheless, the plants themselves should also be taken into consideration, since pesticide uptake can occur directly from the leaves to the fruits or vegetables during the vegetation and florescence period, as in the case of systemic pesticides for instance.

Since 1995, in Austria, the Federal Ministry of Agriculture, Forestry, Environment and Water Management has devised a programme called ÖPUL to promote an environmentally friendly and extensive agriculture that would protect our natural environment [3]. In this sense, a new method of farming, called 'organic farming' is increasingly being used. Defined in the Directive 2092/91/EEC [4], organic farming implies that 'only products composed of substances mentioned in Annex I and Annex II of the Directive 2092/91/EEC i.e. not chemically synthesized substances may be used as plant protection products, fertilizers or soil conditioners'.

This implies the control of pesticide use by farmers by pesticide residue monitoring in the plants during the growing time of the cultures. Although there are no MRLs available for plants, pesticides authorized for agricultural use in Austria are listed in section 11 and section 12 of the pesticide national law [5]. Non-used pesticides are defined for residue concentrations in leaf samples lower than $100\,\mu g\,kg^{-1}$ fresh weight. The list entails many gas-chromatography (GC) amenable pesticides but also an increasing number of pesticides with physico-chemical properties making them more amenable for liquid-chromatography (HPLC).

Whereas apolar and middle polar pesticide residues are commonly analysed with GC coupled to single quadrupole (SQ) and, less frequently, triple quadrupole (TQ) mass spectrometers, polar pesticides in contrast are determined by HPLC. With the use of these polar pesticides, a need for new detection methods has emerged. SQ detectors are easy to use, stable, and cheap but do not offer the possibility of confirmation and can support false-positive results. More elaborate mass spectrometers like TQ, time-of-flight (TOF) and ion-trap (IT) detectors allow this confirmation, since they can carry out tandem spectrometry [6]. Until recently, TQ detectors were the detectors of choice for the quantification of pesticide multiresidues in environmental and food samples, since they are stable, sensitive, and selective, and have a wide linear range in SRM mode [7–14]. The other MS detectors in the market were until recently principally reputed as confirmation and identification systems because they can measure exact masses (TOF) or operate in MS^n mode (IT) but are less sensitive and linear. TOF has been studied in the recent years, mainly as an identification tool to discover unknown pesticides in environmental and food samples, as intensively published by Hernández *et al*. [9] and Thurman *et al*. [15–17]. Regarding IT, several studies have been published on the detection of up to 14 pesticides in water [18, 19], detection of quaternary ammonium pesticides in foodstuff [20–22], and detection of up to 17 pesticides in food samples, especially citrus fruits [14, 23–29].

One of the latest successful foodstuff preparations for the analysis of pesticide multiresidues to our knowledge is the QuECheRS (quick, easy, cheap, rugged, and safe) method [30], which interestingly can be combined with GC but also with LC. It has already been applied to GC-MS for the determination of pesticides from fruit and vegetable matrices, which are difficult to detect and quantify [30–32]. The QuECheRS method principally relies on the extraction of the pesticides from the matrices with acetonitrile followed by a salting effect with $MgSO_4$ and NaCl. The actual clean-up step of the matrices is achieved through the addition of a dispersive sorbent in the bulk liquid followed by its removal.

The goal of our work was to apply the QuECheRS method to leaf samples (apple trees, cornstalks, grapevines, hop plants, strawberry bushes, potato plants, and vegetable plants) in a combination of LC-MS using an IT analyser to develop a multiresidue method for the analysis of 46 polar pesticides.

2. Experimental

2.1 *Reagents and chemicals*

Pesticide standards were purchased either from Dr. Ehrenstorfer (Germany) or from Sigma-Aldrich (USA) with the highest available purity. Ultra-residue reagent acetonitrile, HPLC/MS grade methanol, ultra HPLC/MS grade water and HPLC/MS grade formic acid were purchased from J.T. Baker (USA).

Anhydrous magnesium sulfate, sodium chloride, and sodium citrate dihydrate were purchased from J.T. Baker (USA), di-sodium hydrogen citrate sesquihydrate was provided from Fluka (Germany), and 40 μm Bondesil-PSA was from Varian (Germany).

Single standard stock solutions were prepared by dissolving 10 mg of standard in 10 mL of acetonitrile to yield a concentration of 1000 $\mu g\,mL^{-1}$ and further diluted with acetonitrile down to 10 $\mu g\,mL^{-1}$. Multicompound standard stock solutions were prepared, dissolving 10 mg of each standard in 1000 mL of acetonitrile to yield a concentration of 10 $\mu g\,mL^{-1}$ and further diluted with acetonitrile to achieve concentrations of 5 $\mu g\,mL^{-1}$, 2 $\mu g\,mL^{-1}$, 0.5 $\mu g\,mL^{-1}$, 0.2 $\mu g\,mL^{-1}$, and 0.05 $\mu g\,mL^{-1}$. Matrix-matched standards were obtained by evaporating 10 mL of the acetonitrile multicompound standard and redissolving it in 10 mL of blank leaf matrix extract. Further dilution with the same extract brought the solution to the same concentrations as mentioned above. The single and multicompound standards were stored at 4°C in the dark.

2.2 *Sample preparation*

The real samples were at first analysed in the full scan mode (MS mode) before confirmation in the SRM mode (MS^2 mode) and comparison of the quantification in the full scan and SRM modes. We analysed 1400 leaf samples over a period of 3 months from production sites in Austria originating from organic farming (22%) and conventional farming (88%). They consisted of apple tree leaves (42%), grape tree leaves (38%), cornstalks (9%), potato plant leaves (7%), vegetable plant leaves (cabbage, cucumber, pumpkin) (3%), hop plant leaves (1%), and strawberry-bush leaves (1%).

The samples were prepared with the QuECheRS method described by Anastassiades [30] and adapted for leaf samples. Roughly summarized, it consisted of (1) homogenize around 500 g leaf samples, (2) weigh 10 g previously chopped fresh sample into a 50 mL Teflon centrifuge tube; (3) add 20 mL acetonitrile and shake the sample vigorously for 1 min using a vortex mixer; (4) add 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate dihydrate and 0.5 g di-sodium hydrogen citrate sesquihydrate and vortex immediately for 1 min; (5) centrifuge the extracts for 3 min at 5000 rpm; (6) transfer a 6 mL aliquot of the upper layer into a 15 mL

Teflon centrifuge tube containing 150 mg PSA and 950 mg $MgSO_4$; (7) centrifuge the extracts for 3 min at 5000 rpm; (8) filter through 0.45 μm filter; (9) transfer 1.5 mL of the extract into an autosampler vial for HPLC/MS analysis.

Triphenylphosphate (TPP) was used as internal standard and spiked at the initial step to reach a 0.5 $\mu g\,mL^{-1}$-concentration in the final extract.

2.3 *Analyses*

The high-performance liquid chromatography system was an Agilent Technologies HP-1100 Series (Agilent Technologies, Waldbronn). Chromatographic separation was achieved using a Zorbax SB-C18 analytical column 2.1 × 150 mm (3.5 μm particle size) from Agilent Technologies at a flow rate of 300 $\mu L\,min^{-1}$. The mobile phases consisted of A: H_2O–MeOH, 90–9.95% (v/v) with 0.05% HCOOH and B: H_2O–MeOH, 9.95–90% (v/v) with 0.05% HCOOH. The gradient was 100% A at 0 min, 100% A at 1 min, 0% A at 10 min, 0% A at 17 min, and 100% A at 20 min. The post time was 2 min with 100% A and the stop time 22 min. The HPLC was controlled with the Agilent Technologies Chemstation for LC 3D System Software.

The HPLC system was interfaced to an Agilent Technologies mass spectrometer LC/MSD trap XCT Plus (Agilent Technologies, Waldbronn) equipped with an electrospray ionization (ESI) interface operated in positive mode and controlled with the Agilent Technologies LC/MSD trap software 5.3. Parameters were optimized by continuous injection of a standard solution of 10 $\mu g\,mL^{-1}$ via a syringe pump, at a flow rate of 6 $\mu L\,min^{-1}$, mixed with the mobile phase at 50 $\mu L\,min^{-1}$ by means of a T piece. The IT detector operating conditions were set as shown in table 1. The IT mass detector operated in full scan and SRM modes. The precursor ions (MS mode) were isolated and fragmented with an amplitude of 0.6 V to produce a first set of product ions (MS^2 mode) and so on.

Table 1. IT operating conditions.

ESI source	Nebulizer gas (nitrogen) pressure	40 psi
	Drying gas flow rate	9 $mL\,min^{-1}$
	Drying gas temperature	350°C
	Capillary voltage	4500 V
	Endplate offset	Fixed at −500 V
Detector and block voltages	Multiplier voltage	1900 V
	Dynode voltage	7 kV
	Skimmer block	40.0 V
	Lens 1 block	−200.0 V
	Octopole RF amplitude Block	0 Vpp
	Partition block	12.0 V
	Lens 2 block	0 V
	Capillary exit block	0 V
Ion Charge Control (ICC)	Target (ion counts)	150 000
	Maximum accumulation time	50 ms
	Scan (m/z)	From 50 to 500, from 0 to 15 min From 500 to 1000 from 15 to 22 min
	Scan averages	5

2.4 *Pesticide selection*

We selected a total of 49 pesticides representing 24 insecticides, 11 herbicides, six fungicides, five chitin synthesis inhibitor, two mite growth inhibitors, and one acaricide (tables 2 and 3). Derivates of aldicarb, demeton-S and fention were not selected in these sets of experiments. After the first experiments, the list was reduced to 46 pesticides, as explained in section 3, leaving acephate, benomyl, and chlorfluazuron out of the method.

2.5 *MS optimization*

We first worked with single standards to determine the retention time (RT), the characteristic m/z ions in the MS and SRM modes (i.e. precursor and product ions) of each substance (tables 2 and 3). In the MS mode, we obtained the ions $[M+H]^+$ and $[M+Na]^+$ for each pesticide, as well as the ion $[M+K]^+$ for 20 substances and in 13 cases fragmentation ions, which were product ions also found in the SRM mode, or isotope ions. The most abundant ion (i.e. with the highest intensity(I)) was in 28 cases the ion $[M+H]^+$, in 14 cases the ion $[M+Na]^+$, in four cases another ion, and in three cases a fragmentation ion.

We always used $[M+H]^+$ as precursor ion in the SRM mode when it was the most abundant ion. When $[M+H]^+$ was not the most abundant ion, product ions of the main ions in the MS mode were collected in the SRM mode, and their signal to noise (S/N) ratios were compared. In this way, we finally used $[M+H]^+$ as a precursor ion in the SRM mode in 44 cases, since it produced in each case product ions with a better S/N ratio. For avermectin B1a, demeton-S, and thiofanox, we used $[M+Na]^+$, and for tebufenozide, the fragmentation ion at m/z 297 as precursor ion in the SRM mode. Metobromuron showed two main precursor ions at m/z 259 and m/z 261 due to the presence of the two isotopes of the bromide atom $^{79}Br/^{81}Br$ in the molecule. Since they both presented the same main product ion at m/z 148 (resulting in the loss of a molecule containing the bromide atom) but with a better intensity in the case of the ion at m/z 261, they were selected as the precursor ion in the MS^2 mode.

Consequently, we worked with multicompound standards at decreasing concentrations down to $0.05\,\mu g\,mL^{-1}$ to determine the ratio between the characteristic m/z in the full scan and the SRM modes as well as the LOQ and LOD of each pesticide. The substances were identified, as recommended by the SANCO European Guidelines [33, 34] relying on three ion criteria for permitted substances and four ion criteria for banned substances, i.e. one precursor ion and two product ions with an IT detector. Collecting two product ions was possible for 40 of the substances (only one product ion for acephate, benomyl, carbaryl, dimethoate, fenpyroximate, metamitron, methabenzthiazuron, tebufenozide, and thiofanox) with a capillary voltage of 4500 V and an amplitude fragmentation in MS^2 mode of 0.6 V. The ion ratios in the full scan and SRM modes were compiled in a library and referred to as (first but not sufficient) identification in MS mode and as complete identification (together with the retention time) in MS^2 mode.

2.6 *LC optimization*

Due to the high amount of samples to analyse, we wanted to use the selectivity of the IT detector to develop a short-run-time method as detailed in section 2.3. With the given

Table 2. Pesticide class, most abundant ions and LOQ/LOD in MS mode in matrix-matched standards (μg kg^{-1} fresh weight).[a]

Name	Pesticide class	RT (min)	Molecular weight	$[M+H]^+$ ion (m/z)	I (%)	$[M+Na]^+$ ion (m/z)	I (%)	Other ion (m/z)	I (%)	Fragment ion (m/z)	I (%)	LOQ (μg kg^{-1})	LOD (μg kg^{-1})
Acephate	Insecticide	0.7	183.0	183.8	16.8	205.9	74.3	243.1	100.0			298	90
Acetamiprid	Insecticide	8.6	222.0	222.9	100.0	244.9	47.4	282.0	31.4			54	16
Aldicarb	Insecticide	9.3	190.0			212.9	100.0					56	16
Avermectin B1a	Insecticide	16.7	872.5	873.5	21.1	895.5	52.6			871.7	100.0	396	118
Bendiocarb	Insecticide	9.8	223.0	223.9	67.5	245.9	100.0	283.2	5.5	166.9	97.8	66	20
Benomyl	Fungicide	12.5	290.0	291.0	100.0			313.1	3.5			524	158
Butocarboxim	Insecticide	9.3	190.0			212.9	100.0					48	14
Carbaryl	Insecticide	10.1	201.0	201.9	21.7	223.9	99.2	261.0	32.4	144.9	100.0	92	28
Carbendazim	Fungicide	6.6	191.0	191.9	100.0							44	12
Carbofuran	Insecticide	9.8	221.0	221.9	100.0	243.9	61.1	281.0	54.8			24	7.3
Chlorfluazuron	Chitin synthesis inhibitor	14.6	539.0	539.9	100.0	561.9	60.1	541.9	77.3			20 600	6 180
Clomazone	Herbicide	11	239.0	239.9	100.0	261.9	61.7	299.0	13.3			40	12
Clothianidin	Insecticide	8.1	249.0	249.9	100.0	271.9	92.7			168.9	35.8	46	14
Cyromazine	Chitin synthesis inhibitor	1.4	166.0	166.9	100.0							28	8.5
Demeton-S	Insecticide	11	258.0	258.9	16.0	280.9	100.0	318.0	39.5			40	12
Dictrotophos	Insecticide	7.8	237.0	237.9	73.5	259.9	26.2	297.0	100.0			64	20
Diflubenzuron	Chitin synthesis inhibitor	11.7	310.0	311.0	43.0	332.9	100.0					74	22
Dimethoate	Insecticide	8.4	229.0	229.8	100.0	251.8	94.3	288.9	24.4	198.8	78.3	94	28
Diuron	Herbicide	10.7	232.0	232.9	100.0	254.9	44.6					60	18
Ethiofencarb	Insecticide	10.3	225.0	225.9	28.2	247.9	100.0	285.0	49.1	168.9	41.9	60	18
Ethirimol	Fungicide	8.8	209.0	210.0	100.0							18	5.1
Fenamiphos	Insecticide	11.8	303.0	304.1	100.0	326.0	90.1	363.1	37.6			32	9.4
Fenpyroximate	Acaricide	14.5	421.0	422.3	100.0	444.2	26.6			366.2	13.5	32	9.4
Fenthion	Insecticide	12.4	278.0	278.9	100.0	300.9	90.7					128	38

Flufenoxuron	Chitin synthesis inhibitor	13.4	488.0	489.1	42.1	511.1	100.0					166	50
Furathiocarb	Insecticide	13.4	382.0	383.2	51.5	405.2	100.0	442.2	96.5			26	7.9
Hexythiazox	Mite growth regulator	13.4	352.0	353.1	56.7	375.1	100.0					104	32
Imazalil	Fungicide	10.26	296.0	296.9	100.0							10	2.8
Imidacloprid	Insecticide	8.01	255.0	255.9	100.0	277.9	52.1					104	30
Indoxacarb	Insecticide	12.5	527.0	528.0	41.3	550.0	100.0					244	74
Linuron	Herbicide	11.1	248.0	248.9	100.0	270.9	59.4	250.9	67.4			86	26
Metamitron	Herbicide	8.4	202.0	202.9	100.0							36	12
Methabenzthiazuron	Herbicide	10.7	221.0	221.9	100.0	243.9	11.4					30	10
Metobromuron	Herbicide	10.5	258.0	258.9	94.9	280.9	53.5	260.9	100.0			64	20
Metoxuron	Herbicide	9.3	228.0	228.9	100.0	250.9	32.7	288.0	28.9			36	10
Monocrotophos	Insecticide	7.4	223.0	223.9	91.3	245.9	100.0	283.0	71.4			312	94
Monolinuron	Herbicide	10.3	214.0	214.9	100.0	236.9	40.3					40	12
Omethoate	Insecticide	5.4	213.0	213.9	100.0	235.9	49.4	272.9	28.3			390	118
Oxycarboxim	Fungicide	8.7	267.0	267.9	78.5	289.9	100.0	327.0	29.5			42	12
Paclobutrazol	Mite growth regulator	11.3	293.0	294.0	100.0							14	4.4
Pencycuron	Herbicide	11.8	328.0	329.1	100.0	351.1	56.9	388.1	56.6			42	12
Propamocarb	Herbicide	4.8	188.0	188.9	100.0							58	18
Pyraclostrobin	Herbicide	12.2	387.0	388.1	84.5	410.0	100.0					22	6.7
Rotenone	Insecticide	11.9	394.0	395.1	100.0	417.1	87.4					56	16
Tebufenozide	Insecticide	11.9	352.0	353.1	14.1	375.1	76.5	412.2	80.6	297.1	100.0	26	7.8
Thiabendazole	Fungicide	7.5	201.0	201.9	100.0							46	14
Thiamethoxam	Insecticide	7.2	291.0	291.9	84.5	313.9	100.0	351.0	35.7			260	78
Thiofanox	Insecticide	10.3	218.0	218.9	8.8	240.9	67.2	278.1	100.0	183.9	20.6	58	18
Triflumuron	Chitin synthesis inhibitor	12.1	358.0	359.2	100.0	381.0	3.1					4.8	1.5

[a]The intensity, I (%), was set to 100 for the peak presenting the higher MS ion count response.

