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Analysis of Agrochemical Residues in Tobacco Using Solid Phase Microextraction-Gas Chromatography with Different Mass Spectrometric Techniques

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ABSTRACT : A solid phase microextraction (SPME) method in combination with gas chromatography/mass spectrometric techniques was used for the extraction and quantification of 12 selected agrochemical residues in tobacco. The parameters such as the type of SPME fiber, adsorption/desorption time and the extraction temperature affecting the precision and accuracy of the SPME method were investigated and optimized. Among three types of fibers investigated, polyacrylate (PA), polydimethylsiloxane (PDMS) and polydimethylsiloxane-divinylbenzene (PDMS-DVB), PDMS fiber was selected for the extractions of the agrochemicals. The SPME device was automated and on-line coupled to a gas chromatograph with a mass spectrometer. Mass spectrometry (MS) was used and two different instruments, a quadrupole MS and triple quadrupole MS-MS mode, were compared. The performances of the two GC-MS instruments were comparable in terms of linearity (in the range of 0.01~0.5 μg/mL) and sensitivity (limits of detection were in the low ng/mL range). The triple quadrupole MS-MS instrument gave better precision than that of quadrupole MS system, but generally the relative standard deviations for replicates were acceptable for both instruments (< 15%). The LODs was fully satisfied the requirements of the CORESTA GRL. Recoveries of 12 selected agrochemicals in tobacco yielded more than 80% and reproducibility was found to be better than 10% RSD so that SPME procedure could be applied to the quantitative analysis of agrochemical residues in tobacco.

Key words **: tobacco, agrochemicals, SPME, quadrupole MS, triple quadruopole MS**

INTRODUCTION

Public interest in agrochemical residues in foods and related commodities is increasing. This situation has led to regulation setting maximum residue limits (MRLs) (1) for agrochemical residues in agricultural products, including tobacco. Thus, the rapid multi-residue determination of a wide range of agrochemicals in many samples is required.

Agrochemical residues analysis consists of several steps, extraction by using an organic solvent followed by liquid-liquid partitioning (LLE), clean-up by using solid phase extraction (SPE) or gel permeation chromatography (GPC), chromatographic separation and determination by instrumental analysis. However, these processes employing conventional sample preparation

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techniques, such as LLE, column chromatography and evaporation, are time-consuming, labor-intensive and complex.

Solid-phase micro extraction (SPME), which allows the extraction, concentration, and sample injection steps to be combined into one single step, is a relatively new sample preparation technique. SPME has been applied to the analysis of agrochemical residues in water (2), soil (3) and food samples (4), and has also been introduced for agrochemical residue analysis in tobacco samples (5).

Gas chromatography-mass spectrometry (GC-MS) has been widely used (6) for agrochemical residue analysis, because of its high specificity and sensitivity, and also for its potential of use in multi-residue and multiclass analyses. Another recent technique for agrochemical detection is gas chromatography-tandem mass spectrometry (GC-MS/MS). The tandem MS allows highly specific, sensitive and rapid determination of trace-level analytes (7). Triple quadrupole mass spectrometry can increase the number of co-eluted analytes if it is operated in selected reaction monitoring (SRM) mode that only monitors a few selected product ions per analyte.

Therefore we developed an SPME-based method for selected agrochemicals, choosing GC-MS as the instrumental technique. We compared two different MS instruments, a single quadrupole MS using the selected ion monitoring (SIM) mode and triple quadrupole MS using the MS-MS mode.

MATERIALS AND METHODS

Reagents and Materials

Acetone (pesticide grade) and water (HPLC grade) were obtained from JT Baker (USA). Selected agrochemicals and the internal standards (ISTD, mirex) were obtained from Sigma, Supelco and Reidel-de-Haën (USA). Stock standard solutions of individual compounds with concentration 100 μg/mL were prepared by weighing agrochemical standards dissolving them in 100 mL of acetone, which were then stored in a freezer at -19℃. A multi-compounds working standard solution (approximately 1 μg/mL concentration of each compound) was prepared by making appropriate dilutions of the stock solutions with acetone, and the diluted solutions were stored in a refrigerator at 4℃. Flue-cured tobacco (B1O, Korea) was used in this study.

SPME Procedure

The SPME device was automated and on-line coupled to a gas chromatograph with mass spectrometer and tandem mass spectrometer. Three types of SPME fibers (Sulpelco, USA), 65 μm polyacrylate (PA), 100 μm poly dimethylsiloxane (PDMS) and 65 μm polydimethylsiloxane-divinylbenzene (PDMS-DVB) were tested for an initia lfiber selection. All extractions were conducted in 20 mL amber glass vials, to which 4 mL of water was added. The sample was agitated and the fibers were inserted into the vial in the headspace over the sample. After exposure, the fiber was immediately desorbed in the GC injection port for analysis.

