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Recent advances in pesticide residue analyses in food and environmental samples*

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Introduction

In 1906, Mikhail Tswett separated the pigments of a green leaves extract on a glass tube filled with powdered chalk or alumina. Tswett called this new technique chromatography. The method was improved and gas chromatography (GC) and liquid chromatography (LC) appeared in the middle of 20th century. Both techniques were rapidly used in routine in all laboratories involved in residue analysis with ECD, NPD or FPD detectors for GC and UV, DAD or Fluorescent detectors with LC. One century after the discovery of Tswett, chromatography entered a new era dedicated to mass spectrometry (MS) detection. Indeed, MS is an universal, highly selective and sensitive detection technique. Furthermore, it is possible to use it for screening and confirmation and can be easily coupled to GC and LC. Therefore, MS is ideally suited for residue analysis in food or environmental samples. Quadrupole (Q), ion traps (Trap), time of flight (ToF) and their combinations such as triple quadrupoles (QqQ), QTrap or QToF are now in common use. Each instrument offers advantages, such as quantitative capability, high scanning speed or accurate mass measurement. All these new technologies revolutionized the manner of working in laboratories. Instead of developing numerous analytical methods for a specific compound or family of substances, the trend is to use generic samples preparations and multiresidue methods with generic separation and MS or MSMS detection. There are two major trends in residue analysis today. First, the use of GC-MSMS or LC-MSMS with QqQ detectors which gives highly selective analysis using acquisition in multiple reaction monitoring (MRM) modes for targeted analysis (1-10). Excellent quantitative performances are obtained but on the other hand, only known or suspected compounds could be monitored. The second approach is

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to use more sensitive and faster MS in full scan acquisition mode to perform non targeted acquisition. This could be done with ion trap detectors or more recently with ToF instruments and specific data treatments (10–13). ToF has also the advantages of high resolution measurement enable to identify an unknown contaminant.

Experimental

Our laboratory is involved in pesticides residues analysis since more than 30 years. Since then, methodology of analysis has changed (see fig. 1). After a generic extraction and eventually purification by GPC or MSPD, two multiresidue methods are applied in order to screened samples for more than 400 different pesticides and metabolites in less than 24 hours The first method use a QqQ coupled to LC for the targeted monitoring of ~250 polar pesticides. Complementary, we developed a second multiresidue method by GC coupled to ion trap MS and operated in full scan mode. Data processing involves deconvolution of mass spectra with automated library search and gives the opportunity to detect unexpected compounds. Detected residues are quantified using external standard calibration and compared with maximal residue limits fixed in Swiss legislation. In case of residue approaching or exceeding MRLs, a confirmation analysis is carried out by GC-MS, ECD or NPD or by LC-MSMS. At least two extracts are freshly prepared from the original sample and quality control samples (QC) are included in each series.



Figure 1 Flow chart of pesticides residue analysis



Figure 2 LC-MSMS system

The method consists of a reverse phase liquid-chromatography coupled to triple quadrupole MS (QuattroMicro Waters, fig. 2) as was described in a previous publication (1). For method development, optimisation of ionisation and fragmentation parameters was carried out for each compound. This requires obtaining commercial reference compounds which is often difficult for metabolites. During infusion of standard solution at 10 μ g/ml, the first step is to determine the most sensitive parent ion and the optimal cone voltage for the ionisation. The parent ion, usually the molecular protonated ion M+H⁺, is then selected on the first mass filter (Q1) and after fragmentation, using argon as collision gas, a daughter ion scan is recorded (see fig. 3).



Figure 3 Optimisation of MSMS parameters

As requested by the Council Directive 2002/657/EEC, at least two fragment ions should be used to reach 4 identification points in order to ensure the criteria of selectivity. After determination of MSMS parameters, the retention times of new compounds are determined and the most sensitive transition is dispatched in the corresponding acquisition group (see fig. 4). This strategy enables to monitor a greater number of pesticides in the same run while keeping high acquisition frequency. Considering a dwell time of 40 msec for each transition and interscan delay of 20 msec, the longest cycle time is around 2 sec which always gives enough points for a good peak shape definition. The method is very easy to increment when new pesticides come on the market or in case of crisis.