Table 3. Product ions and LOQ/LOD in MS^2 mode in matrix-matched standards ($\mu g\,kg^{-1}$ fresh weight).[a]

Name	RT (min)	Precursor ion (m/z)	Product ion (m/z)	I (%)	Product ion (m/z)	I (%)	Product ion (m/z)	I (%)	LOQ ($\mu g\,kg^{-1}$)	LOD ($\mu g\,kg^{-1}$)
Acephate	0.7	183.8	143.1	100					8.1	2.4
Acetamiprid	8.6	222.9	126.1	100	196.1	55.9	187.1	48.7	50	14
Aldicarb	9.3	212.9	212.9	100	98.0	22.4	89.1	7.5	24	7.0
Avermectin B1a	16.7	895.5	751.5	100	607.5	50.7	752.5	11.1	210	64
Bendiocarb	9.8	223.9	167.1	100	109.2	8.5	391.4	0.1	38	12
Benomyl	12.5	291.0	192.1	100					42	12
Butocarboxim	9.3	212.9	212.9	100	155.8	33.3	75.2	24.2	24	7.2
Carbaryl	10.1	201.9	145.1	100					14	4.0
Carbendazim	6.6	191.9	159.9	100	192.9	31.8			20	6.2
Carbofuran	9.8	221.9	165.1	100	123.2	8.7			24	7.4
Chlorfluazuron	14.6	539.9	382.9	100	383.8	13.6			32	9.4
Clomazone	11	239.9	124.9	100	127.9	22.3	239.9	10.3	28	8.3
Clothianidin	8.1	249.9	169.1	100	168.1	41.9	132.1	35.0	30	9.2
Cyromazine	1.4	166.9	125.2	100	85.4	81	139.1	37.9	80	24
Demeton-S	11	280.9	253.0	100	281.1	83.5	89.3	78.7	32	9.6
Dictrotophos	7.8	237.9	193.1	100	112.3	41.4			32	9.8
Diflubenzuron	11.7	311.0	157.9	100	140.9	19.4			42	12
Dimethoate	8.4	229.8	199.0	100					14	4.0
Diuron	10.7	232.9	232.9	100	72.3	15.2			30	8.8
Ethiofencarb	10.3	225.9	168.9	100	107.0	42.9	163.9	18.4	36	12
Ethirimol	8.8	210.0	210.0	100	211.0	41			22	6.6
Fenamiphos	11.8	304.1	262.1	100	234.1	76.1	217.1	71.4	52	16
Fenpyroximate	14.5	422.3	366.3	100					18	5.6
Fenthion	12.4	278.9	247.0	100	169.0	22.4			40	12

Flufenoxuron	13.4	489.1	158.1	100	141.1	36			20	6.2
Furathiocarb	13.4	383.2	194.8	100	251.9	98.9	383.1	76.9	88	26
Hexythiazox	13.4	353.1	227.8	100	270.8	48.3	167.8	14.2	44	14
Imazalil	10.26	296.9	255.1	100	201.0	63.3	163.1	31.2	24	7.0
Imidacloprid	8.01	255.9	209.1	100	175.1	64.6	210.1	55.1	40	12
Indoxacarb	12.5	528.0	496.2	100	249.1	71	223.1	58.2	62	18
Linuron	11.1	248.9	182.0	100	160.0	48.3	249.1	18.5	46	14
Metamitron	8.4	202.9	202.9	100					20	5.7
Methabenzthiazuron	10.7	221.9	164.9	100					30	8.9
Metobromuron	10.5	260.9	147.9	100	171.8	28.6	258.9	28.2	24	7.0
Metoxuron	9.3	228.9	228.9	100	229.8	50.1	72.3	39.7	40	12
Monocrotophos	7.4	223.9	193.0	100	98.3	21	167.1	13.5	3.9	1.2
Monolinuron	10.3	214.9	214.8	100	147.9	39.9	125.8	15.5	30	9.0
Omethoate	5.4	213.9	183.0	100	196.1	20.7	143.1	11.4	10	2.9
Oxycarboxim	8.7	267.9	175.0	100	120.2	13.3			26	7.6
Paclobutrazol	11.3	294.0	294.0	100	206.9	4.6			38	12
Pencycuron	11.8	329.1	329.1	100	124.8	81.8	217.8	70.7	46	14
Propamocarb	4.8	188.9	144.2	100	102.3	66.1			12	3.3
Pyraclostrobin	12.2	388.1	194.1	100	296.1	90.5	164.1	38.1	22	5.8
Rotenone	11.9	395.1	395.1	100	213.1	55.9	192.1	16.8	60	18
Tebufenozide	11.9	297.1	132.9	100					20	5.8
Thiabendazole	7.5	201.9	175.0	100	205.1	11.9			24	7.3
Thiamethoxam	7.2	291.9	211.1	100	210.1	27.6			10	3.2
Thiofanox	10.3	240.9	183.8	100					30	9.1
Triflumuron	12.1	359.2	156.0	100	139.1	26.9			16	4.9

[a]The intensity, I (%), was set to 100 for the peak presenting the higher MS ion count response.

flow and gradient, we achieved a good separation of all substances except for aldicarb and butocarboxim. These two substances have the same retention time and are different only in regard to the position of one methyl group (figure 1). Although they have different intensities, they present the same product ions (m/z 213, m/z 156, m/z 116, and m/z 98) in the SRM mode. The only difference is the presence of the ion at m/z 89 only produced from aldicarb and the ion at m/z 75 only produced from butocarboxim. Since only ions with an m/z larger than around one-third of the precursor ion m/z can be efficiently stored in the IT for MS^2 detection [6, 14, 18], the two ions at m/z 75 and m/z 89 are not reliable for the quantification of aldicarb and/or butocarboxim. This forced us to develop a method with a lower flow rate (200 μL min^{-1}) and a smoother gradient (100% A at 0 min, 100% A at 3 min, 0% A at 25 min, 0% A at 35 min, 100% A at 40 min; post-time 5 min with 100% A; stop time 45 min), which allowed their partial separation and quantification (with the product ion at m/z 156) when necessary. This gradient was only applied in case aldicarb and/or butocarboxim were suspected.

2.7 *Validation study*

The method was validated for the 46 substances in the MS mode and for ten selected substances (i.e. those found in real samples) in the SRM mode based on the European SANCO Guideline [33] testing the method for sensitivity, recovery, and precision. Linearity was studied for the standards in acetonitrile and in the matrix by analysing in quintuplicate six concentration levels between 0.05 and 10 μg mL^{-1}.

The limits of detection (LOD) and limits of quantification (LOQ) were estimated for the ion with an m/z at the highest intensity (denoted as I in tables 2 and 3) as the lowest concentration injected that yielded to an S/N ratio of 3 and 10, respectively.

The accuracy and precision (i.e. repeatability expressed in term of relative standard deviation (RSD,%)) of the method were tested with recovery experiments, performed with seven replicates of leaf blank samples spiked at the LOQ and 10 times the LOQ (after obtaining the LOQ as explained below). The spiked samples were allowed to stand for 30 min before extraction to allow the pesticides to penetrate into the matrix. Two different blank matrices were used for the fortification experiments.

2.8 *Quantification of real samples*

For substances with a linear calibration curve, the quantification was done within the linear range including the origin in the calibration curve. For substances with a quadratic calibration curve, a linear interpolation between two calibrations around the sample concentration was achieved, as recommended elsewhere [28].

3. Results and discussion

3.1 *Validation of the method*

3.1.1 Linearity of the standard curve and matrix matched standards. Table 4 shows that most of the 46 substances were linear, covering a range between 0.05 and 5 μg mL^{-1} or

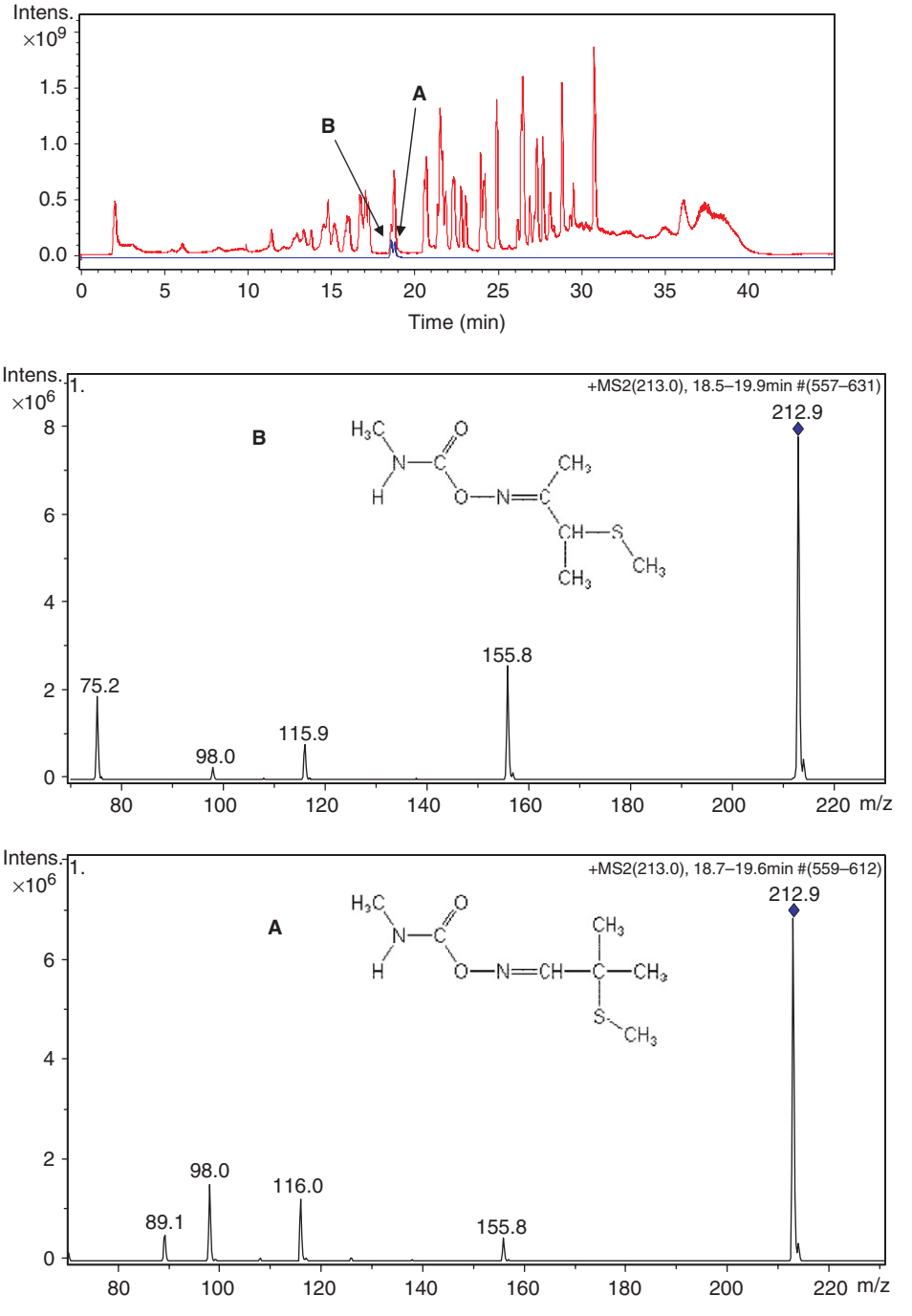

Figure 1. Total ion chromatogram (TIC) and extract ion chromatogram (EIC) *m*/*z* 213 operated with the smoother gradient coupled to MS^2 spectra of a 5 μg mL^{-1} multicompound standard containing (a) aldicarb and (b) butocarboxim.

Table 4. Linearity of the method in the MS and SRM mode in solvent and in matrix-matched standards ($\mu g\,mL^{-1}$).

Substance	m/z	Function	Linear range ($\mu g\,mL^{-1}$)	R^2 solvent MS mode	R^2 matrix MS mode	Matrix/solvent MS mode	R^2 solvent SRM mode	R^2 matrix SRM mode	Matrix/solvent SRM mode
Acetamiprid	222.9	Linear	0.05–5	0.9998	0.9990	1.018			
Aldicarb	212.9	Linear	0.05–5	0.9992	0.9942	0.947			
Avermectin B1a	895.5	Linear	0.2–10	0.9940	0.9971	0.933			
Bendiocarb	245.9	Linear	0.05–2	0.9923	0.9946	0.995			
Butocarboxim	212.9	Linear	0.05–2	0.9939	0.9966	0.928			
Carbaryl	223.9	Linear	0.05–5	0.9992	0.9961	0.907			
Carbendazim	191.9	Linear	0.05–5	0.9993	0.9984	0.998	0.9994	0.9991	0.990
Carbofuran	221.9	Linear	0.05–5	0.9997	0.9961	0.938			
Clomazone	239.9	Linear	0.05–5	0.9998	0.9958	0.905			
Clothianidin	249.9	Linear	0.05–5	0.9973	0.9981	0.930			
Cyromazine	166.9	Linear	0.05–5	0.9987	0.9960	0.859			
Demeton-S	280.9	Linear	0.05–5	0.9953	0.9971	0.976			
Dicrotophos	297.0	Linear	0.08–7.5	0.9992	0.9977	0.912			
Diflubenzuron	332.9	Linear	0.05–2	0.9924	0.9965	1.015	0.9966	0.9984	0.904
Dimethoate	229.8	Linear	0.05–5	0.9970	0.9946	0.922			
Diuron	232.9	Linear	0.05–5	0.9981	0.9973	0.972			
Ethiofencarb	247.9	Linear	0.05–5	0.9931	0.9972	0.861			
Ethirimol	210.0	Linear	0.05–5	0.9984	0.9979	0.904			
Fenamiphos	304.1	Linear	0.05–2	0.9707	0.9967	0.976			
Fenpyroximate	422.3	Linear	0.05–2	0.9971	0.9993	0.643	0.9999	0.9973	0.813

Fenthion	278.9	Linear	0.08–3.5	0.9998	0.9986		0.873			
Flufenoxuron	511.1	Linear	0.05–2	0.9910	0.9402		0.974	0.9987	0.9993	0.895
Furathiocarb	405.2	Quadratic	0.05–10		0.9955	–				
Hexythiazox	375.1	Linear	0.05–2	0.9987	0.9978		1.126	0.9994	0.9978	0.918
Imazalil	296.9	Linear	0.05–5	0.9975	0.9955		0.963			
Imidacloprid	255.9	Linear	0.05–5	0.9985	0.9936		0.896	0.9995	0.9922	0.784
Indoxacarb	550.0	Quadratic	0.05–10		0.9989	–		–	0.9975	–
Linuron	248.9	0.05–5	0.05–5	0.9996	0.9976		0.950			
Metamitron	202.9	Linear	0.05–5	0.9987	0.9986		0.951			
Methabenzthiazuron	221.9	Linear	0.05–5	0.9976	0.9979		1.002			
Metobromuron	260.9	Linear	0.05–5	0.9991	0.9971		0.992			
Metoxuron	228.9	Linear	0.05–5	0.9982	0.9979		0.923			
Monocrotophos	245.9	Linear	0.2–5	0.9987	0.9986		0.982			
Monolinuron	214.9	Linear	0.05–5	0.9995	0.9969		0.957			
Omethoate	213.9	Linear	0.2–5	0.9994	0.9963		0.893			
Oxycarboxin	289.9	Quadratic	0.05–10		0.9977	–				
Paclobutrazol	294.0	Linear	0.05–5	0.9985	0.9859		0.885			
Pencycuron	329.1	Linear	0.05–2	0.9953	0.9988		1.095			
Propamocarb	188.9	Linear	0.05–5	0.9957	0.9978		0.594	0.9890	0.9994	0.592
Pyraclostrobin	410.0	Quadratic	0.05–10		0.9907	–		–	0.9952	–
Rotenone	395.1	Linear	0.05–2	0.9956	0.9937		1.026			
Tebufenozide	297.1	Linear	0.05–2	0.9974	0.9978		0.972	0.9980	0.9980	1.004
Thiabendazole	201.9	Linear	0.05–5	0.9983	0.9988		0.988			
Thiamethoxam	313.9	Linear	0.1–5	0.9985	0.9968		0.957			
Thiofanox	240.9	Linear	0.05–2	0.9976	0.9933		0.980			
Triflumuron	359.2	Linear	0.05–2	0.9910	0.9825		0.815			

between 0.05 and 2 $\mu g\,mL^{-1}$. Avermectin B1a, which had a higher LOQ, was linear between 0.2 and 10 $\mu g\,mL^{-1}$. Most correlation coefficients, R^2, were higher than 0.99 in both modes. Furathiocarb, indoxacarb, oxycarboxim, and pyraclostrobin did not show any linear range but quadratic functions. Linear ranges of three orders of magnitude have even been reported in MS^3 mode for pesticides in orange matrix-matched standards [14]. Nevertheless, it seems that TQ detectors are more linear, although many studies report linear ranges of two orders of magnitude, too [7, 8, 18].

The influence of the matrix on the detector response for the substances with linear calibration functions was also studied. For 23 of the 46 substances, the difference for the signal in solvent and in the matrix was less than 5%. For hexythiazox and pencycuron, the signal in the matrix was enhanced by 12.6 and 9.5%, repectively. For 21 substances, we observed a decrease in the signal between 5.0 and 40.6%. Most of them presented a decrease between 5 and 15% except fenpyroximate, propamocarb, and triflumuron with signal decreases of 35.7, 45.6, and 18.5%, respectively. The influence of the matrix relies on the competition between the analyte ions and the matrix components [26, 28] and is seemingly more obvious when working with IT [14] than with TQ. As a consequence, we used matrix-matched standards.

3.1.2 LOD and LOQ. The LOD and LOQ are given in $\mu g\,kg^{-1}$ product (fresh weight). Chlorfluazuron could hardly be detected at a 10 $\mu g\,mL^{-1}$ $\mu g\,mL^{-1}$ (20 000 $\mu g\,kg^{-1}$) level (LOD and LOQ in full scan 6180 $\mu g\,kg^{-1}$ and 20 600 $\mu g\,kg^{-1}$, respectively), which was far off the necessary authorized value of 100 $\mu g\,kg^{-1}$, and was consequently removed from the present method.