The parameters that affect the SPME process were optimized for the type of fiber, the extraction time, the exposure time of the fiber, and the thermal desorption time of fiber.

Sample Preparation

Tobacco samples were dried in an oven set at 40℃ for 5 h to approximately 5% moisture (by mass) after drying. Those samples were ground and sieved through a 2 mm mesh, taking care to avoid heating above 40℃. The sieved samples were stored in sealed containers in the dark until use.

The tobacco sample (0.25 g) was exactly weighed into a 20 mL headspace amber vial, and 50 μL of ISTD solution and 4mL of water were added. The vial was then placed in SPME device.

GC-MS and MS/MS analysis

The GC-MS analysis was carried out on an Agilent 6890 gas chromatograph with a 5973 inert mass spectrometer (USA). GC-MS/MS analysis was conducted on a Varian 3800 gas chromatograph with a 1200L triple quadrupole mass spectrometer (MS/MS). Both instruments were equipped with a CTC CombiPAL autosampler (CTC Analytics, USA), which automated the SPME analysis. An analytical capillary column used was VF-5ms (30m × 0.25mm i.d., 0.25 μm film thickness, Varian, USA). Helium was used as the carrier gas at flow rate of 1.0 mL/min. Argon was used as the collision gas in MS-MS mode. The injector temperature was set at 270℃ and 1 μL was injected in the splitless mode for 1 min. The GC oven temperature program was used as follows: initial temperature 50℃(hold for 3 min), ramp to 150℃ at 20℃ min and finally ramp to 280℃ at 2℃ min and held for 5 min. The ion source and transfer line temperature were set at 230℃ and 250℃ respectively.

The MS operated in selected-ion monitoring (SIM) mode and electron impact energy was 70 eV. From the mass spectra of individual compounds, two characteristic fragments were identified and were used for quantification purposes. The ions monitored by GC-MS were shown in Table 1.

The triple quadrupole MS was operated in the selected reaction monitoring (MS/MS) mode. To optimize the MS/MS conditions, a specific precursor ion which is most abundant in the single MS spectrum of each agrochemical. Collision energy was adjusted in order to optimize the signal of the product ions for quantification. The product ion of each agrochemical was chosen for the purposes of quantification and for the reduction of interference. The optimized MS/MS acquisition was carried out with the transitions reported in Table 1.

Quantitative analysis

The calibration curves were obtained by analyzing the solutions spiked with different levels of the agrochemicals and then they were subjected to the overall treatment, i.e. extraction, adsorption and desorption from the fibers mentioned above. The calibration curves were linear over the range of 0.01 to 0.05 μg/mL. These linearity tests were repeated on three different days to obtain mean values.

The limit of detection (LOD) for the procedure was calculated on a signal-to-noise basis of 3:1.

Recovery tests were carried out at three spiking levels and 3 replicates. An analyte solution was spiked to the tobacco to achieve concentrations of 0.05, 0.5 and 1 μg/g for each agrochemical. Extraction procedures were performed as described in the SPME methods.

RESULTS AND DISCUSSION

Optimization of MS and MS/MS conditions

To optimize the MS conditions, fragmentation of agrochemicals was performed by using single quadrupole MS in the full scanning mode. From the mass spectra of each analyte, qualification and quantification ions were identified (Table 1). Two ions were screened in SIM mode.

To optimize the MS-MS conditions, fragmentation of each analyate was conducted by collisions of precursor ions. Product ions of each agrochemical were chosen for the purposes of quantification and reduction of interferences. The product ions chosen were the ions (*m/z*) with the highest intensities. The optimized MS/MS acquisition was carried out with the transitions reported in Table 1. MS/MS acquisition could detect the low levels of the agrochemicals without interference peaks. The gas chromatographic separation of the target analytes in selected reaction monitoring (SRM) mode was not very important because this MS/MS acquisition was able to determine several co-eluated analytes at the same time.