otal Run Time: 24.50		10 20mins
Туре	Information	Time
Q	MRM of 9 mass pairs, Time 2.00 to 5.20, ES+	
G	MRM of 7 mass pairs, Time 4.60 to 6.30, ES+	a service as a service to the present of the protocol of the
C	MRM of 10 mass pairs, Time 5.70 to 8.00, ES+	
G	MRM of 9 mass pairs, Time 7.30 to 9.50, ES+	
C	MRM of 12 mass pairs, Time 8.70 to 10.80, ES+	
	MRM of 7 mass pairs, Time 9.90 to 12.00, ES+	
Ø	MRM of 15 mass pairs, Time 11.00 to 13.50, ES+	
C	MRM of 16 mass pairs, Time 12.10 to 14.10, ES+	
C	MRM of 18 mass pairs, Time 13.30 to 15.30, ES+	and the second
Ø	MRM of 435.00->330.00, Time 13.60 to 15.20, ES-	
B	MRM of 21 mass pairs, Time 14.10 to 16.80, ES+	
C	MRM of 11 mass pairs, Time 15.50 to 18.50, ES+	and the second contraction of the second
C	MRM of 12 mass pairs, Time 18.00 to 24.50, ES+	

Figure 4 Multiple reaction monitoring in 13 acquisition groups

Optimisation of parameters has been carried out for more than 250 pesticides including fungicides, insecticides, acaricides and herbicides. Typical pesticides analysed by LC-MSMS are carbamates (e.g. carbendazim, diethofencarb, iprovalicarb, and thiophanate), conazoles (e.g. imazalil, fenamidone, myclobutanil, and tebuconazol), sulfonylureas (e.g. amidosulfuron, foramsulfuron, and triasulfuron), triazines (e.g. atrazine, simazine, and terbuthylazine), phenylureas (e.g. diuron, linuron, and chlorbromuron), and strobilurins (e.g. azoxystrobin, trifloxystrobin), nicotinoids (e.g. acetamiprid, imidacloprid, thiacloprid, thiametoxam) and their metabolites. Actually, many of these compounds are difficult to analyse by GC due to their polarity, their high evaporation temperature and/or their degradation caused by high temperature in GC injector. The selected reaction monitoring (SRM) mode was used for quantitation of pesticides. According to the number of substances to monitor, the detection is carried out in two different runs and using only one MSMS transition for each compound. The first run achieved the detection of 146 fungicides, insecticides and acaricides and the second one for 88 herbicides and metabolites. An example of standard chromatogram is shown in figure 5. If required after the screening, a confirmation analysis is carried out. Two sample extracts and QC samples are freshly prepared and a specific acquisition program is set using all known fragments but at least two.



Figure 5 Chromatograms of standard mix at 0.1 µg/ml for herbicides, fungicides, insecticides and acaricides analysis

GC-MS

The GC-MS system (fig. 6) consist in a classical GC system (TraceGC Ultra – Finnigan) equipped with a splitless injector and ZB-5ms column (30 m, 0.25 id, 0.25 µm). The MS is an ion trap detector (PolarisQ – Finnigan) operated in full scan mode. Unlike quadrupole, ion trap scans very rapidly and a typical spectrum from 50 to 500 m/z takes approximately 250 msec. The sensitivity is also very good and can be compared to sensitivity obtained in SIM mode on a single quadrupole. Typical sensitivity (~100 pg injected) enables to reach limits of detection ~0.01 mg/kg for pesticides in fruits and vegetables. For complex matrices such as baby food, spices or fatty food, limits of detection could be higher. Typical pesticides analysed by GC are organochlorines (e.g. dieldrine, DDT, lindane), organophosphorous (e.g. diazinon, dichlorvos, malathion), pyrethroids (e.g. cypermethrin, deltamethrin, fenvalerate), aromatic fungicides (e.g. chlorothalonil, quintozene) and dicarboximide fungicides (e.g. iprodione, procymidone, folpet).







An example of chromatogram obtained for a lemon extract is given in figure 7. As full scan chromatograms could be difficult to interpret, a data processing using AMDIS deconvolution software is performed. The software extracts and cleans mass spectra and then automatically search in library if spectra correspond to target compounds. In the example (fig. 7), the software found possible 2769 components

in the extract and one was matching with the reference spectra of dicofol giving an unambiguous identification. An extract ion chromatogram is then generated for quantification.

Results and discussion

Food control

Sample preparation for multiresidue method must be adapted for a large number of substances. The extraction should be fast, efficient, easy, robust but not selective. For food samples, an ethyl acetate extraction was chosen. After homogenisation and pH adjustment, an ethyl acetate extraction is performed. In some cases of complex matrices, purification by matrix solid phase dispersion or gel permeation chromatography is performed. The extract is evaporated and reconstituted in appropriate solvent before injections in GC-MS and LC-MSMS.