LOD and LOQ are presented in tables 2 and 3 in MS mode and SRM mode, respectively. The lowest LOD and LOQ were as low as 1.4 $\mu g\,kg^{-1}$ and 4.8 $\mu g\,kg^{-1}$, respectively in the MS mode and 1.2 $\mu g\,kg^{-1}$ and 4.0 $\mu g\,kg^{-1}$, respectively in the SRM mode. In the full scan mode, 45 substances showed LODs lower than 100 $\mu g\,kg^{-1}$, i.e. satisfying the required 100 $\mu g\,kg^{-1}$ fresh leaves for organic farming. Only three substances (avermectin B1a, benomyl and omethoate) gave LODs higher than 100 $\mu g\,kg^{-1}$. In the same way, 37 substances were below 100 $\mu g\,kg^{-1}$ and nine substances were between 100 and 520 $\mu g\,kg^{-1}$. The LOD and LOQ in SRM mode were better, as illustrated with 47 substances with an LOD lower than 30 $\mu g\,kg^{-1}$ and only avermectin B1a with a LOD of 63 $\mu g\,kg^{-1}$. The LOQ in the SRM mode was below 100 $\mu g\,kg^{-1}$ for 47 substances. Only avermectin B1a showed an LOQ higher than 100 $\mu g\,kg^{-1}$ (at 210 $\mu g\,kg^{-1}$). To decrease the LOD and LOQ and match the 10 $\mu g\,kg^{-1}$ required for foodstuff, we are now trying to optimize our IT detector. Another idea is the improvement in the clean-up step, although this could be quite tedious and time-consuming.

Since benomyl was rapidly converted to carbendazim [35, 36] and acephate, which is thermally labile, pH-labile, very polar, not retained on the column (RT: 0.7 min), and rapidly undetectable in the standard solution, these two substances were removed from the present method, which finally resulted in a multiresidue method for 46 pesticides.

3.1.3 Recovery. The recovery of the method was tested at the two fortification levels LOQ and 10 times the LOQ in MS mode (figure 2a and b) for all the substances and in SRM mode for ten of them (figure 2c). The method was found to be precise and accurate, with recoveries between 70 and 110% for almost all the substances and a

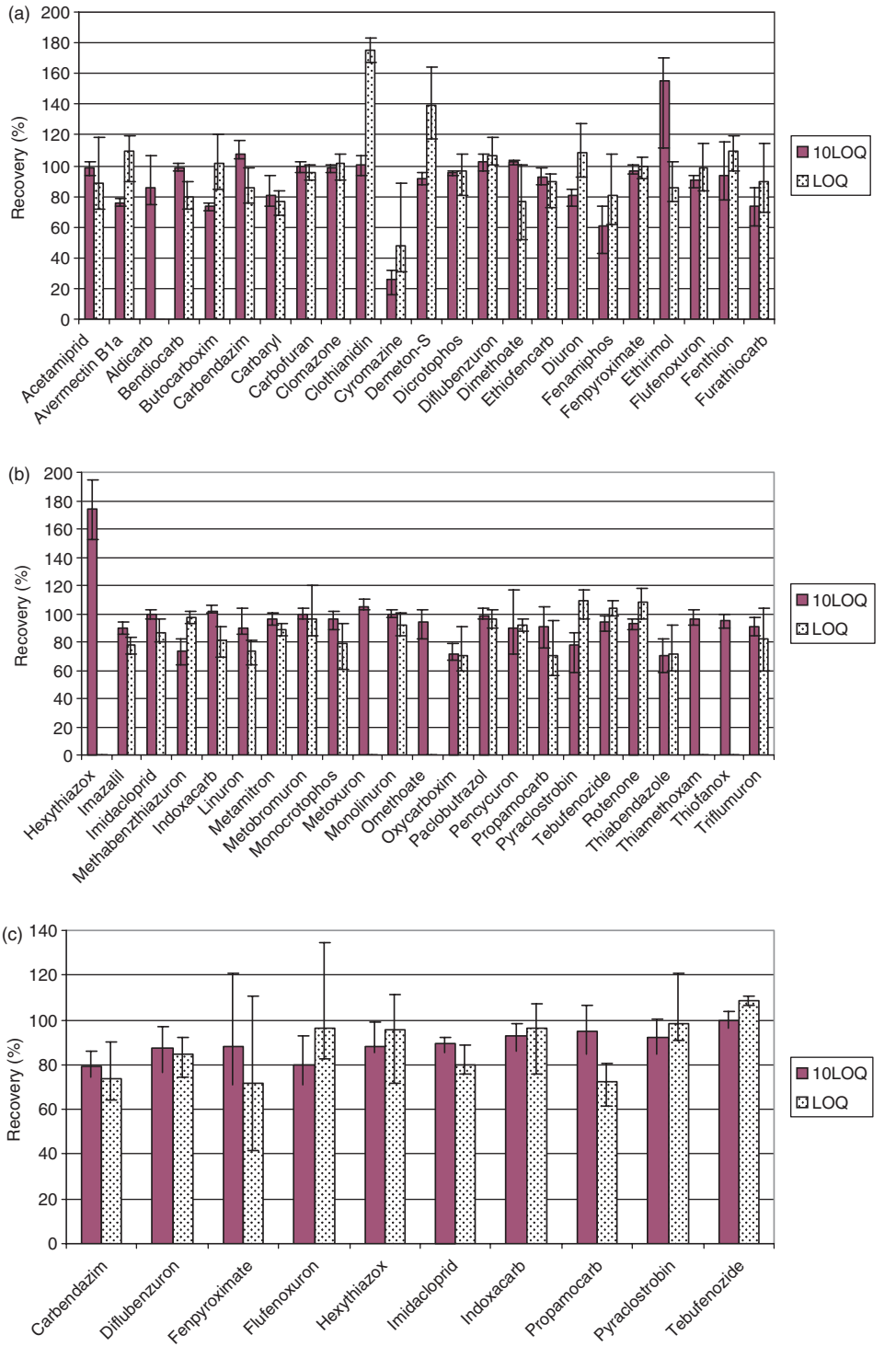

Figure 2. Recovery (average value and min/max in%) of the method in (a) and (b) MS mode at LOQ and 10LOQ and (c) SRM mode ($n = 7$).

relative standard deviation (RSD) lower than 16% at both modes and fortification levels.

At the LOQ fortification level in MS mode, we found recoveries between 70.4% (thiabendazole) and 107.8% (carbaryl) and repeatabilities between 1.1% (dicrotophos) and 16.0% (pencycuron) for all the substances except for fenpyroximate (154.9%, RSD 30.9%), hexythiazox (174.3%, RSD 30.5%), ethirimol (60.5%, RSD 6.1%) and cyromazine (26.1%). At ten times the LOQ fortification level, we obtained recoveries between 70.1% (propamocarb) and 109.7% (aldicarb) coupled to repeatabilities between 2.4% (methabenzthiazuron) and 14.9% (ethiofencarb) except for clothianidin (174.9%, RSD 9.9%), demeton-S (139.2, RSD 20.7%), and cyromazine (48.2%, RSD 9.6%).

The two blank matrices used for the two fortification levels presented different interference peaks of the same m/z ratio and RT than the analytes, resulting in recoveries for these substances higher than 110% in the full scan mode as shown for hexythiazox in figure 3. It could be certified in the SRM mode that these interfering substances in the blank matrices were not our analytes.

It is stated in figure 3(b) that the identification of hexythiazox in MS mode can be easily achieved in an interference-free sample but is not relevant in an interference-containing blank. When considering this same interference-containing blank in the SRM blank, the interference can be completely removed and the right concentration measured. This was confirmed when analysing the fortification samples at LOQ in SRM mode, where the recoveries for fenpyroximate and hexythiazox decreased to 71.9% (RSD 15.8%) and 95.5% (RSD 16.0%), respectively. In the SRM mode at LOQ, we achieved recoveries between 71.9% (fenpyroximate) and 108.4% (tebufenozide) with repeatabilities ranging from 1.5% (tebufenozide) to 17.9% (flufenoxuron). At ten times the LOQ, we obtained recoveries between 79% (carbendazim) and 100% (tebufenozide) coupled with repeatabilities between 2.1% (imidacloprid) and 14.9% (fenpyroximate).

Cyromazine showed at both fortification levels poor recoveries (26.1% at LOQ and 48.2% at ten times the LOQ). It is a small polar basic molecule ($pK_a = 5.22$), easily hydrolysed at an extreme pH owing to an ionic behaviour. Because of its particular properties, only a few analytical methods have been reported. Sancho *et al.* [13] recommends not only an acidic extraction solvent to promote its protonation and thus increase the extraction efficiency but also ion-pair reversed-phase liquid chromatography for its analysis. We obtained the same m/z precursor ion 167 and product ions 125, 85, and 139 at RT 1.4 min, but the extraction took place at pH 5.5, which can thus deplace cyromazine as its weak base and reduce its recovery.

3.2 *Application to real samples*

All the organic farming samples were below LOD in the full scan mode against 88% of the conventional farming samples. Of the remaining conventional samples, 12% presented pesticide residues above LOQ in full scan mode; 74% of the contaminated samples (residues > LOQ) contained one residue; 21% contained two residues; and 5% contained three residues. Samples with residue concentrations outside the linear range were diluted with acetonitrile before the second analysis. Figure 4 shows the occurrence of the pesticide residues in the contaminated samples after confirmation in the SRM mode. Indoxacarb was the most encountered pesticide (51.5% of the

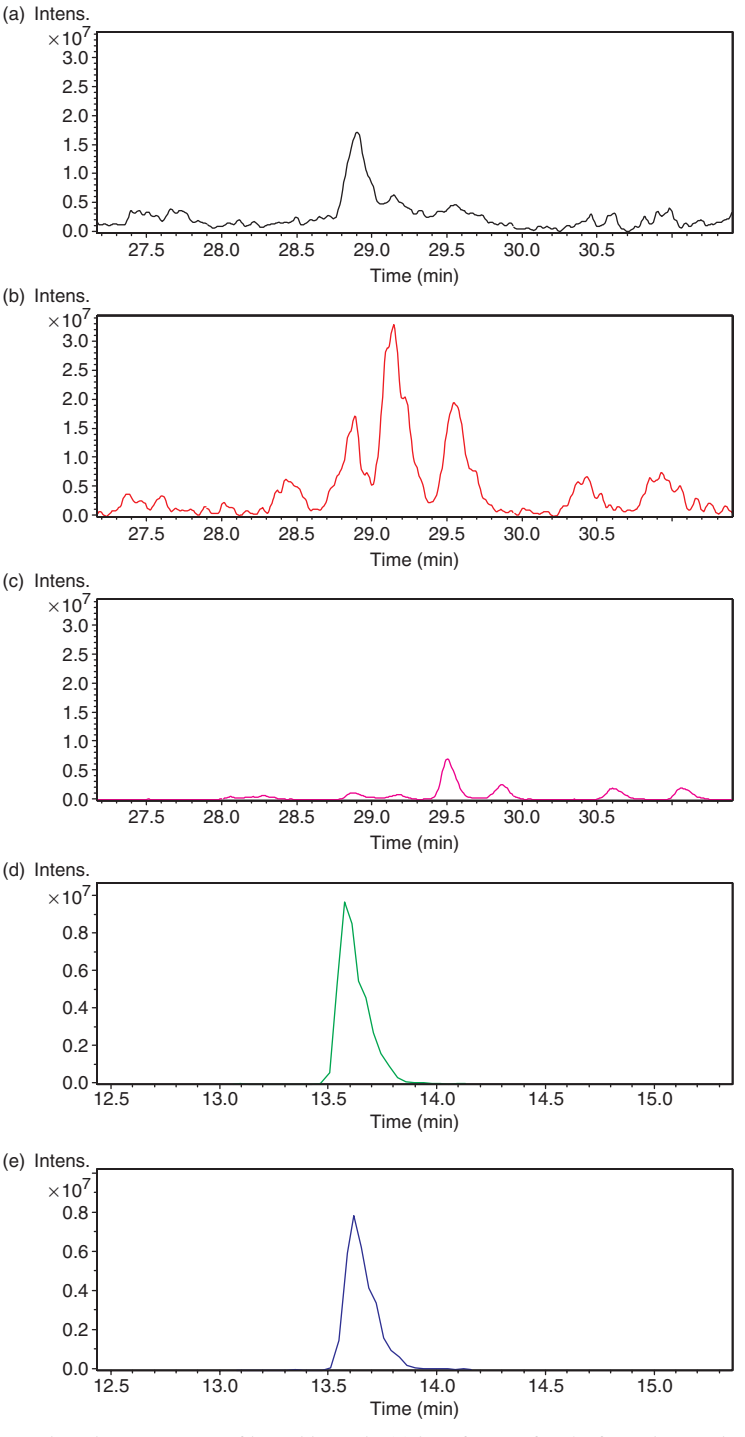

Figure 3. Extract ion chromatogram of hexythiazox in (a) interference-free leaf matrix-matched standard at 0.5 μg mL^{-1} in MS mode, (b) leaf blank with interference peak at ca 0.5 μg mL^{-1} in MS mode, (c) interference-free leaf blank in MS mode, (d) interference-free leaf matrix-matched standard at 0.5 μg mL^{-1} in MS^2 mode, and (e) leaf blank with interference peak at ca 0.5 μg mL^{-1} in MS^2 mode.

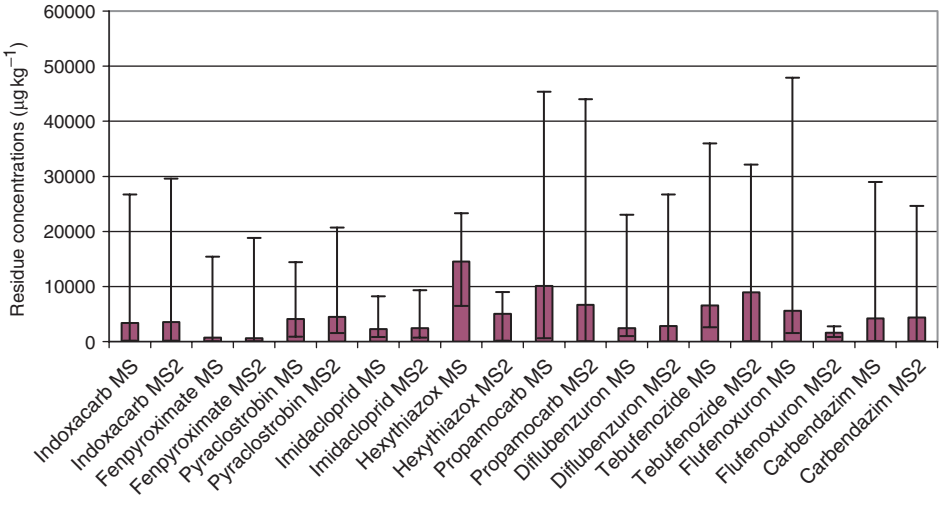

Figure 4. Occurrence of the pesticide residues in the contaminated leaf samples (minimum concentration and maximum concentration in bars; mean concentration in blocks) ($\mu g\,kg^{-1}$).

contaminated samples) followed by fenpyroximate (17.6% of the contaminated samples) and tebufenozide (15.2% of the contaminated samples). The seven other detected pesticides represented between 2 and 11% of the contaminated samples. Fenpyroximate was found in grape and in apple leaves; indoxacarb, tebufenozide, and pyraclostrobin were found only in grape leaves; diflubenzuron, carbendazim, and flufenoxuron were typical for apple leaves; potato and vegetable leaves were only contaminated with propamocarb, whereas hexythiazox and imidacloprid were characteristic for hop leaves. As already mentioned, there is no MRL established for pesticide residues in leaves but there is a list of pesticides authorized for use. As a matter of fact, none of the samples analysed presented unauthorized residues.

For hexythiazox and flufenoxuron, the quantification in the SRM mode gave concentrations always much lower than in the full scan mode as seen when comparing the min value, max value, and median value. The explanation is the same as that for the fortification experiments, since some samples showed interference peaks at the same ion and RT than hexythiazox and flufenoxuron, respectively (figure 3). In addition, we suspected nine samples of being contaminated with flufenoxuron based on the MS results, which was confirmed in the SRM mode only in three cases.

4. Conclusions

This study presents a multiresidue method for the analysis of 46 non-easily GC amenable pesticides in leaf samples with HPLC-IT-MS. The method is repeatable and accurate in full scan and SRM mode, and allows the quantitative and qualitative analysis of samples within 24 min. The method showed good recoveries at the LOQ and 10 times the LOQ fortification levels. When applied to around 1400 real leaf samples, it showed a good correlation for the concentrations in MS and SRM modes, apart

for hexythiazox and flufenoxuron due to the presence of interfering componds in MS mode, implying a possible quantification of the samples in both modes. Unfortunately, for some pesticides this method shows LOD and LOQ values (up to 218 $\mu g\,kg^{-1}$ and 725 $\mu g\,kg^{-1}$ in full scan, respectively) that are still too high to match the authorized values of 100 $\mu g\,kg^{-1}$ fresh weight required for organic farming leaf samples in the ÖPUL programme. Future work will involve (1) decreasing the sensitivity of the method by optimizing the IT detector; (2) testing the method for other matrices as recommended in the SANCO European Guidelines [33] for high-water-content matrices, high-sugar-content matrices, high-acidic matrices and high-sulfur-content matrices; and (3) broadening the spectrum of pesticide analysed with this method.

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Comparison of four extraction methods for the analysis of 24 pesticides in soil samples with gas chromatography–mass spectrometry and liquid chromatography–ion trap–mass spectrometry

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Abstract

With a view to analyse multiresidues of pesticides in soil samples, a new ultrasonic solvent extraction (USE) was compared to the European Norm DIN 12393 for foodstuff (extraction with acetone, partitioning with ethylacetate/cyclohexane and clean-up with gel permeation chromatography (GPC)), the QuECheRS method and a pressurised liquid extraction (PLE) method. Pesticides were analysed with both GC-MS and HPLC-MS/MS. The reference materials were the EUROSOIL 7 and its subsoil SO26 as well as a sea sand. All the substances were observed to be linear in the range of 4–800 ng g^{-1} for the European Norm DIN 12393, 7–1400 ng g^{-1} for the USE method and 20–4000 ng g^{-1} for the QueCheRS and the PLE methods. Limits of detection (LOD) and limits of quantification (LOQ) were with HPLC between 0.006 and 0.23 ng g^{-1} and between 0.022 and 0.77 ng g^{-1}, respectively, with the exception of diuron (LOD up to 11.8 ng g^{-1}; LOQ up to 39.2 ng g^{-1}). With GC they ranged from 3.0 to 87.5 ng g^{-1} and from 10 to 292 ng g^{-1}, respectively. All substances could be recovered with USE as well as with the QuECheRS method; the European Norm DIN 12393 could not recover carbendazim and metamitron; the PLE carbendazim, metamitron and monolinuron. For the remaining substances, recoveries at a 500 ng g^{-1} fortification level ranged from 10.9 to 96.3% with the USE. In comparison, the QuECheRS method was the most efficient extraction method with recoveries from 27.3 to 120.9%. It was followed by the European Norm DIN 12393 with recoveries between 6.8 and 108.1% and the PLE with recoveries from 12.2 to 153.2%. Recoveries were higher from the EUROSOIL 7 than from the SO 26. The repeatability expressed in term of standard deviation was below 20% for all substances and all materials.