Table 1. Mass ions for qualification and quantification by GC-MS and GC-MS/MS

Active ingredient	Retention Time	MS		MS-MS	
		Fragmentation ions *(m/z)*	Precursor ions *(m/z)*	Product ions *(m/z)*	CE (eV)
1 Trifluralin	15.876	306, 206	306	264	-15
2 Benfluralin	16.006	292, 264	292	160	-25
3 Diazinon	19.095	304, 137	179	121	-10
4 Chlorpyrifos methyl	21.532	286, 125	286	93	-30
5 Tolclofos methyl	21.933	265, 267	265	93	-25
6 Fenchlorphos	22.463	285, 287	285	270	-25
7 Aldrin	24.164	263, 293	263	193	-30
8 Butralin	25.237	266, 295	266	174	-30
9 Bromophos methyl	25.366	331, 125	331	316	-30
10 Isopropalin	25.790	280, 238	280	238	-25
11 Pendimethalin	26.066	252, 281	252	162	-30
12 Prothiofos	29.46	309, 267	309	239	-30
IS Mirex	40.273	272, 237	306	264	-25

Optimization of SPME conditions

The headspace SPME conditions were optimized to obtain the maximum sensitivity. The conditions for headspace SPME were tested by using standard solutions of the 12 agrochemicals, and the following parameters were adjusted to optimize extraction: fiber selection, extraction temperature, extraction time, desorption temperature and desorption time. The samples were continuously agitated at 500 rpm.

Comparison of fiber types

Three kinds of SPME fibers were tested polyacrylate (PA) which is a polar coating phase, polydimethylsiloxane (PDMS) which is currently most popular non-polar phase coating, and polydimethylsiloxane-divinylbenzene (PDMS-DVB) which is a mixed-phase fiber consisting of porous polymer particles divinylbenzene (DVB) suspended in a matrix of PDMS, having complimentary properties to those of DVB. All fibers were conditioned under helium in the bake out station in the CTC CombiPal autosampler according to instructions provided by the supplier.

The adsorption efficiencies of the three different SPME fibers were then determined for the 12 agrochemicals (Fig. 1). The PDMS fiber, mainly used for the extraction of nonpolar agrochemicals such organochlorines and some nonpolar organophosphorous compounds (8), gave the best extraction efficiencies for most of the selected agrochemicals. The PA fiber generally gave lower extraction efficiencies than that of PDMS fiber. This fiber is usually used for the extraction of polar and semivolatile compounds. The PDMS/DVB fiber showed higher affinity than PDMS for two compounds (trifluralin, bromophos methyl), but a lower affinity for the rest ten agrochemicals. Goncalves *et al.* (9) reported that the 65 ㎛ PDMS-DVB fiber had the lowest extraction abilityfor organochlorine, pyrethroid, organophosphorous and triazine agrochemicals.

As a result of adsorption efficiency, the PDMS fiber seems to be more effective than the PDMS-DVB for extraction of the selected agrochemicals, and also it gave better extraction efficiencies than those of PA. The 100 ㎛ PDMS fiber was used for the remainder of this study.

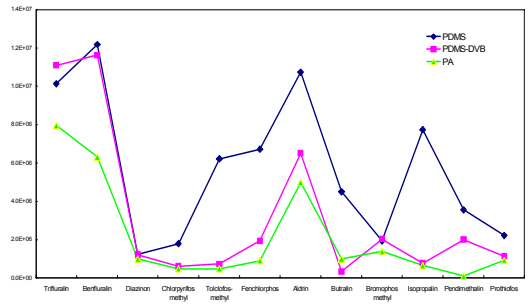

Fig. 1. Responses of the three SPME fibers to 12 agrochemicals.

Extraction temperature

Since the SPME technique involves the adsorption of analytes from a liquid sample into the polymeric phase according to their partition coefficients, it is important to determine the optimum temperature and time required to reach this equilibrium for each analyte. In order to study the effect of temperature on the extraction process, the samples were heated to temperatures ranging from 40℃ to 90℃ for 10 min, with continuous agitation. Fig. 2. shows the effect of extraction temperature on extraction efficiency. The extraction efficiency for most of the agrochemicals tested increased with increasing temperature, up to 80℃ with no further improvement at 90℃. Therefore an extraction temperature of 80℃ was selected for further experiments.

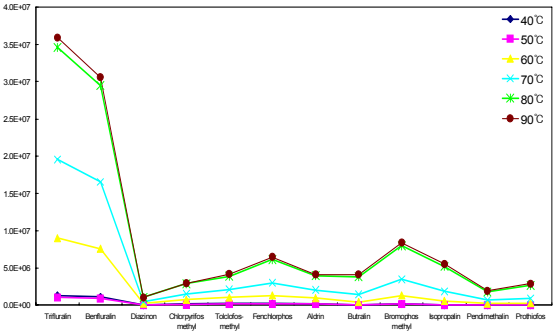

Fig. 2. Extraction temperature profile of standard solution mixture of 12 agrochemicals.