Over this last 5 years, more than 7500 samples have been controlled, as a part of the pesticide monitoring program of the SPCo in Geneva (see table 1). Overall, 38.9% of samples were blank, 53.5% contained residue but in lower amount than maximum residue limits. Finally, 7.6% were not compliant according to maximum residue limits established in Swiss legislation (Swiss regulation 817.021.23). This corresponds to approximately 15000 identifications and quantifications considering that multiple residues are frequently found in the same sample. Indeed, almost 22 % of samples contained one pesticide, 27% contained between 2 and 4 and finally 12% had five or more residues. Results for berries or citrus fruits demonstrate and confirm that pesticides (mainly fungicides) are intensively used with more than 80% of positive samples. For citrus fruits, post-harvest treatment with thiabendazol or imazalil is done almost systematically (14). Majority of problems on fruits with MRL exceeding occurred out of production season with imported products. For vegetables, leafy or fruiting vegetables, almost 60% of samples contained one or more residues with respectively 9 and 6% of non compliant samples. As demonstrated before, Swiss production contained usually less residues (15). Fresh herbs are more contaminated than other foodstuffs with 30% of non compliant samples. Exceeding of MRL occurred frequently but also numerous illegal uses of pesticides are observed. Each year, some specific themes are study. In 2004, Swiss and foreign wines were analysed to determine their content in residues (16). Study has demonstrated that fenhexamide is very persistent from grapes into wine even after all vinification process. Today, solutions are searched in collaboration with Agroscope Changins to find alternative treatments resulting in lower residue in wine.

FRUITS	Nb of samples	Without residue	With residue <mrl< th=""><th>With residue >MRL</th></mrl<>	With residue >MRL
Pome fruits (eg. apple, pear)	429	134 (31%)	290 (68%)	5 (1%)
Stone fruits (eg. apricot, peach, cherry)	496	153 (31%)	334 (67%)	9 (2%)
Berries and small fruits (eg. strawberry, grape, raspberry)	1249	242 (19%)	892 (71%)	115 (9%)
Citrus fruits (eg. lemon, orange, grapefruit)	587	94 (16%)	450 (77%)	43 (7%)
Exotic Fruits (eg. mango, banana, kiwi)	269	171 (63 %)	88 (33%)	10(4%)
VEGETABLES	Nb of samples	Without residue	With residue <mrl< td=""><td>With residue >MRL</td></mrl<>	With residue >MRL
Root vegetables (eg. potatoes, carrot, radish)	93	75 (81%)	17 (18%)	1 (1%)
Leafy vegetables (eg. salad, spinach, cabbage)	1095	453 (41%)	550 (50%)	92 (9%)
Fresh herbs (eg. basil, spearmint, parsley)	584	271 (46%)	141 (24%)	172 (30%)
Fruiting vegetables (eg. tomatoe, eggplant, sweet pepper)	1529	653 (43%)	777 (51%)	99 (6%)
Legume vegetables (eg. bean, peas, soja)	73	53 (73%)	19 (26 %)	1 (1%)
Miscellaneous vegetables (eg. asparagus, celery, onion)	116	107 (92%)	8 (7%)	1 (1%)
MISCELLANEOUS	Nb of samples	Without residue	With residue <mrl< td=""><td>With residue >MRL</td></mrl<>	With residue >MRL
Fruits Juice Baby food Fat, Butter, Oil Wine	50 127 49 354	34 (68 %) 109 (86 %) 39 (80 %) 59 (17 %)	16 (32%) 14 (11%) 8 (16%) 277 (78%)	4 (3 %) 2 (4 %) 18 (5 %)
Mineral water Honey Tea Jam	31 198 52 25	13 (42%) 136 (69%) 21 (40%) 16 (64%)	18 (58 %) 59 (30 %) 29 (56 %) 9 (36 %)	- 3 (1%) 2 (4%) -
TOTAL	7644	2971 (38.9 %)	4088 (53.5%)	s (3 %) 585 (7.6 %)

Table 1 Results of the food monitoring program from January 2002 to September 2006

Water analysis

As for food samples, a generic sample preparation has been developed for water samples. 500 ml are extracted by solid phase extraction on an OasisHLB cartridge (Waters) and concentrated at 100 µl. This high concentration factor (5000x) enables to reach detection limits at 1 ng/L for 72% of pesticides, between 5 and 20 ng/L for 21% and between 20 and 100 ng/L for 7%. The SPCo is active in pesticides monitoring in Geneva lake since numerous years in collaboration with the "Commission Internationale pour la Protection des Eaux du Léman" (CIPEL) (17). The main role of the CIPEL is to survey the evolution of the water quality of lake Geneva and its tributaries. It issues recommendations to Swiss and French governments, encouraging them to take measures to fight sources of pollution. Indeed, Geneva lake water accounts for 80% of the drinking water resources for the Canton of Geneva and for 600 000 inhabitants around the lake. Sampling and analysis is done two times a year at the different depths between Lausanne and Evian. Results have changed these last years with the use of LC-MSMS multiresidue method. Besides finding well known compounds such as triazines and their metabolites and few organochlorinated compounds, new contaminants such as foramsulfuron and metalaxyl have been identified in the lake in important concentrations (81 ng/L and 52 ng/L respectively in October 2005). Further studies have been conducted and proved the industrial dumping of these substances upstream in the Rhône. Figure 8 shows a selection of some positive and blank chromatograms found in the lake in October 2005. 36 compounds have been identified for a total concentration above 400 ng/L which is near from the maximal residue limit for drinking water fixed at 500 ng/L for the sum or 100 ng/L for a single compound. A study was carried in collaboration of the "Service Industriel de Genève" which is the only water producer in Geneva. Pesticides residues have been search from the lake water until the final product, tap water. After the first step of processing including preoxydation, acidification, flocculation and sand filtration a decrease of ~25% of residues was observed. After the ozonation step, 2/3 of the initial residues have disappeared and finally, the charcoal filtration leads to a water free of residue. But on the other hand, the situation is not so good everywhere as half of water processing station around the lake use neither ozonation nor charcoal filtration!