Keywords: Pesticides; Soil; Gas chromatography; Liquid chromatography; Mass spectrometry

1. Introduction

The increasing worldwide need for food demands a higher agricultural productivity, which can only be achieved by an extensive use of pesticides. Unfortunately pesticides contaminate the environment through intensive or inappropriate use [1]. Although organochlorine insecticides like DDT and its metabolites, lindane, aldrin or dieldrin for instance have been banned years ago in many countries based on their mutagenic, carcinogenic and endrocrine disrupting properties, they still can be found in environmental samples due to their persistence and lipophilic properties [2–6]. Organophosphorus insectices (like chlorpyrifos, chlorpyrifos-methyl or chlorfenvinphos) and triazine herbicides (like atrazine, simazine, metribuzine) are the

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most commonly used pesticides around the world; they and their metabolites are detected in the environment although several members of these classes have been banned for years [4–6]. Among the different groups of pesticides, herbicides are more likely to pollute soils. In 1997, Barceló and Hennion [7] published valuable data about herbicides use: herbicides have been extensively used in the world for over 40 years representing 45% of the total market value in 1993 with more than 80% of herbicide use localised in North America, Western Europe and East Asia; 22% of the total herbicides are also used for non-agricultural purposes. Phenylurea and urea herbicides (e.g. linuron, diuron or metamitron) are in a sense emerging herbicides in recent years, but are already considered in EU list of priority substances containing some endocrine disruptors [8] and monitored in environmental samples [9]. Therefore, the monitoring of trace levels of pesticides in environmental samples is imperative because of their widespread use in agriculture but the sample preparation methods for the monitoring of multiresidues of pesticides in soil are scarce. The reason is the wide range of physico-chemical properties of the pesticides of interest that implies a comprehensive extraction and furthermore an expensive equipment such as GC-MS and HPLC-MS [10].

Ideally, a sample preparation should be rapid, simple, cheap, environmentally friendly and provide clean extracts. Traditionally pesticide analyses in soils were prepared by the time- and solvent-consuming Soxhlet extraction [11], which is more and more replaced by more environmentally friendly procedures including ultrasonic solvent extraction (USE) [2,4,9,12–17], pressurised liquid extraction (PLE) [3,18–22], shake-flask extraction [23–25], microwave assisted extraction (MAE) [26–28] or supercritical fluid extraction (SFE) [28] followed in some cases by a clean-up step with solid-phase extraction (SPE) [8,21,23,24,27] or solid-phase microextraction (SPME) [13,14,26].

Liquid–solid extractions (LSE) have the disadvantage of being time-, solvent-consuming and tedious. The new strategies are more environmentally friendly with advantages like rapidity, automation, selectivity and low consumption of solvents but lack sensitivity and selectivity [10]. Among them the ultrasonic extraction of contaminants from solid samples is becoming more and more favoured. USE and MAE are reported to improve the extraction efficiency but due to their limited selectivity and simultaneous co-extraction of soil and sediment components together with the target analytes, they often require a further clean-up step [4,23]. Usually, USE is operated with ultrasonic baths [2,4,9,13,15–17]. In the last years, a norm using a horn-type device equipped with a titanium tip was published for the extraction of nonvolatile and semivolatile organic compounds from solids such as soils, sludges, and wastes [29]. Recently, a more efficient system using a cylindrical ultrasonic probe for the sonication of soil samples was developed, described and applied to the dispersion of soil [12,14]. PLE and SFE are sample-volume restricted; in addition the recoveries of polar and/or thermolabile pesticides can be critical [4].

In addition to the techniques devoted to pesticide analysis in soil, some other procedures can be adopted from the analysis of pesticide multiresidues in foodstuff. To that purpose two main methods have been employed in the past and recent years: the European Norm DIN 12393 method [30,31] is applied in many laboratories as reference methods for the analysis of apolar and middle polar pesticides in non-fatty food samples and the QuECheRS multimethod has been developed recently and implemented in numerous laboratories for the analysis of apolar, middle polar and polar pesticides in non-fatty food samples [32–34]. Although until now these reference methods for foodstuff are rarely used for the extraction of soil samples, it is interesting to state whether these methods could be applied to soil analysis.

The goal of this study was the application of a new ultrasonic system, based on an ultrasonic cylindrical probe, for the extraction of pesticides from soil samples and to compare its efficiency to different extraction methods such as a PLE or methods conventionally applied to food analysis, i.e. the Norm method DIN 12393 and the QuECheRS method, a recent but well-known and quick method, for the determination of apolar, middle polar and polar pesticides with gas chromatography (GC) coupled to single quadrupole mass selective detector (MS) and high-performance liquid chromatography (HPLC) combined to ion trap (IT) mass spectrometry.

2. Material and methods

2.1. Reagents and chemicals

Pesticide standards were purchased either from Dr. Ehrenstorfer or from Sigma–Aldrich with the highest available purity.

Magnesium sulfate anhydrous, sodium chloride and sodium citrate dihydrate were purchased from J.T.Baker, di-sodium hydrogen citrate sesquihydrate was provided from Fluka and Bondesil-PSA (Primary Secondary Amine) 40 μm was from Varian.

Ultra-residue reagent acetone, ultra-residue reagent ethylacetate, ultra-residue reagent acetonitrile, HPLC-MS grade methanol, ultra HPLC-MS grade water and HPLC-MS grade formic acid were purchased from J.T.Baker.

Individual stock solutions were prepared dissolving 10 mg of standard in 10 ml acetonitrile and further diluted with acetonitrile to 10 $\mu g\,ml^{-1}$. Multicompound stock standard solutions were prepared dissolving 10 mg of each standard in 1000 ml acetonitrile reaching 10 $\mu g\,ml^{-1}$ and further diluted with acetonitrile to achieve concentrations of 5, 2, 0.5, 0.2 and 0.05 $\mu g\,ml^{-1}$. The solution used to spike the samples was prepared dissolving 10 mg of each standard in 100 ml acetone reaching 100 $\mu g\,ml^{-1}$ and further diluting 10 ml of this multicompound solution in 2000 ml petroleum ether to reach a 0.5 $\mu g\,ml^{-1}$ concentration. The single and multicompound standards were stored at 4 °C in the dark.

2.2. Pesticide selection

24 pesticides reported as soil pollutants in the literature [2–7,9] were selected. Preliminary experiments were carried out to find the best chromatographic technique for the few substances that can be analysed with GC and LC like atrazine and its

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Table 1
Physico-chemical properties (MW: molecular weight, solubility in water, K_{OW}: octanol/water coefficient, K_{OC}: organic carbon sorption constant) of the selected pesticides

Substance	Pesticide class	MW (g mol^{-1})	Water solubility (mg l^{-1})	log K_{OW}	K_{OC} (cm^3 g^{-1})
Atrazine	Triazine herbicide	215.10	35	2.7	100
Carbendazim	Carbamate fungicide	191.07	8	1.48	400
Chlorfenvinphos	Organophosphorus insecticide	357.97	145	3.8	680
Chloroxuron	Phenylurea herbicide	290.08	3.7	3.4	2,820
Chlorpyrifos	Organophosphorus insecticide	348.93	1.4	4.7	6,925
Chlorpyrifos-methyl	Organophosphorus insecticide	320.89	2.6	4.24	4,645
Deltamethrin	Pyrethroid insecticide	502.97	0.0002	4.60	460,000
Desethylatrazine	Metabolite of atrazine	187.63	n.a.	n.a.	n.a.
Desisopropylatrazine	Metabolite of atrazine	176.61	n.a.	n.a.	n.a.
Dieldrin	Organochlorine insecticide	377.87	0.14	3.7	12,000
Diuron	Phenylurea herbicide	232.02	35.6	2.87	1067
Flufenoxuron	Phenylurea herbicide	488.04	0.0043	4.01	3,200
Isoproturon	Phenylurea herbicide	206.14	70.2	2.5	139
Lindane	Organochlorine insecticide	287.86	8.52	3.69	1,100
Linuron	Phenylurea herbicide	248.01	63.8	3.0	620
Metamitron	Triazinone herbicide	202.09	1700	0.83	242
Methabenzthiazuron	Urea herbicide	221.06	60	2.64	527
Metobromuron	Phenylurea herbicide	258.0	330	2.41	197
Metoxuron	Phenylurea herbicide	228.07	678	1.6	120
Monolinuron	Phenylurea herbicide	214.05	735	2.2	200
Pencycuron	Phenylurea herbicide	328.13	0.3	4.68	6,207
Simazine	Triazine herbicide	201.08	5	2.3	130
Trifluralin	Dinitroaniline herbicide	335.11	0.221	5.27	8,765
Vinclozoline	Dicarboximide fungicide	285.0	3.4	3.01	100

n.a.: not available.

derivates, simazine or flufenoxuron for instances. On the ground of their peak shape/response and LOD/LOQ, this resulted in the analyses of 12 GC-amenable and 12 LC-amenable herbicides (dinitroaniline, phenylurea, urea, triazine and triazinone) and other fungicides/insecticides (carbamate, dicarboximide, organochlorine, organophosphorus and pyrethroid). Table 1 presents some of their physico-chemical properties [35].

2.3. Soil selection and preparation

The soils used in this study were the European reference material EUROSOIL 7 and its subsoil SOIL SO26 from the European Commission Environment Institute, Joint Research Center, ISPRA as well as a sea sand, purified by acid and calcinated, from Merck. The soils have been selected since they represent 24% of the arable land in Austria. The main physico-chemical properties of the 2 soils are given in Table 2 [36].

600 g of each solid material was contaminated with 600 ml of the 0.5 μg ml^{-1} multicompound standard solution, air-dried at room temperature for 7 days to obtain "aged soil" samples [10].

Table 2
Physico-chemical properties of the soils

Parameter	EUROSOIL 7	SO26	Sea sand
pH ($CaCl_2$)	4.4	4.6	5.5
Organic matter	11.52	1.81	0
Sand (w/w%)	46.0	64.3	100
Slit (w/w%)	35.2	31.1	0
Clay (w/w%)	18.8	4.6	0
$CaCO_3$	0.15	0.13	0

After the bulk of the solvent was evaporated, the materials were finally dried overnight at 30 °C. Sensitivity, recovery and precision of the methods were tested as assigned for foodstuff by the SANCO European Guideline [37] at a 500 ng g^{-1} soil fortification level for 7 replicates. The linearity of the methods was tested for 5 standards in acetonitrile in the range 0.010–2 μg ml^{-1} (i.e. 4–800 to 20–2000 ng g^{-1} depending on the extraction method). LOD and LOQ were assessed for the target ion (GC) and most important fragmentation ion (HPLC) as the lowest concentration that yielded to a signal to noise ratio of 3 and 10, respectively.

2.4. Soil sample extraction

Four extraction methods were assessed and compared in this study: (i) a new ultrasonic extraction method; (ii) a pressurised liquid extraction; (iii) the European Norm DIN 12393 [30] as multiresidue method for the gas chromatographic determination of pesticide residues in non-fatty foodstuff; (iv) the QuECheRS method [32] for the analysis of pesticide multiresidue in non-fatty foodstuff. The solvent composition and ratio used for PLE and USE were the same as in the QuECheRS method in order to compare the results.

2.4.1. Ultrasonic solvent extraction (USE)

USE experiments were carried out with 20 g of sample extracted with 60 ml of a water/acetonitrile (1:2, v/v) solution in a 200 ml glass beaker. The device design and set-up used in this study is given in details elsewhere [12,14]. The samples were homogenised with a small magnetic stirring bar during the ultrasonic extraction with a Sonoplus HD 2200 from Ban-

delin equipped with a cylindrical probe US 70 T with a diameter of 12.7 mm. The sonication took place for 2 min at 20 kHz; the vibration amplitude was 35 μm; the energy value was 7.8 J ml^{-1} and the insertion depth 10 mm. A second set-up with a vibration amplitude of 60 μm and an energy value of 13.6 J ml^{-1} was investigated to test the influence of these parameters on the extraction of the pesticides. The extract was filtered through 0.45 μm and 1.8 ml aliquot was evaporated under a gentle stream of nitrogen and collected in 400 μl acetonitrile/acetone (1:1, v/v) for the soil samples and 700 μl acetonitrile/acetone (1:1, v/v) for the sea sand samples, respectively. Triphenylphosphate (TPP) was used as internal standard and spiked before the extraction to reach a concentration of 0.5 μg ml^{-1} in the final extract.

The first main difference between the system used in this study [12,14] and the system recommended by the US EPA method 3550C [29], is the definition of the input energy that is to be at least 300 W in the US EPA method 3550C. The application as defined inhere (insertion depth, vibration amplitude and energy value) reflects the real input energy received by the samples since the vibration amplitude, i.e. the energy has been calibrated, and is measured and controlled through stain gauges of a feedback system of the ultrasound amplifier [12,14]. This owns the particularity of delivering a constant vibration amplitude (lower than 0.01%).

The second difference is the recommended use of a pulse mode in the US EPA method 3550C for low concentrations to be extracted. This is not necessary with our cylindrical probe since its geometry guaranties a strong circular laminar flow that is enhanced through the use of a magnetic stirring device.

The extraction took place only once, and not three times as recommended by the US EPA method 3550C, due to the relatively high fortification concentration of 500 ng g^{-1}.

2.4.2. Pressurised liquid extraction (PLE)

For the PLE experiments, 5 g of sample was mixed with 1 g silica gel and introduced in a 10 ml steel column. The accelerated solvent extractor was an ASE 100 from Dionex. In preliminary experiments the best set-up for the extraction with water/acetonitrile (1:2, v/v) was found to be 110 bar and 140 °C during 20 min with 3 PLE cycles. The collected extract (ca. 40 ml) was evaporated as far as it could, i.e. almost to dryness with rotary evaporator at 40 °C, further dissolved in 10 ml acetonitrile/acetone (1:1, v/v), filtrated through 0.45 μm and 1.5 ml were filled in 2 autosampler vials for GC-MS and HPLC-MS analysis. TPP was used as internal standard and spiked before extraction to reach a concentration of 0.5 μg ml^{-1} in the final extract.

2.4.3. European Norm DIN 12393

Although traditionally applied to foodstuff, this method [30] was used here for the extraction of soil samples. This conventional method consists of an extraction step of 25 g of the sample with 50 ml water and 100 ml acetone (1:2, v/v) followed by partitioning with ca. 100 ml ethylacetate/cyclohexane (1:1, v/v). After evaporation to dryness with rotary evaporator at 40 °C, the samples were collected in 10 ml ethylacetate/cyclohexane (1:1, v/v), filtered through 0.45 μm and 1.5 ml of the extract were cleaned-up by gel permeation chromatography (GPC). The extracts were consequently evaporated almost to dryness with rotary evaporator at 40 °C and diluted to 1.5 ml acetonitrile/acetone (1:1, v/v) prior to analysis with GC-MS and HPLC-MS. Aldrin, which is forbidden to use since over 20 years, was used as internal standard spiked at the partitioning step.

2.4.4. QuECheRS method

The QuECheRS method described by Anastassiades [32] is based on the extraction of 10 g of sample with 20 ml acetonitrile followed by a salting-out step with 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate dihydrate and 0.5 g di-sodium hydrogen citrate sesquihydrate. The clean-up step of the samples was carried out with 150 mg Bondesil-PSA and 950 mg $MgSO_4$ before filtration of the sample through 0.45 μm filter and transfer of 1.5 ml of the extract into 2 autosampler vials for GC-MS and HPLC-MS analysis. TPP was used as internal standard and spiked before extraction to reach a concentration of 0.5 μg ml^{-1} in the final extract.

2.5. Apparatus and analytical conditions

2.5.1. GC-MS

The GC-MS analyses were performed on a Hewlett-Packard (Agilent Technologies, Waldbronn, Germany) GC-MS Model 6890N Series gas chromatography coupled to a 5973N mass selective detector. A HP 5 MS (30 m × 0.25 mm i.d.) (Agilent Technologies, Waldbronn, Germany) fused silica capillary column with a 0.25 μm film thickness was used with helium as carrier gas at a constant pressure daily adjusted (chlorpyriphos-methyl RT relocked to 16.596 min). One microliter of the sample was injected in the splitless mode at 280 °C with a splitless time before opening the injector valve of 2 min. The GC oven was operated with the following temperature program: initial temperature 70 °C held for 2 min, ramped at 25 °C/min to 150 °C not held, followed by a ramp of 3 °C/min to 200 °C not held, followed by another ramp of 8 °C/min to 280 °C held for 10 min and finally ramped to 320 °C at 15 °C/min held for 2.47 min. The total run time was 47 min, the interface was kept at 320 °C, the ion source at 250 °C, the quadrupole at 150 °C and the mass spectra were obtained at an electron energy of 70 eV. The analyses were operated in simultaneous full scan/SIM mode method presented elsewhere [19,23]. The target ions and qualifiers used for quantification are presented in Table 3. The Agilent Chemstation Software G1701DA version D.02.00.237 was used for data analysis.

2.5.2. HPLC-MS/MS

The high-performance liquid chromatography system was an Agilent Technologies HP-1100 Series (Agilent Technologies, Waldbronn). Chromatographic separation was achieved using a Zorbax SB-C18 analytical column 2.1 × 150 mm, 3.5 μm particle size from Agilent Technologies at a flow rate of 300 μl min^{-1}. The mobile phases consisted of A: H_2O–MeOH, 90%–9.95% (v/v) with 0.05% HCOOH and B: H_2O–MeOH, 9.95%–90% (v/v) with 0.05% HCOOH. The gradient was 100% A at 0 min, 100% A at 1 min, 0% A at 10 min, 0% A at 17 min,

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Table 3
Target ions and qualifier ions for the GC-MS

Substance	R.T. (min)	Target ion (*m/z*)	Qualifier ion (*m/z*)	Qualifier ion (*m/z*)	Qualifier ion (*m/z*)
Atrazine	13.5	200	215	173	202
Chlorfenvinphos	21.6	267	269	323	325
Chlorpyrifos	19.2	314	197	97	258
Chlorpyrifos-methyl	16.6	286	288	323	290
Deltamethrin	36.2	181	253	251	255
Desethylatrazine	11.7	172	187	174	145
Desisopropylatrazine	11.5	158	173	145	175
Dieldrin	23.8	279	277	237	345
Lindane	13.6	219	254	181	
Simazine	13.3	186	201	173	158
Trifluraline	11.5	306	264	290	307
Vinclozoline	16.8	285	198	189	241

100% A at 20 min. The post time was 2 min with 100% A and the stop time 22 min. The HPLC was controlled with the Agilent Technologies Chemstation for LC 3D System Software.

The HPLC system was interfaced to an Agilent Technologies mass spectrometer LC/MSD trap XCT Plus (Agilent Technologies, Waldbronn) equipped with an electrospray ionisation (ESI) interface operated in positive mode and controlled with the Agilent Technologies LC/MSD trap software 5.3. The nebulizer gaz (nitrogen) pressure was 40 psi, the drying gas flow rate was 8 ml/min and the drying gas temperature was 325 °C. The capillary voltage was −4500 V, the endplate offset was fixed at −500 V. The ion trap was operated in the ion charge control (ICC) mode with a target ion count of 150 000 and a maximum accumulation time of 50 ms. The quantification was done in the selected reaction monitoring (SRM) mode with a fragmentation voltage of the $[M+H]^+$ ion set at 0.6 V. The precursor and fragmentation ions selected for quantification are presented in Table 4.

3. Results and discussion

3.1. Optimization of the method

Figs. 1–3 present the recoveries achieved for the 3 soil materials with the 4 extraction methods. In preliminary tests with USE, the influence of the energy value was tested at 2 levels (7.8 and 13.6 J ml^{-1}) and concluded to be insignificant since the experiments produced comparable results (not shown). USE was further carried out with the parameters given in Section 2.4.1.

For all the methods and samples, the recovery of the internal standard was between 90 and 100%. First of all, it can be noticed that only the QuECheRS and the USE methods allowed the recovery of all the substances. Carbendazim and metamitron were recovered neither with the European Norm DIN 12393 nor with PLE as well as additionally monolinuron were unable to be extracted with PLE. When considering their soil sorption coefficient (K_{OC}), carbendazim, metamitron and monolinuron were not expected to present any problem as to their extraction from the materials since they present K_{OC} between 200 and 400 $cm^3\ g^{-1}$. Nevertheless, they own the lowest octanol–water partition coefficient (K_{OW}) of all the selected substances between 0.8 and 2.2 implying a possibly high repartition in the water phase and as a consequence low concentration in the analysed organic phase. Overall the substances often reported for their strong binding to soil [10] like lindane, trifluralin, dieldrin or deltamethrin for instances (i.e. those with the highest K_{OC}) were always recovered.

3.2. Validation of the method

3.2.1. Linearity of the calibration curve, LOD and LOQ

The linearity, plotted as MS response area vs. concentration, as well as the achieved LOD and LOQ with GC-MS and HPLC-MS, estimated for the target ion and the parent ion, respectively,

Table 4
Target ions and qualifier ions for the HPLC-MS/MS

Substance	R.T. (min)	Precursor ion (*m/z*)	Fragmentation ion (*m/z*)	Fragmentation ion (*m/z*)
Carbendazim	6.2	191.9	159.9	
Chloroxuron	11.5	291.1	163.9	
Diuron	10.7	232.9	232.9	72.3
Flufenoxuron	13.2	489.1	158.1	141.1
Isoproturon	10.6	207.1	207.1	72.1
Linuron	11.1	248.9	182.0	160.0
Metamitron	8.3	202.9	202.9	
Methabenzthiazuron	10.6	221.9	164.9	
Metobromuron	10.5	258.9	147.9	
Metoxuron	9.1	228.9	228.9	
Monolinuron	10.2	214.9	214.8	
Pencycuron	12.3	329.1	329.1	

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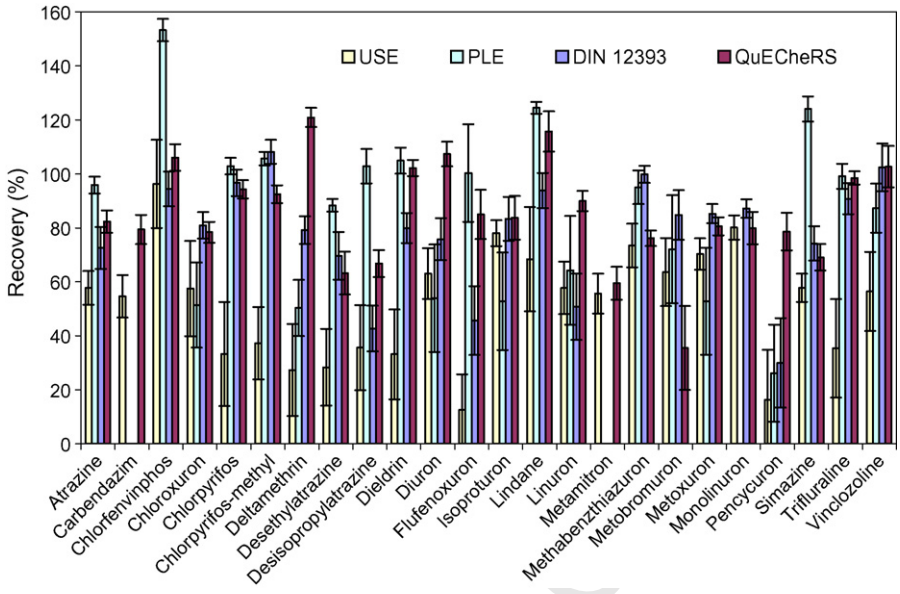

Fig. 1. Recovery (%) of the pesticides at 500 ng g^{-1} from EUROSOIL 7 ($n = 7$) with error bars representing the standard deviation.

as the lowest concentration injected that yielded to a S/N ratio of 3 (LOD) and 10 (LOQ), for the selected substances, are presented in Table 5.

All the substances presented a linear behaviour with GC-MS and LC-MS/MS analysis in the standard concentration range of 0.010–2 μg ml^{-1} corresponding to a soil concentration range of 4–800 ng g^{-1} for the European Norm DIN 12393, 7–1400 ng g^{-1} for the USE method and 20–4000 ng g^{-1} for the QueCheRS and the PLE methods. The lowest LOD/LOQ were achieved with the European Norm DIN 12393 and the highest with the QueCheRS and the PLE methods. With the present methods the LC-amenable substances presented LOD and LOQ in the low ng g^{-1} range between 0.006 ng g^{-1} (carbendazim) and 0.23 ng g^{-1} (flufenoxuron) and from 0.022 to 0.77 ng g^{-1}, respectively, except for diuron with LOD from 3.1 to 11.8 ng g^{-1} and LOQ from 10.5 to 39.2 ng g^{-1}. Unlikely,

Table 5
Linearity, LOD and LOQ of the selected pesticides with GC-MS and HPLC-MS/MS

Substances	Linearity	QuECheRS		DIN 12393		PLE		USE	
	R^2	LOD (ng g^{-1})	LOQ (ng g^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})
Atrazine	0.9952	13	43	5	17	4	12	7	23
Desethylatrazine	0.9996	13	43	5	17	4	12	7	23
Desisopropylatrazine	0.9959	11	38	5	15	3	10	6	20
Carbendazim	0.9995	0.02	0.08	0.01	0.03	0.006	0.02	0.01	0.04
Chlorfenvinphos	0.9970	22	73	8.7	29	6	19	12	39
Chloroxuron	0.9996	0.10	0.33	0.04	0.13	0.03	0.09	0.05	0.17
Chlorpyrifos	0.9937	24	79	10	32	6	21	13	42
Chlorpyrifos-methyl	0.9946	37	125	15	50	10	33	20	67
Deltamethrin	0.9962	14	47	6	20	3.8	13	8	25
Dieldrin	0.9990	88	292	35	117	23	78	47	156
Diuron	0.9956	12	39	5	16	3	11	6	21
Flufenoxuron	0.9969	0.23	0.77	0.09	0.30	0.06	0.21	0.12	0.41
Isoproturon	0.9990	0.17	0.56	0.07	0.20	0.04	0.15	0.09	0.30
Lindane	0.9962	13	42	5	17	3	11	7	22
Linuron	0.9992	0.09	0.30	0.04	0.12	0.02	0.08	0.05	0.16
Metamitron	0.9992	0.05	0.16	0.02	0.07	0.01	0.04	0.03	0.09
Methabenzthiazuron	0.9986	0.17	0.58	0.07	0.23	0.05	0.16	0.09	0.31
Metobromuron	0.9994	0.12	0.42	0.05	0.17	0.03	0.11	0.07	0.22
Metoxuron	0.9999	0.08	0.26	0.03	0.10	0.02	0.07	0.04	0.14
Monolinuron	0.9988	0.10	0.35	0.04	0.14	0.03	0.09	0.06	0.19
Pencycuron	0.9952	0.09	0.30	0.04	0.12	0.02	0.08	0.05	0.16
Simazine	0.9928	14	48	6	19	4	13	8	25
Trifluraline	0.9965	20	65	8	26	5	17	10	35
Vinclozoline	0.9949	20	68	8	27	5	18	11	36

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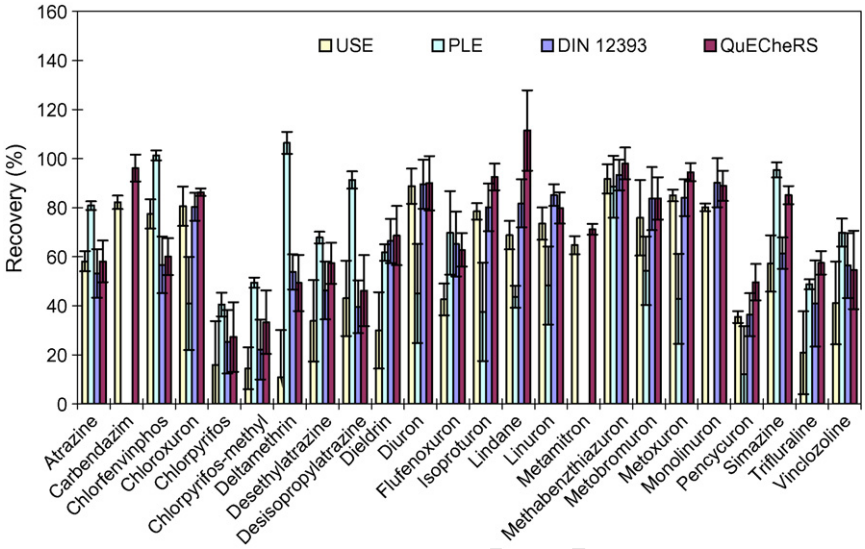

Fig. 2. Recovery (%) of the pesticides at 500 ng g^{-1} from SO 26 ($n = 7$) with error bars representing the standard deviation.

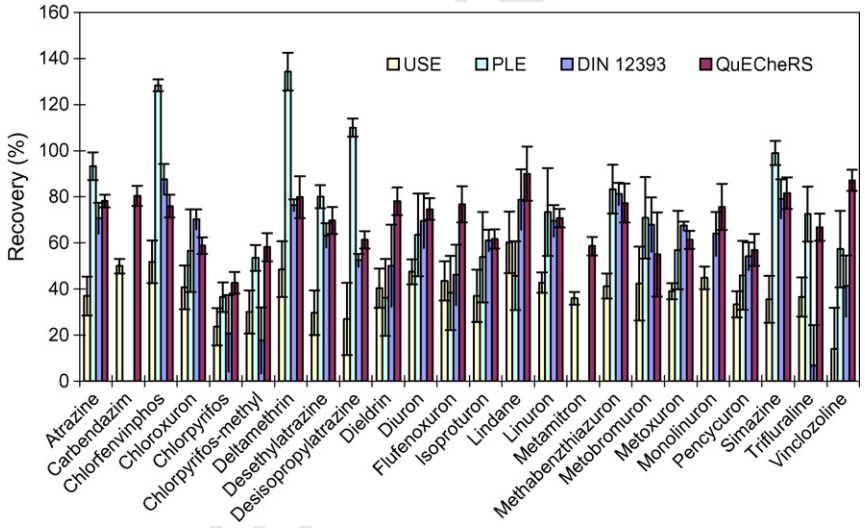

Fig. 3. Recovery (%) of the pesticides at 500 ng g^{-1} from sea sand ($n = 7$) with error bars representing the standard deviation.

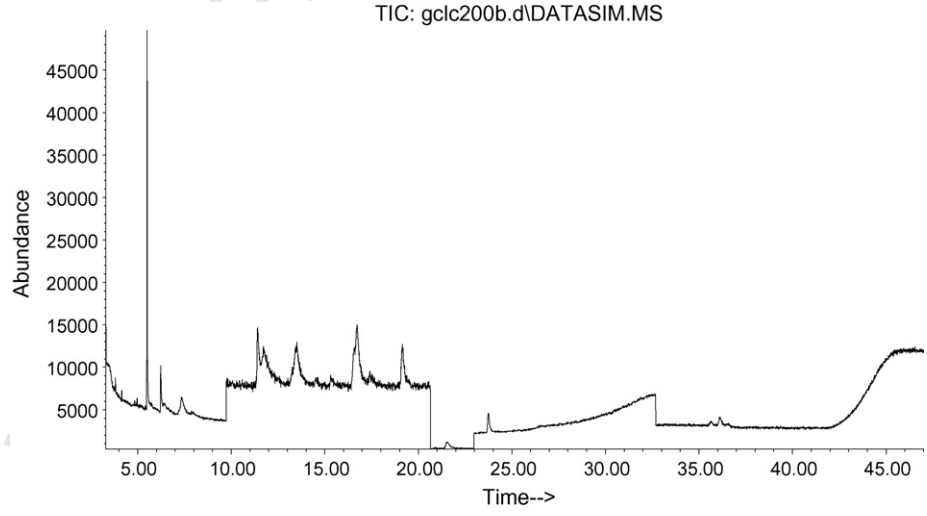

Fig. 4. SIM mode GC-MS chromatogram of a 0.060 μg ml^{-1} standard corresponding to 25 ng g^{-1} sample with the European Norm DIN 12393, 40 ng g^{-1} with USE and 120 ng g^{-1} with the QuECheRS and PLE methods.

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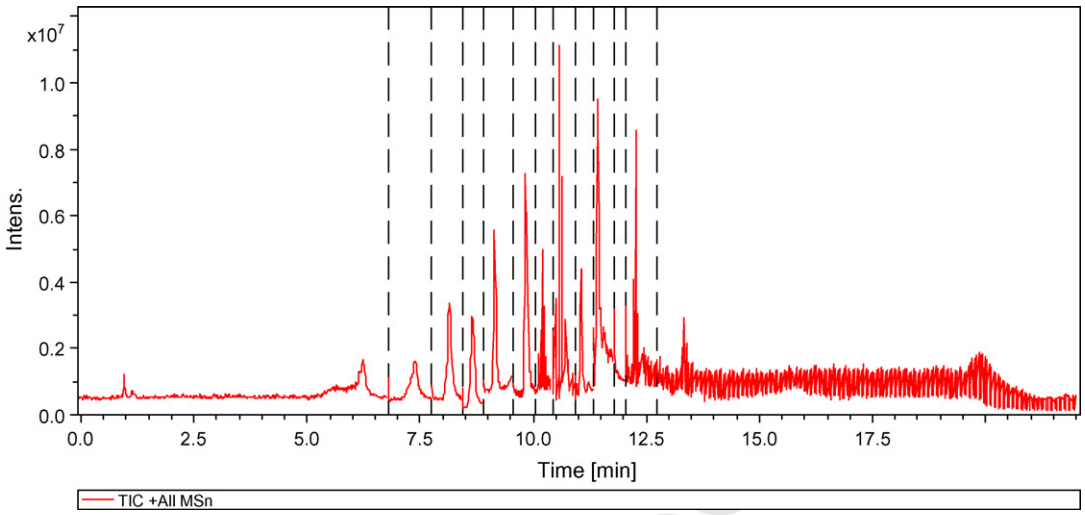

Fig. 5. LC-MS/MS chromatogram of a 0.010 μg ml^{-1} standard corresponding to 0.4 ng g^{-1} with the European Norm DIN 12393, 0.7 ng g^{-1} with USE and 2 ng g^{-1} with the QuECheRS and PLE methods.

GC-amenable analytes and in particular chlorpyrifos-methyl and dieldrin presented higher LOD and LOQ in the range 3.0 ng g^{-1} (desisopropylatrazine) to 87.5 ng g^{-1} (dieldrin) and 10 to 292 ng g^{-1}, respectively. Similar LOD and LOQ were reported in the literature [2,4,9,10,13,15–17]. Figs. 4 and 5 also shows a GC-MS chromatogram of a 0.060 μg ml^{-1} standard and a LC-MS/MS chromatogram of a 0.010 μg ml^{-1} standard, respectively.

3.2.2. Recovery and precision

When considering only the substances that were recovered from the materials, the lowest recoveries were obtained with the USE (between 10.9 and 96.3% with a median recovery of 57.0% for the 3 materials). On the contrary the highest recoveries were obtained with the QuECheRS method (between 27.3 and 120.9% with a median recovery of 72.7% for the 3 materials). The remaining method produced similar results: European Norm DIN 12393 (between 6.8 and 108.1% with a median recovery of 65.7% for 3 materials) and PLE (between 12.2 and 153.2% with a median recovery of 63.5% for 3 materials). The QuECheRS method was the method with the highest rate of substances (around 50%) in the 3 matrices satisfying the 70–110% recovery range.

The repeatability was similar and acceptable below 20% for all the methods: USE (S.D. between 1.5 and 19.3% with a median repeatability of 8.5%); PLE (S.D. between 1.8 and 20.2% with a median repeatability of 6.4%); QuECheRS method (S.D. between 1.5 and 18.3% with a median repeatability of 5.4%) and European Norm DIN 12393 (S.D. between 1.9 and 17.8% with a median repeatability of 7.7%). Nevertheless, it was slightly higher for the USE and the PLE.