Figure 8 Examples of chromatograms for a selection of compounds found in Geneva lake sample

Conclusion

Multiresidue methods are the required tools for risk assessment and monitoring of a great number of various chemical compounds achieving comprehensive pesticides residue analysis. The use of new generation MS such as triple quadrupole or ion trap detectors are ideally suited for multiresidue analysis. This new tools revolutionized the manner of working in laboratories. Instead of developing numerous analytical methods, generic samples preparations coupled to highly selective and sensitive MS or MSMS detection are carried out. Two multiresidue methods (LC-MSMS and GC-trap) were developed in the SPCo for the detection of pesticides residues in food and water samples. Both methods were applied successfully over these last 5 years for the monitoring of more than 7500 food samples. Samples were screened for more than 400 different pesticides resulting to more than 60% of positive samples overall. The LC-MSMS methods also lead to the detection of contaminants in Geneva lake water which were never found before.

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Summary

The use of new generation mass spectrometer such as triple quadrupole or ion trap detectors are ideally suited for multiresidue analysis. These new tools revolutionized the manner of working in laboratories. Instead of developing numerous analytical methods, generic sample preparations coupled to highly selective and sensitive MS or MS-MS detection are carried out. Two multiresidue methods were developed in the SPCo for the detection of pesticides residues in food and water samples. Both methods were applied successfully over the last 5 years for the monitoring of more than 7500 food samples. Samples were screened for more than 400 different pesticides resulting in more than 60% positive samples overall. The LC-MSMS methods also lead to the detection of contaminants in Geneva lake water which were never found before.

Zusammenfassung

Massenspektrometer der neuen Generation wie der Triple Quadruple oder Iontrap werden idealerweise für Mehrfachrückstandsanalysen angewendet. Diese neuen Werkzeuge haben die Arbeitsweisen in den Laboratorien revolutioniert. Anstatt zahlreiche analytische Methoden zu entwickeln, werden Probenvorbereitungen gemacht, verschiedene Trennungen zusammengefügt und eine äusserst selektive, empfindliche Detektion mit MS oder MS-MS durchgeführt. Zwei Rückstandsmethoden für die Feststellung von Pestizidrückständen in Lebensmitteln und Gewässern sind im SPCo entwickelt worden. Beide Methoden sind erfolgreich für die Überwachung von mehr als 7500 Nahrungsmittelproben während den letzten fünf Jahren angewendet worden. Es wurden über 400 verschieden Pestizide in den Proben untersucht, wobei mehr als 60% positiv waren. Die LC-MS-MS-Methode ist ebenfalls für Analysen von Rückständen im Genfersee eingesetzt worden. Neue Schadstoffe, die nie zuvor detektiert worden waren, konnten identifiziert werden.

Résumé

Les spectromètres de masse de nouvelle génération tel que le triple quadripôle ou la trappe ionique sont idéalement appropriés aux analyses multirésidus. Ces nouveaux outils ont révolutionné la façon de travailler dans les laboratoires. Au lieu de développer de nombreuses méthodes analytiques, des préparations d'échantillons et séparations génériques couplées avec une détection extrêmement sélective et sensible en MS ou MS-MS sont effectuées. Deux méthodes multirésidus ont été développées au SPCo pour la détection des résidus de pesticides dans les denrées alimentaires et les eaux. Les deux méthodes ont été appliquées avec succès ces 5 dernières années pour la surveillance de plus de 7500 échantillons alimentaires. Plus de 400 pesticides différents ont été recherchés dans ces échantillons avec au final plus de 60 % de positifs en général. La méthode LC-MSMS a également été appliqué à la recherché de résidus dans le lac Léman. De nouveaux contaminants qui n'avaient jamais été décelés auparavant ont pu être identifiés.

Key words

Chromatography, mass spectrometry, pesticide residues, food, fruits, vegetables, waters, environment, multiresidue screening

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