It is known that organochlorine pesticides have a high affinity to organic humic substances of soil matrices (high K_{OW}) with which they develop chemical bonds [10,38,39]. Since the bond energy between two atoms is higher than the Van der Wals energy, which is related to surface processes, or than ion exchange energy, the energy produced by the ultrasonic dispersion (40 W) is too weak to break down the created C–C bonds (348 kJ mol^{-1}) between organo-mineral complexes [14]. This is valid for chlorpyriphos, chlorpyriphos-methyl, deltamethrin and dieldrin. As far as lindane is concerned, it owns the highest water solubility and the lowest soil sorption coefficient, which can explain the better recovery than with the other organochlorine pesticides [40]. The secondary and tertiary amine pesticides (phenylureas, triazines and their metabolites) tend to adsorb on the soils' inter-crystalline layers of clay minerals [41] that cannot be reached with ultrasonic vibration and makes this extraction less efficient with these substances [14].

Higher recoveries were achieved with the EUROSOIL 7 than with the SO 26 and in any case than with the sea sand whatever the extraction method. Since the adsorption of pesticides increases with the organic matter content [9,38,39], pesticides should adsorb better to the EUROSOIL 7 than to its subsoil SO 26 and consequently be possibly harder to desorb from the materials. The recoveries obtained with the sea sand were in contradiction with the expected results since the material was not expected to retain the substances. An explanation could be that the samples were dried overnight at 30 °C and analytes can build bonds to soil aggregates and solid matter that do not take place with sea sand.

Especially for chlorpyrifos and chlorpyrifos-methyl far better recoveries were obtained for the EUROSOIL 7 than for the SO 26 with all extraction methods. Since recoveries as high as 150% (chlorfenvinphos, deltamethrin, lindane and simazine) were obtained with PLE, a clean-up step is strongly recommended. An explanation for these recoveries higher than 100% could be the interference of the matrix also known as "matrix induced chromatographic response enhancement" effect as detailed by Molins et al. [42] and Mol et al. [43]. Trifluralin, chlorpyriphos and chlorpyriphos-methyl presented extreme low recoveries from sea sand with the European Norm DIN 12393 although this method has been demonstrated to be effective for these analytes but for other matrices. The adsorption of ionisable substances like atrazine and its metabolites is reported to increase with

decreasing pH [10,38,39], which explains the rather low recoveries of atrazine, desethylatrazine and desisopropylatrazine with less acidic extraction methods.

The European Norm DIN 12393 has been developed for the analysis of GC-amenable substances and is known to be low efficient in the recovery of polar pesticides like phenylureas or carbamates. Lambropoulou et al. [13] noticed that water, acetone, acetonitrile and methanol showed similar results for the extraction of vinclozoline but that acetone was significantly more efficient in the case of dicloran. Tor et al. [2] recognized acetone, a more polar solvent, as best solvent for the break-up and disintegration of aggregates leading to a better contact between particles and solvent and an improvement of the extraction especially in the case of organochlorine pesticides. By studying the USE of tetramethrin and chlorpropham, Babić et al. [16] noticed that acetonitrile was the only solvent out of 7 that was not able to recover these substances, whereas Gonçalves and Alpendurada [4] recommend acetonitrile for the extraction of organochlorine pesticides from soil samples. Nevertheless most of the studies approve the use of water:solvent mixture since better recoveries are reported [22]. The use of acetone might favor the extraction of analytes from soil matrices but together with that of co-eluents inherent to the materials. This results in less clean extracts leading to the drawbacks of higher LOD and LOQ and the need for a clean-up step, which is always critical in the case of multiresidue methods.

4. Conclusions

The lowest LOD for the analysis of the selected pesticides from soil samples were achieved with the European Norm DIN 12393 and the highest with the QueCheRS and the PLE methods. The LODs achieved with LC-MS/MS were much lower than those achieved with GC-MS, namely in the low ng g^{-1} range between 0.006 ng g^{-1} (carbendazim) and 0.23 ng g^{-1} (flufenoxuron) for the LC-MS/MS against values in the range 3.0 ng g^{-1} (desisopropylatrazine) to 87.5 ng g^{-1} (dieldrin) for the GC-MS. The investigation of a new ultrasonic extraction method for analysis of 24 herbicides and insecticides showed that this new USE was successful to recover all the selected substances with a good repeatability (S.D. between 1.5 and 19.3% with a median repeatability of 8.5%) in comparison with the European Norm DIN 12393 that could recover neither carbendazim nor metamitron and the PLE that could not recover carbendazim, metamitron and monolinuron at a 500 ng g^{-1} fortification level. Nevertheless, the QuECheRS method presented the highest recoveries (median recovery of 72.7%) followed by the European Norm DIN 12393 (median recovery of 65.7%) and the PLE (median recovery of 63.5%) whereas the USE showed the lowest recovery (median recovery of 57.0%) of the four selected methods at a 500 ng g^{-1} fortification level. Especially the pesticides with a water solubility lower than 5 mg l^{-1} could not be extracted properly. The QuECheRS method was the most adapted method with around 50% of the substances with recoveries in the recommended range of 70–110%. Some substances presented recoveries as high as 150% with PLE implying the need for a cleaning step. The ultrasonic energy seems to be too low to extract the substances that either create bonds with humic substances or adsorb on the inter-crystalline layers of clay minerals. The European Norm DIN 12393 and the PLE were proven to be not adapted for the extraction of polar pesticides. The EUROSOIL 7 presented the highest recoveries and the sea sand the lowest. For some substances (chlorpyrifos and chlorpyrifos-methyl for instances) the recoveries with the EUROSOIL 7 were much higher than with the SO 26. Atrazine, desethylatrazine and desisopropylatrazine presented low recoveries with almost all the methods.

This new USE method is accurate as monitoring method for the extraction of the selected pesticides from soil but cannot be implemented as currently applied as quantification method due to its low recovery for chorpyrifos, chlorpyrifos-methyl, deltamethrin, desethylatrazine, desisopropylatrazine, dieldrin, flufenoxuron, pencycuron and trifluraline. The QuECheRS method seems so far to be the most adapted method for these analyses. Nevertheless, another solvent like acetone for instances should be investigated with USE to increase the recoveries.

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Pesticide Residues in Fruit and Vegetable Samples: Analytical Results of 2 Year´s Pesticide Investigations

Pestizidrückstände in Obst- und Gemüseproben: Analytische Ergebnisse einer zweijährigen Pestiziduntersuchung

C. LESUEUR, M. GARTNER, P. KNITTL, P. LIST, S. WIMMER, V. SIELER, M. FÜRHACKER

Summary

During the last two years around 3300 fruit and vegetable samples produced under organic farming and conventional farming conditions were analysed for apolar and middle polar pesticide residues with gas chromatography (GC/MS). 90 % of the organic farming products were below the limit of detection (LOD) and the percentage of organic farming products exceeding 10 µg/kg product (fresh weight) was 3.9 % in 2004 and 8.2 % in 2005. The number of conventional farming products with detectable pesticide residues was 62 % in 2004 and 54 % in 2005. 8.4 % (2004) and 7.7 % (2005) of the conventional farming samples presented residues exceeding maximum residue levels (MRLs). 37 % (2004) and 32 % (2005) of the conventional farming samples presented more than one pesticide residue. Still some conventional samples containing a cocktail of up to 13 pesticide residues were found. Finally some samples with residues exceeding MRL showed multiple residues (up to four) over MRL.

Keywords:
pesticides, fruits, vegetables, GC/MS

Zusammenfassung

Während der letzten zwei Jahre wurden 3300 biologische und konventionelle Obst- und Gemüseproben auf unpolare und mittelpolare Pestizide mittels GC/MS untersucht. Die Pestizidkonzentrationen von 90 % der biologisch produzierten Proben lagen unter der Nachweisgrenze. Der Anteil an biologischen Proben, mit Pestizidgehalten über 10 µg/kg Produkt lag 2004 bei 3,9 % und 2005 bei 8,2 %. 62 % (2004) und 54 % (2005) der konventionell produzierten Proben zeigten nachweisbare Pestizidrückstände. 8,4 % (2004) und 7,7 % (2005) der konventionell produzierten Proben zeigten Höchstwertüberschreitungen. 37 % (2004) und 32 % (2005) der konventionell produzierten Proben wiesen mehr als einen Wirkstoff auf. Außerdem gab es konventionell produzierte Proben in denen bis zu 13 Wirkstoffe nachgewiesen werden konnten. Schließlich gab es Proben, die bis zu vier Höchstwertüberschreitungen aufwiesen.

Kennwörter:
Pestizide, Obst, Gemüse, GC/MS

1. Introduction

The US Environmental Protection Agency (EPA) [1] defines a pesticide as "*any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest including weeds, insects, rodents, fungi, bacteria, or other organisms.*" Pesticide is a general term for insecticides, herbicides, fungicides, acaricides or nematicides *(Table 1)*. Insecticides kill or prevent the growth of insects. Herbicides control or destroy plants. Fungicides control or destroy fungi. Acaricides control or destroy mites. Nematicides control or destroy nematodes.
Unfortunately pesticides are not only destroying pests, they are also potentially harmful to human beings. The World Health Organization (WHO) [2] estimates pesticide poisonings occurring every year between one and five million cases, resulting in several thousands of fatalities. In 1973, it established a classification of pesticides that distinguishes between the more and the less hazardous forms of each pesticide, which is ever since yearly up-dated [3]. In 1993, the US EPA, in coordination with the International Agency for Research on Cancer (IARC) and the European Union (EU) listed seventy possible carcinogenic pesticides [4]. In 2005 this list was extended to 160 potential pesticide carcinogens and published in a briefing paper of the Pesticide Action Network (PAN) UK [5]. Furthermore, some pesticides are suspected of being endocrine disruptors [6].

These facts and figures made pesticides a more and more discussed issue in the last years since some of the pesticides are persistent, accumulate in the food chain and contaminate the environment. In Europe, this forced the European Community to establish directives and maximum residue levels (MRLs). In Austria the European Foodstuff Directive 90/642/EEC [7] has been implemented under the Bundesgesetzblatt BGBl. II 441/2002 [8] which is yearly up-dated [9].
The MRLs are values established for pesticide residues in food. It is based on the assumption that good

agricultural practice is applied at the use of pesticides in farming. It is assumed that the product has been used in an appropriate manner and suitable withdrawal periods have been permitted. Since residues of pesticides may be metabolised in tissues, MRLs are expressed either in terms of parent compound or toxic metabolites.

To minimise the uptake of pesticides people, who are becoming more and more aware of potential health problems, prefer products of a new way of farming known as "organic farming". Organic farming is defined in the Directive 2092/91/EEC [11], which states that *"only products composed of substances mentioned in Annex I and Annex II of the Directive 2092/91/EEC i.e. not chemically synthesised substances may be used as plant protection products, fertilisers or soil conditioners"*. Organic farming is assigned to an absence of pesticides defined in praxis as a pesticide residue concentration lower than 10 µg/kg product for any synthetic pesticide.

Concerning the analysis of pesticides in foodstuffs nowadays two methods are used. Apolar and middle polar pesticides are essentially analysed with gas chromatography (GC) [12-19] whereas polar pesticides are in most of the cases appraised with high-performance liquid chromatography (HPLC) [20-27]. The detection of choice is largely becoming the mass spectrometry (MS) due to its selectivity and sensitivity, contributing to the decrease of the limits of detection (LOD) [12-27]. The foodsuff sample preparation and determination of apolar and middle polar pesticides in non fatty products i.e. fruits and vegetables is regulated by the DIN Norm 12393 part 1 to 3 [28-30] adapted from the German Norm DFG S19 [31].

In this study over 3300 fruit and vegetable samples from the Austrian and foreign agriculture were extracted and analysed based on the method DFG-S19 during a period of two years. This very broad database allowed an estimation of the gc-amenable pesticide contamination of the fruits and vegetables on the Austrian market based on the products and not on the potential risk for human beings.

2. Experimental

2.1. Materials

Table 1 presents the analysed substances. The listed pesticides have been selected based on their occurrence in foodstuff, health relevance as well as according to the analytical ability to be analysed with GC. Each analyte was provided either from Sigma-Aldrich or from Ehrenstorfer with the highest available purity. The solid standards were at first dissolved in acetone to prepare stock solutions of around 1000 ng/µl used for the preparation of a multicompound standard of 10 ng/µl, 5 ng/µl, 2 ng/µl, 1 ng/µl and 0.2 ng/µl.

2.2. Sample preparation

Around 500 g of unwashed, unpealed fruit or vegetable samples were homogenised as purchased with a chopper. 50 g of the mixed samples were extracted according to the DFG S19 method [31]. This conventional method consists of an extraction step with water/acetone (1:2, v/v) followed by partitioning with ethylacetate/cyclohexane (1:1, v/v), clean-up by gel permeation chromatography (GPC) and analysis with GC-MS. Aldrin, which is forbidden to use since over twenty years, was used as internal standard spiked at the partitioning step. This extraction step provides enriched and purified samples concentrated by a factor 20 through the different extraction and cleaning steps. In the last two years, we analysed some 3300 organic farming and conventionally produced samples. They were distributed as follows: 1500 samples in 2004 meaning 600 organic farming products and 900 conventional products, and 1800 samples in 2005 implying 450 organic farming samples and 1350 conventional samples. We quantified one hundred different pesticides corresponding to a total of 1500 and 2100 pesticide residues in 2004 and 2005, respectively.

2.3. Analyses

The analyses were performed on three Hewlett-Packard (Agilent Technologies) GC/MS Model 6890N Series gas chromatography coupled to 5973N and 5975 mass selective detectors. Details concerning the devices and operating conditions have already been published elsewhere [32]. The Agilent Chemstation Software G1701DA version D.02.00.237 was used for data analysis. During the first one and a half year we were i) operating a screening of the samples in full scan mode by acquiring a total ion chromatogram (TIC), ii) confirming our sample qualification with a deconvolution reporting software which achieved a computer-supported screening of the samples in a database of over 560 pesticides and iii) repeating the measurements in the SIM mode to quantify the samples. Since summer 2005 we have been working with the new software of Agilent Technologies and developed a method capable of acquiring a full scan parallel and simultaneous to SIM data as already reported in [32]. This allows the simultaneous screening and quantification of a sample as well as its confirmation through a coupled systematic deconvolution reporting software in a single run. The identification and confirmation of the pesticides were realised like recommended by the EU [33]. In its point, the basic premise is that a correct identification by single quadropole-MS requires 3-ion criteria (one target ion and two qualifiers) for permitted substances and 4-ion criteria (one target ion and three qualifiers)

Analyte	LOQ Scan (µg/kg)	LOD Scan (µg/kg)	LOQ SIM (µg/kg)	LOD SIM (µg/kg)	Lowest MRL (µg/kg)	Pesticide class
Aldrin	2.9	0.9	0.4	0.1	20	insecticide
Alpha endosulfan	41.3	12.4	5.8	1.7	50	acaricide
Atrazin-desethyl	5.3	1.6	3.9	1.2	100	herbicide
Azoxystrobin	8.9	2.7	1.4	0.4	50	fungicide
Benalaxyl	5.9	1.8	3.2	0.9	50	fungicide
Beta endosulfan	48.7	14.6	6.5	1.9	50	acaricide
Bifenthrin	1.5	0.4	0.2	0.1	50	insecticide, acaricide
Biphenyl	1.2	0.4	0.4	0.1	10	fungicide
Bitertanol	3.7	1.1	1.2	0.4	50	fungicide
Bromopropylate	3.4	1.0	1.1	0.3	50	acaricide
Bupirimate	3.7	1.1	0.4	0.1	1000	fungicide
Buprofezin	9.4	2.8	1.6	0.5	500	inhibitor
Captan	100.6	30.5	49.8	15.1	100	fungicide
Carbaryl	11.3	3.4	1.1	0.3	100	insecticide; acaricide
Carboxin	12.3	3.7	6.7	2.0	50	fungicide
Chlorethaldimethyl	1.3	0.4	0.3	0.1	100	herbicide
Chlorfenvinphos	7.8	2.3	7.4	2.2	50	insecticide; acaricide
Chlorothalonil	11.7	3.5	3.2	1.0	50	fungicide
Chlorpropham	2.2	0.7	0.7	0.2	50	herbicide
Chlorpyriphos	5.5	1.7	0.2	0.1	50	insecticide
Chlorpyriphos methyl	2.2	0.7	0.2	0.1	50	insecticide; acaricide; nematicide
Cyfluthrin	103.1	30.9	69.1	21.0	20	insecticide
Cypermethrin	59.9	18.0	37.8	11.3	50	insecticide; acaricide
Cyproconazole	10.0	3.0	0.4	0.1	50	fungicide
Cyprodinil	1.2	0.37	1.0	0.3	50	fungicide
Cyromazin	5.1	1.5	3.6	1.1	50	inhibitor
Deltamethrin	25.9	7.8	3.2	1.0	50	insecticide
p,p'-DDD	3.1	1.0	0.8	0.2	50	insecticide; acaricide
p,p'-DDE	0.8	0.3	0.3	0.1	50	insecticide; acaricide
o,p-DDT	3.1	0.9	2.2	0.7	50	insecticide; acaricide
p,p'-DDT	4.8	1.4	2.8	0.8	50	insecticide; acaricide
Diazinon	7.6	2.3	1.3	0.4	20	insecticide; acaricide
Dichlorvos	3.0	0.9	1.5	0.4	100	insecticide; acaricide
Dicloran	9.5	2.9	0.6	0.2	10	herbicide
Dicofol	3.5	1.1	0.2	0.1	20	acaricide
Dieldrin	12.8	3.9	3.8	1.2	10	insecticide
Difenoconazole	12.1	3.6	1.5	0.4	20	fungicide
Dimethoate	6.9	2.1	8.4	2.5	50	insecticide; acaricide
Dimethomorph	11.9	3.6	0.6	0.2	50	fungicide
Diphenylamine	3.4	1.0	0.1	0.1	50	acaricide
Endosulfan sulfate	59.1	17.7	14.3	4.3	50	acaricide
Ethion	5.5	1.7	1.5	0.4	50	insecticide; acaricide
Ethoprophos	3.2	1.0	2.1	0.6	20	insecticide; nematicide
Etofenprox	1.8	0.5	0.3	0.1	10	insecticide
Fenarimol	5.3	1.6	1.5	0.5	20	fungicide
Fenbuconazole	7.2	2.2	6.7	2.0	10	fungicide
Fenhexamid	45.7	13.7	10.1	3.0	50	fungicide
Fenitrothion	6.0	1.8	0.3	0.1	50	insecticide
Fenoxycarb	7.4	2.2	0.6	0.2	50	insecticide
Fenpropathrin	6.8	2.0	1.1	0.3	20	insecticide; acaricide
Fenvalerate	26.2	7.9	14.8	4.4	20	insecticide; acaricide
Fludioxonil	4.4	1.3	0.3	0.1	50	fungicide
Fluquinconazole	4.1	1.2	0.9	0.3	50	fungicide
Flusilazole	1.6	0.5	0.1	0.1	50	fungicide
Fluvalinate-tau	33.6	10.1	14.4	4.3	50	insecticide; acaricide
Folpet	99.8	30.2	47.2	14.3	100	fungicide

Tab. 1: List of pesticides with LOD and LOQ, lowest MRL, pesticide class

Analyte	LOQ Scan (µg/kg)	LOD Scan (µg/kg)	LOQ SIM (µg/kg)	LOD SIM (µg/kg)	Lowest MRL (µg/kg)	Pesticide class
Heptachlor-endo-epoxid (trans)	11.5	3.5	5.2	1.6	20	insecticide
Heptachlor-exo-epoxid (cis)	4.3	1.3	0.8	0.2	20	insecticide
Heptenophos	3.4	1.0	1.8	0.5	100	insecticide; acaricide
Imazalil	19.7	5.9	4.2	1.2	20	fungicide
Iprodione	26.2	7.8	7.7	2.3	50	fungicide
Kresoxim-methyl	1.7	0.5	0.2	0.1	50	fungicide
lambda-Cyhalothrin	5.9	1.8	0.8	0.2	20	insecticide
Lindan	3.6	1.1	2.0	0.6	10	insecticide; acaricide
Malathion	3.4	1.0	0.2	0.1	50	insecticide; acaricide
Mecarbam	15.1	4.5	14.3	4.3	50	insecticide; acaricide
Metalaxyl	8.0	2.4	0.4	0.1	50	fungicide
Metconazole	11.4	3.4	5.9	1.8	20	fungicide
Methamidophos	63.5	19.0	25.9	7.8	50	insecticide; acaricide
Methidathion	6.9	2.1	0.2	0.1	20	insecticide
Methiocarb	5.6	1.7	0.5	0.2	50	insecticide; acaricide
Monocrotophos	10.2	3.1	1.0	0.3	50	insecticide; acaricide
Myclobutanil	9.5	2.8	0.3	0.1	20	fungicide
Ofurace	20.2	6.1	3.5	1.1	10	fungicide
Omethoate	76.5	22.9	22.2	6.7	50	insecticide; acaricide
o-Phenyphenol	1.4	0.4	0.4	0.1	10	fungicide
Oxadixyl	9.8	2.9	9.5	2.9	50	fungicide
Paclobutrazole	3.8	1.1	3.5	1.1	10	inhibitor
Parathion	4.5	1.3	1.7	0.5	50	insecticide; acaricide
Parathion methyl	10.1	3.0	7.6	2.3	100	insecticide
Penconazole	2.8	0.9	0.3	0.1	50	fungicide
Pendimethanil	6.4	1.9	2.4	0.7	50	herbicide
Permethrin	3.4	1.0	0.6	0.2	50	insecticide; acaricide
Phosalone	4.2	1.3	1.1	0.32	20	insecticide; acaricide
Piperonyl butoxide	3.9	1.2	0.3	0.1	500	inhibitor
Pirimicarb	1.7	0.5	0.4	0.1	50	insecticide
Primiphos-methyl	1.2	0.4	0.8	0.2	50	insecticide; acaricide
Prochloraz	14.6	4.4	2.2	0.7	50	fungicide
Procymidone	6.1	1.8	0.5	0.2	20	fungicide
Propamocarb	19.0	5.7	2.3	0.7	100	fungicide
Propargite	11.7	3.5	4.3	1.3	1000	acaricide
Propiconazole	14.6	4.4	5.2	1.6	50	fungicide
Propyzamide	3.9	1.2	0.2	0.1	20	herbicide
Pyridaben	5.0	1.5	1.0	0.3	10	insecticide; acaricide
Pyrimethanil	1.5	0.5	0.7	0.2	50	fungicide
Pyriproxyfen	1.5	0.5	0.4	0.1	20	insecticide
Quinalphos	4.6	1.4	1.2	0.4	50	insecticide; acaricide
Quinoxyfen	2.7	0.8	0.5	0.2	20	fungicide
Quintozene	8.1	2.4	0.5	0.2	10	fungicide
Tebuconazole	6.0	1.8	3.1	0.9	50	fungicide
Tecnazene	3.5	1.1	1.2	0.4	50	fungicide
Terbutryne	3.4	1.0	0.5	0.2	50	herbicide
Tetradifon	13.9	4.2	4.5	1.3	50	acaricide
Tetramethrin	4.1	1.2	0.8	0.2	10	insecticide
Thiabendazole	8.3	2.5	1.0	0.3	50	fungicide
Tolclofos-methyl	0.8	0.2	0.4	0.1	50	fungicide
Tolylfluanid	26.9	8.1	2.6	0.77	20	fungicide
Triadimefon	7.5	2.3	0.8	0.2	100	fungicide
Triadimenol	6.7	2.0	0.7	0.2	100	fungicide
Triazophos	9.7	2.3	7.3	2.2	20	insecticide; acaricide; nematicide
Trifloxystrobin	5.1	1.5	0.8	0.2	20	fungicide
Trifluralin	1.2	0.4	0.7	0.2	100	herbicide
Vinclozoline	5.6	1.7	0.7	0.2	50	fungicide

Tab. 1: List of pesticides with LOD and LOQ, lowest MRL, pesticide class

for banned substances [34]. The list of retention time, target ion and qualifiers for each analyte is presented in [32]. All the results presented in this paper are given for fresh samples i.e. in µg/kg wet weight. The LOD and LOQ were estimated for the ion with an m/z at the highest intensity at the lowest concentration injected that yielded to a S/N ratio of 3 and 10, respectively. With the present method we achieved limits of detection (LOD) and limits of quantification (LOQ) as low as 0.2 and 0.8 µg/kg product in full scan and 0.03 and 0.1 µg/kg product in SIM mode, respectively. The LOD in full scan and LOQ in SIM mode were in each case below the lowest MRLs for conventional foodstuff except for cyfluthrin *(Table 1)*. Since cyfluthrin (LOD: 103 µg/kg in full scan and LOQ: 69.1µg/kg in SIM mode) is usually found in peaches and lettuce (MRL: 500 µg/kg), in grapes and sweet peppers (MRL: 300 µg/kg), in apples and cherries (MRL: 200 µg/kg) and in tomatoes (MRL: 50 µg/kg) [35], we can still safely screen and quantitate the substance. For the organochlorine insecticide endosulfan (alpha-, beta- and sulphate), the phthalimide fungicides captan and folpet, the pyrethroid insecticides cyfluthrin and cypermethrin, the anilide fungicide fenhexamid, the phosphoramidothiate insecticide methamidophos and the organothiophosphate acaricide omethoate, we achieved LOD in full scan between 12 and 31 µg/kg product i.e. higher than the MRL of 10 µg/kg for organic farming foodstuff. We are aware of this problem and presently working at achieving at least LOD below 10 µg/kg product. The results were compared to MRL in consideration of the measurement uncertainty of the method, which is 40 % in our lab. The measurement uncertainty was estimated with control chart of the most important pesticides and the recovery of the internal standard. The measurement uncertainty was added to the measured value before comparison to the MRL for organic farming and conventional farming products.

3. Results and discussion

Figure 1 and *Table 2* present pesticide residue findings for the years 2004 and 2005. It is demonstrated that 90 % of the organic farming products were below LOD, which means for organic farming samples in any case below the 10 µg/kg MRL in the product. It also showed that the total number of samples with detectable pesticide residues decreased from 62 to 54 % from 2004 to 2005.

Figure 1 also states for the conventional farming products that the number of samples with one pesticide residue decreased from 24 to 22 % and the numbers of samples with two and more pesticide residues was reduced from 37 to 32 % between 2004 and 2005.

These data also show that some samples were still very contaminated not only in the concentration but also in the number of different pesticide residues. Still 14 % of conventional produced samples contained from four to 13 pesticides in 2004 and in 2005. Less than 2 % of the samples, which mean 25 samples per year, contained between ten and even 13 pesticide

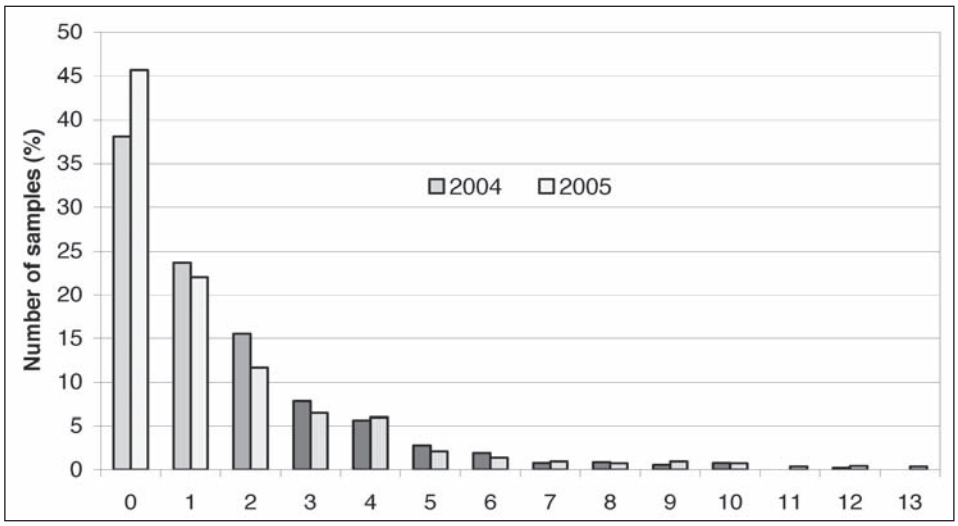

Fig. 1: Percentage (%) of conventional farming products contaminated with pesticides in 2004 and 2005

Year	Type of product	Number of samples	Total number of residues	Number of pesticide residues per sample													
	Org. / Conv.			0	1	2	3	4	5	6	7	8	9	10	11	12	13
2004	Organic farming	604	65	553	41	8	2	0	0	0	0	0	0	0	0	0	0
	Conventional	889	1411	343	213	140	71	51	25	17	7	8	5	7	0	2	0
	Sum	1493	1476	896	254	148	73	51	25	17	7	8	5	7	0	2	0
2005	Organic farming	440	90	391	32	8	3	3	1	0	0	2	0	0	0	0	0
	Conventional	1336	2018	610	294	156	87	80	28	19	13	10	13	10	5	6	5
	Sum	1776	2108	1001	326	164	90	83	29	19	13	12	13	10	5	6	5

Tab. 2: Number of samples below LOD and samples with pesticide residues in 2004 and 2005 (in number of samples)

residues. This study over two years allowed us to build a database in which each foodstuff could be associated to typical pesticide residues and reciprocally. This is developed in *Table 3* and *Table 4*. It came to evidence that products like onions, garlic, apricots, courgettes, eggplants, leeks, carrots and broccoli were rarely contaminated.
On the other hand some products, when contaminated, contain just one characteristic pesticide like:

- figs, for instances, were only contaminated with dichlorvos, a pre-harvest insecticide sprayed on the plants. It should be noticed that the maximum residue concentration found was 41 µg/kg product, which is still below the MRLs for figs (100 µg/kg) [8]:
- potatoes contained only chlorpropham, which is used for potatoes as post-harvest sprout inhibitor.

Our results showed that samples like grapes, sweet pepper and salads were very often contaminated and presented sometimes multiresidues up to 13 pesticides.

Finally, we could establish a list of typical pesticides for some product classes like pipfruits (apples and pears), citrus fruits (lemons, oranges, clementines, grapefruits), berries (raspberries, red currants and strawberries), exotic fruits (papayas, kiwis, pineapples and mangos), herbs (dill and parsley) or stone fruits (apricots, peaches and cherries). This allowed the establishment of a "top 20" table of the most frequent pesticide hits in 2004 and 2005 (*Figure 2*). We could figure out that:

- **procymidone** was mainly found in tomatoes, grapes and sweet peppers, where it is used as pre-harvest fungicide during the florescence,
- **cyprodinil** was typical for berries, grapes and salads, as fungicide sprayed during the florescence,
- **chlorpyriphos** was found in several products as pipfruits, citrus fruits, stone fruits, grapes and herbs where it is employed as pre-harvest insecticide spread on the plant leaves,
- **metalaxyl** was found in salads and is used as pre-harvest fungicide,
- **iprodione** was quite often found in berries, stone

Foodstuff	2004		2005	
	Number of samples	Pesticide (name and hits)	Number of samples	Pesticide (name and hits)
Apple	23	Chlorpyriphos: 12 Carbaryl :4 Diphenylamine: 4 Fenitrothion: 3 Folpet: 2	34	Chlorpyriphos: 8 Fenoxycarb: 6 Pyrimethanil: 3 Cyprodinil: 2 Diphenylamine: 2
Apricot	7	Lambda-Cyhalothrin: 1 Myclobutanil: 1	11	Lambda-Cyhalothrin: 2 Cypermethrin: 1
Banana	3	-	11	-
Cherry	10	Cyprodinil: 2 Diazinon: 2 Fludioxonil: 2	19	Fenhexamid: 5 Dimethoate: 3
Clementine	8	Chlorpyriphos: 7 Imazalil: 4 o-Phenylphenol: 4 Dicofol: 3 Malathion: 3	13	Chlorpyriphos: 9 Imazalil: 9 o-Phenylphenol: 8 Dicofol: 5 Malathion: 4
Fig	4	-	7	Dichlorvos: 5
Grape	98	Procymidone: 60 Cyprodinil: 48 Lambda-Cyhalothin: 33 Quinoxyfen: 33 Fludioxonil: 32 Chlorpyriphos: 26 Metalaxyl: 26 Pyrimethanil: 26 Propargite: 18 Triadimenol: 18 Azoxystrobin: 17 Capermethrin: 15 Penconazole 15 Trofloxystrobin: 15 Myclobutanil: 14 Iprodione: 13 Chlorpyriphos-methyl: 10	139	Procymidone: 70 Cyprodinil: 54 Pyrimethanil: 50 Fludioxonil: 43 Lambda-Cyhalothrin: 43 Chlorpyriphos: 31 Quinoxyfen: 31 Triadimenol: 27 Iprodione: 24 Metalaxyl: 23 Propargite: 23 Azoxystrobin: 22 Penconazole: 22 Trifloxystrobin: 19 Cypermethrin: 15 Fenhexamid: 14 Quinalphos: 14

Tab. 3: Typical pesticide residues for fruits from conventional farming (pesticides classified per hit frequency)

Foodstuff	2004		2005	
	Number of samples	Pesticide (name and hits)	Number of samples	Pesticide (name and hits)
Grapefruit	4	Chlorpyriphos: 3 Metalaxyl: 2 o-Phenylphenol: 2	-	-
Kiwi	16	Iprodione: 6 Vinclozoline: 3	16	Iprodione: 2 Vinclozoline: 2 Chlorpyriphos-methyl: 2
Lemon	19	o-Phenylphenol: 9 Chlorpyriphos: 6 Imazalil: 6 Bromopropylat: 3 Methidathion: 2	24	Bromopropylate: 7 Chlorpyriphos: 4 Imazalil: 3 Methidathion: 3 o-Phenylphenol: 3
Lime	6	-	3	-
Mango	4	-	6	-
Melon	14	Endosulfan: 7 Procymidone: 4 Buprofezin: 3 Pyridaben: 3	18	Endosulfan: 4
Nectarine	27	Iprodione: 4 Cyprodinil: 3 Chlorpyriphos: 2 Procymidone: 2	25	Fenbuconazole: 6 Chlorpyriphos: 5 Etofenprox: 3 Procymidone: 3
Orange	22	Imazalil: 13 Chlorpyriphos: 10 Thiabendazole: 4 Dicofol: 2 o-Phenylphenol: 4	25	Imazalil: 7 Chlorpyriphos: 5 o-Phenylphenol: 4 Thiabendazole: 4 Methidathion: 2
Papaya	4	Prochloraz: 1	5	Prochloraz: 5
Peach	15	Chlorpyriphos: 3 Iprodione: 3 Tebuconazole: 3	22	Chlorpyriphos: 6 Iprodione: 2 Lambda-Cyhalothrin: 2
Pear	21	Chlorpyriphos: 5 Diphenylamine: 4 Bromopropylate: 3 Kresoxim-Methyl :3 Captan: 2 Cyprodinil: 2 Procymidone: 2	31	Chlorpyriphos: 7 Carbaryl: 6 Diphenylamine: 6 Procymidone: 5 Iprodione: 4 Fenoxycarb: 3 Kresoxim-methyl: 3
Pineapple	6	Triadimenol: 6 Tridimefon: 4	13	Tridimefon: 12 Triadimenol: 11 Prochloraz: 6 Pyridaben: 3 Diazinon: 2
Raspberry	8	Iprodione: 3 Fenhexamid: 2 Procymidone: 2	19	Procymidone: 5 Cyprodinil: 4 Fenhexamid: 3 Iprodione: 3 Myclobutanil: 3 Fludioxonil: 2
Red Currant	4	Endosulfan: 6 Fenhexamid: 2	10	Fenhexamid: 7 Lambda-Cyhalothrin: 4 Fludioxonil: 3 Cyprodinil: 2 Phosalone:2 Tolylfluanid: 2
Strawberry	57	Cyprodinil: 24 Fludioxonil: 13 Fenhexamid: 12 Myclobutanil: 10 Azoxystrobin: 8 Triadimenol: 7 Procymidone: 6	51	Fludioxonil: 17 Cyprodinil: 10 Tolylfluanid: 8 Azoxystrobin: 6 Fenhexamid: 6 Kresoxim.methyl: 6 Penconazole: 4

Tab. 3: Typical pesticide residues for fruits from conventional farming (pesticides classified per hit frequency)

Foodstuff	2004		2005	
	Number of samples	Pesticide (name and hits)	Number of samples	Pesticide (name and hits)
Avocado	7	-	3	-
Broccoli	9	Endosulfan: 3	11	Etofenprox: 1
Cabbage	7	Iprodione: 1	10	Difenoconazole: 2 Iprodione: 2 Cypermethrin: 1 Etofenprox: 1 Tebuconazole: 1
Carrot	6	Azoxystrobin: 1	12	Chlorpyriphos: 1
Corn salad	13	Iprodione: 5	19	Iprodione: 7
Courgette	9	Endosulfan: 2	10	Procymidone: 2
Cucumber	16	Azoxystrobin: 4 Cyprodinil: 3 Metalaxyl: 3	50	Cyprodinil: 13 Metalaxyl: 6 Procymidone: 3 Buprofezin: 2 Fludioxonil: 2
Dill	11	Pendimethalin: 4 Cypermethrin: 3 Chlorpyriphos-methyl: 2 Propyzamide: 2	18	Chlorpyriphos: 6 Pendimethalin: 4 Cypermethrin: 2 Difenoconazole: 2
Eggplant	10	Procymidone: 1	10	Procymidone: 1
Endive	38	Endosulfan: 12 Metalaxyl: 7 Procymidone: 5 Vinclozoline: 3	7	Procymidone: 2 Tolclofos-methyl: 2 Bifenthrin: 1 Iprodione: 1 Tolylfluanid: 1
Garlic	25	Piperonilbutoxide: 2	9	-
Iceberg lettuce	29	Metalaxyl: 7 Cyprodinil: 6 Endosulfan: 5 Procymidone: 4 Deltamethrin: 3	44	Metalaxyl: 6 Procymidone: 3 Tolylfluanid: 3 Cyhalothrin: 2 Iprodione: 2
Leek	9	Azoxystrobin: 1	5	-
Lettuce	16	Iprodione: 10 Chlorpyriphos: 7 Cyprodinil: 7 Deltamethrin: 2 Metalaxyl: 2	25	Dicloran: 6 Iprodione: 6 Cyprodinil: 5 Procymidone: 5 Fludioxonil: 3
Onion	16	-	16	-
Sweet pepper	91	Endosulfan: 14 Procymidone: 13 Cypermethrin: 12 Pirimiphos-methyl :9 Pyridaben: 8 Bifenthrin: 6 Chlorpyriphos: 6 Pyrimethanil: 6 Buprofezin: 5 Cyprodinil: 5	156	Pirimiphos-methyl: 35 Procymidone: 33 Endosulfan: 29 Methiocarb: 20 Cyprodinil: 18 Pyridaben: 18 Triadimenol: 18 Cypermethrin: 16 Fludioxonil: 15 Bifenthrin: 13
Parsley	10	Chlorpyriphos: 2 Lambda-Cyhalothrin: 2 Cypermethrin: 2 Procymidone: 2	15	Difenoconazole: 4 Azoxystrobin: 2 Chlorothalonil: 1 Cyfluthrin: 1 Cypermethrin: 1 Flusalizole: 1
Pole bean	-	-	10	Procymidone: 2 Cyhalothrin: 1 Dimethoate: 1 Iprodione: 1
Potato	29	Chlorpropham: 10	101	Chlorpropham: 40
Ruccola	53	Deltamethrin: 13 Dicloran: 12 Iprodione: 9	19	Bifenthrin: 3 Deltamethrin: 2 Iprodione: 2
Salat	16	Procymidone: 4 Cypermethrin: 3 Metalaxyl: 3	29	Iprodione: 8 Cyprodinil: 4 Procymidone: 3
Tomato	47	Cyprodinil: 9 Azoxystrobin: 6 Procymidone: 4 Chlorothalonil: 3 Fenhexamid: 3 Endosulfan: 2	96	Procymidone: 16 Chlorothalonil: 13 Endosulfan: 9 Cyprodinil: 8 Pyriproxyfen: 8 Triadimenol :8

Tab. 4: Typical pesticide residues for vegetables from conventional farming (pesticides classified per hit frequency)

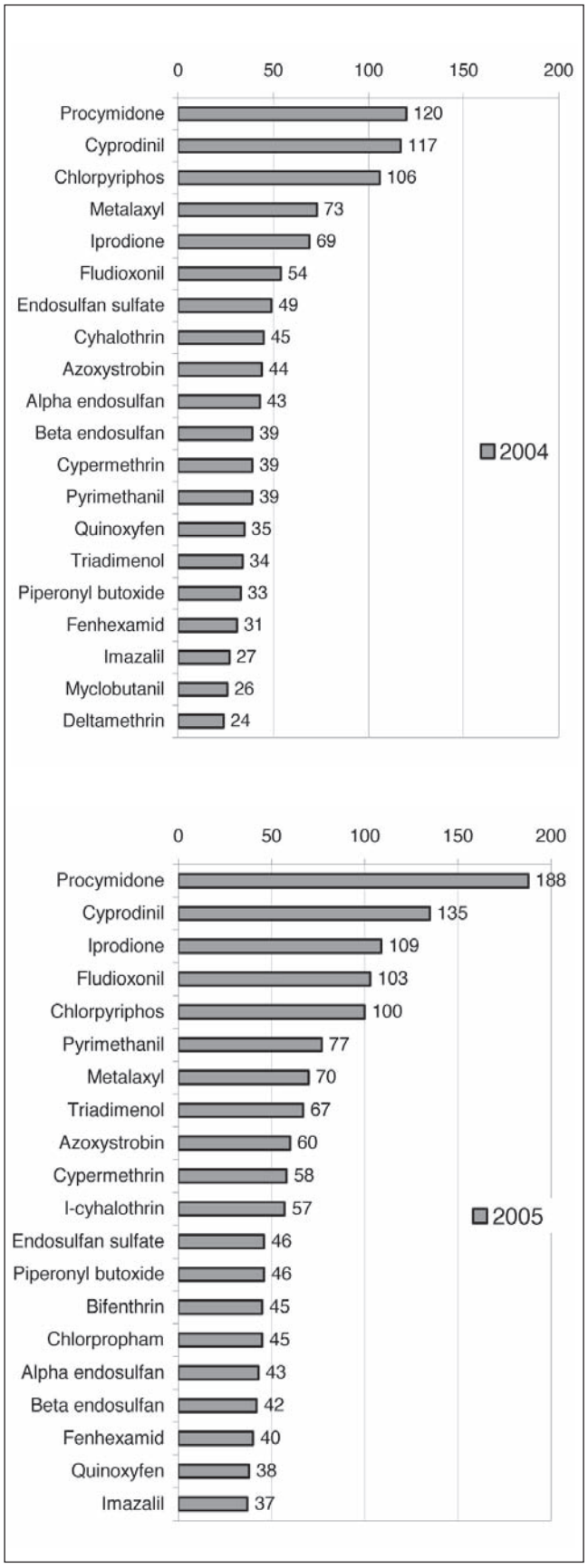

Fig. 2: Most frequent pesticides all products gathered in 2004 and 2005

fruits, salads and to less extend in exotic fruits as pre-harvest fungicide used during florescence,

- **fludioxonil** was in particular found in berries and grapes and is a pre-harvest fungicide,
- **endosulfan (alpha-, beta- and sulfate)** was the major contaminant for sweet peppers and less occurring in tomatoes and salads; it is sprayed as pre-harvest acaricide,
- **lambda-cyhalothrin**, a common residue in grapes and stone fruits, is a pre-harvest insecticide,
- **azoxystrobin** frequently detected in berries, tomatoes and grapes counts for a pre-harvest fungicide,
- **cypermethrin** was found in herbs, grapes and sweet peppers where it is spread during the florescence as pre-harvest insecticide,
- **pyrimethanil** and **quinoxyfen** were mainly found in grapes, where they are applied as pre-harvest fungicides,
- **triadimenol** and **triadimefon** were found in exotic fruits and grapes, and, only in 2005, in tomatoes and sweet peppers. They are classified as post-harvest fungicides in exotic fruits and as pre-harvest fungicides in the others noted cultures.
- **piperonylbutoxide** was found in different cultures since it is a synergist for natural pyrethroids,
- **fenhexamid** was found in berries and grapes, where it is sprayed as pre-harvest fungicide,
- the fungicide **imazalil** was most frequently found in citrus fruits, like the fungicides **thiabendazole** and **o-phenylphenol** and the insecticide **malathion**, where they are used as post-harvest surface preservative pesticides,
- **myclobutanil** was found in grapes and berries and used as pre-harvest fungicide,
- **deltamethrin** was mainly found in salads and employed for this cultures as pre-harvest insecticide,
- **bifenthrin** became more frequent in 2005 and was found in grapes and sweet peppers, where it is sprayed as pre-harvest insecticide.

Furthermore we could state additional information concerning typical residues in products:

- the post-harvest acaricide **diphenylamine** and the pre-harvest insecticide **carbaryl** were only found in pipfruits in 2005 and mainly in pipfruits but also in some grapes and salads in 2004,
- the pre-harvest acaricide **dicofol** and **bromopropylate** were principally found in citrus fruits and to a far less extend in grapes,
- the post-harvest fungicide **prochloraz** was frequent in ananas and papayas,

- the pre-harvest insecticide **diazinon** was a particular residue in ananas but also sometimes in pears and grapes,
- the pre-harvest fungicide **vinclozoline** was especially detected in kiwis and salads,
- the pre-harvest fungicide **chlorothalonil** became a recent residue in tomatoes only since 2005,
- the pre-harvest herbicide **pendimethalin** was exclusively found in dill,
- the pre-harvest insecticide **propargite** was only found in grapes,
- the pre-harvest insecticides **pirimiphos-methyl** and **methiocarb** were typical for sweet peppers,
- the pre-harvest insecticide **pyridaben** was mainly a pesticide residue found in sweet peppers but also in some melon samples in 2004 and more and more in ananas and tomatoes since 2005,
- the pre-harvest herbicide **dicloran** was only found in salads.

Interesting is also the percentage of samples exceeding MRLs as shown in *Table 5*. There was an increase of the number of organic farming samples containing a pesticide residue exceeding 10 µg/kg (3.9 % in 2004 and 8.2 % in 2005). On the other hand we observed a decrease of samples with residue exceeding MRLs within the conventional farming products from 8.4 % in 2004 to 7.7 % in 2005. We found 38 organic farming products with single residue exceeding the 10 µg/kg level (22 in 2004 and 16 in 2005), twelve organic farming products with two residues exceeding the 10 µg/kg level (two in 2004 and ten in 2005), six organic farming products with three residues exceeding the 10 µg/kg level in 2005 and finally four organic farming products with four residues exceeding the 10 µg/kg level in 2005.
We declared 145 conventional products with single residue exceeding the MRLs (69 in 2004 and 76 in 2005), 24 conventional products with two residues exceeding the MRLs (four in 2004 and twenty in 2005), six conventional products with three residues exceeding the MRLs (three in 2004 and three in 2005) and finally four conventional products with four residues exceeding the MRLs in 2005.
The most frequent pesticide residues exceeding the 10µg/kg level and the MRLs are shown in *Figure 3* for organic farming products and in *Figure 4* for conventional farming products, respectively.
For organic farming products **piperonylbutoxide** was the most frequent residue exceeding the 10 µg/kg level and found in twelve celery and tomato samples. Piperonylbutoxide is a principally allowed synergist for natural pyrethrins but the discussion about its presence is still open. The use of pyrethrins is allowed in organic farming production [36]. Piperonylbutoxide is not a pesticide in the strict sense of the definition. It was followed by **procymidone** in eight tomato and grape samples, **azoxystrobin** in five courgette, grape and tomato samples, **dichlorvos** in five fig samples and **metalaxyl** in four grape and potato samples.
In the "top list" of pesticides exceeding MRLs for conventional products we found **pyridaben** (in 21 sweet pepper and seven tomato samples, MRL: 10 µg/kg), **iprodione** (in 18 nectarine, MRL: 5000 µg/kg; peach MRL: 5000 µg/kg and salad MRL: 10 000 µg/kg samples), **dicloran** (in 13 salad samples, MRL: 10 µg/kg), **chlorpropham** (in 13 potato samples, MRL: 5000 µg/kg), **methiocarb** (in ten sweet pepper samples, MRL: 50 µg/kg), **etofenprox** (in eight various samples, MRL: 10 µg/kg) or **endosulfan** (in eight diverse samples). Less frequent pesticide exceeding MRLs were **imazalil** (in six grape samples, MRL: 20 µg/kg), **prochloraz** (in four ananas, MRL: 50 µg/kg and two lemon samples, MRL: 50 µg/kg), fenhexamid (in six miscellaneous samples), piperonyl butoxide (in five grape samples MRL: 500 µg/kg), **bifenthrin** (in four miscellaneous samples, MRL: 50 µg/kg), **procymidone** (in five diverse samples) or **fenvalerate** (in four grape samples).
Since dicloran is banned in Austria, its MRL is decreased to the organic farming MRL of 10 µg/kg. Furthermore etofenprox and pyridaben are still not yet regulated in Austria meaning also a MRL of 10 µg/kg.

Year	Type of product	Number of samples	Samples exceeding MRLs (%)	Samples with residues exceeding			
				1 MRL	2 MRLs	3 MRLs	4 MRLs
2004	Organic farming	608	3,9	22	2	0	0
	Conventional	900	8,4	69	4	3	0
	Sum	1508	6,6	91	6	3	0
2005	Organic farming	440	8,2	16	10	6	4
	Conventional	1336	7,7	76	20	3	4
	Sum	1776	7,8	92	30	9	8

Tab. 5: Samples with residue concentrations exceeding different MRLs

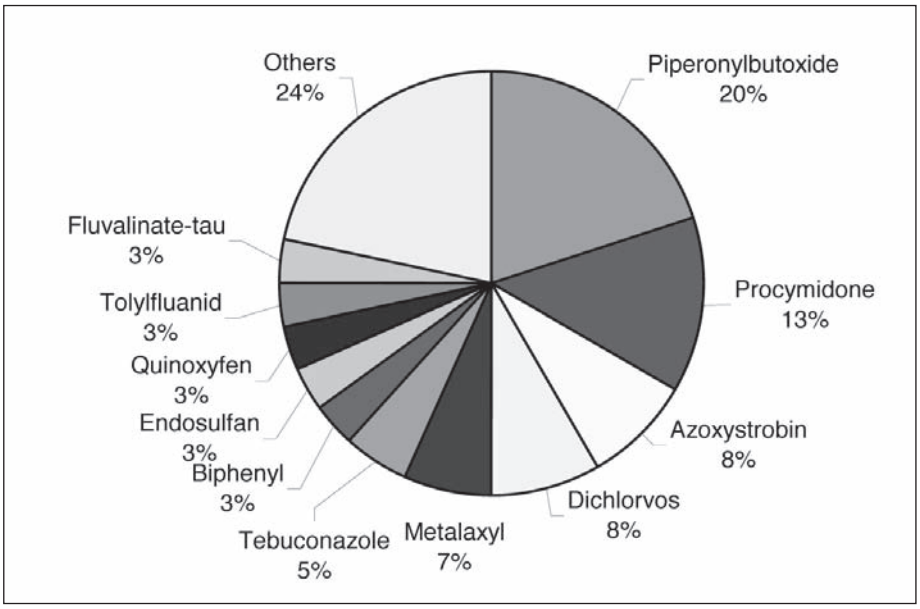

Fig. 3: Pesticide residues exceeding the 10 µg/kg level in organic farming products

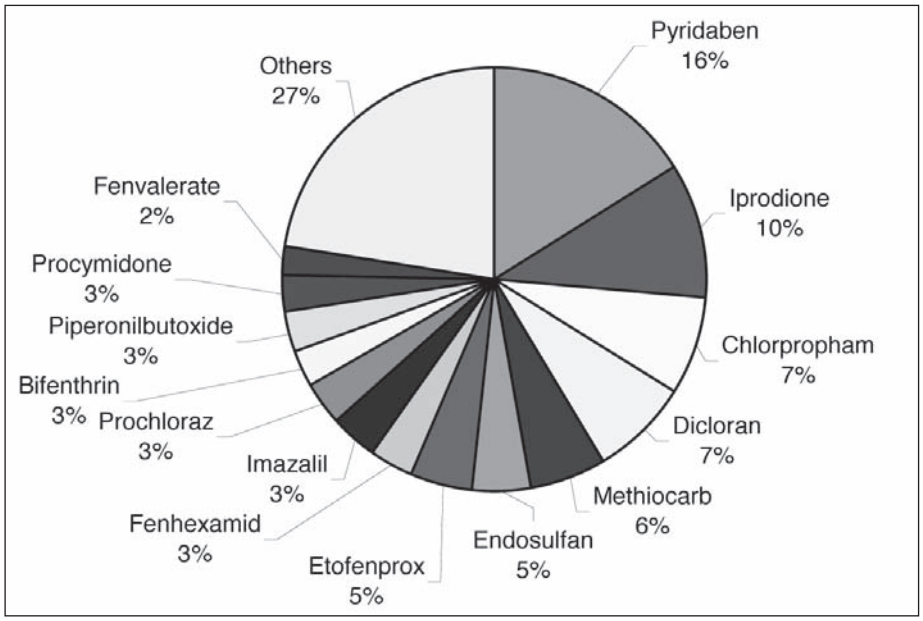

Fig. 4: Pesticide residues exceeding MRLs in conventional farming products

Iprodione is a fungicide, which is often used in the production of stone fruits and salads explaining its residue frequency and unfortunately quite regular exceed of MRLs. Since chlorpropham is a post-harvest sprout growth inhibitor for potatoes it is very frequently found as residue and residue exceeding MRL. When potatoes are washed and peeled the chlorpropham residues can be rinsed out since chlorpropham is a surface pesticide.

Imazalil and piperonil butoxide are not in the "top 20" most frequent found residues in grapes but since they are allowed in quite low residue concentration for this culture in comparison with other pesticides, they happen to be the most frequent substances exceeding MRLs for grapes.

Most of the samples with residues exceeding MRLs were on the limit of the MRL values. But some samples like potatoes for instance contained up to 80 000 µg/kg chlorpropham, which is actually far more than the MRL of 5000 µg/kg. Some sweet pepper samples presented a pyridaben concentration 20-fold higher than the 10 µg/kg MRL. Some grape samples contained imazalil up to 300 µg/kg for a MRL of 20 µg/kg or piperonil butoxide up to 40 000 µg/kg for a MRL of 500 µg/kg.

4. Conclusions

During these two years we noticed a decrease of the number of samples contaminated with pesticides and the number of samples with residues exceeding MRLs. During the same period the organic farming samples with residues exceeding MRLs increased by a factor 2 from 2004 to 2005. A significant amount of conventional farming samples were found with multi-residue contaminations up to 13 pesticides. It is alarming that some samples presented very high contamination exceeding the authorized MRLs several times.

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