Extraction time

The time profile of adsorption was studied by monitoring the area of each peak as a function of exposure time under the same conditions used for the fiber tests. The extraction time ranged from 10 min to 60 min. Fig. 3 shows the adsorption time profiles obtained for the selected agrochemicals. It is apparent that the equilibrium time is compound-dependent. Ten compounds reached equilibrium after 30 min (trifluralin, benfluralin, diazinon, chlorpyrifos methyl, fenchlorphos, aldrin, butralin, bromophos methyl, isopropalin, pendimethalin), while the rest two compounds required 60 min or more. The relative peak response of some agrochemicals (benfluralin, butralin) decreased after longer extraction time.

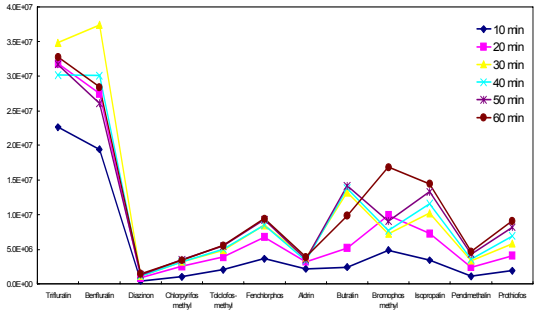

Fig. 3. Extraction time profile of standard solution mixture of 12 agrochemicals.

Desorption temperature

The analyte can be desorbed effectively under a higher temperature in a shorter time, but the stability and the life-time of the fiber will be also affected. Likewise, the analyte may be decomposed if the desorption temperature is too high. Fig. 4 shows the profile of the analyte desorption according to increasing temperature (250℃ to 300℃). A desorption temperature of 270℃ showed no carryover effects.

Desorption time

Another important parameter is the desorption time of the analytes from the SPME fiber in the

injection port of the GC. There are many factors that affect desorption behavior, such as the compound's boiling point, the partition coefficient of the analyte, the thickness of the stationary phase and the desorption temperature. We tested a range of desorption time from 1 min to 10 min to determine the optimal condition. Fig. 5 shows the desorption time profile of the analytes. One minute of desorption time was sufficient to allow the complete desorption of analytes from the fiber.

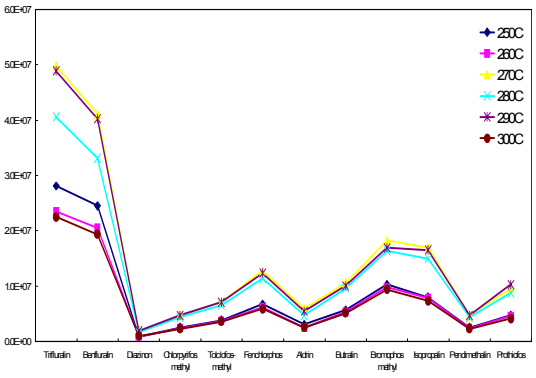

Fig. 4. Desorption temperature profile of standard solution mixture of 12 agrochemicals.

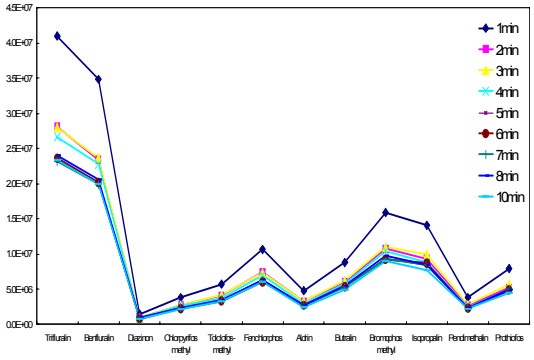

Fig. 5. Desorption time profile of standard solution mixture of 12 agrochemicals.

Linearity, Limit of Detection and Precision

The linearity of the method was checked over the range of 0.01 μg/mL to 0.5 μg/mL. Each concentration was analyzed five times. Both instruments gave good linearities for most of the agrochemicals over this range (Table 2).

The precision of the method was calculated on a single-day basis. The relative standard deviations, calculated by using the mean of the peak areas of five consecutive SPME extractions of the same standard solutions, varied from 2.7%

Table 2. Linearity, LOD, precision and recovery rate by using the proposed method

Active ingredient	Linearity (R^2)		LOD (μg/mL)		Precision (RSD%)		Recovery (%)	
	MS	MS/MS	MS	MS/MS	MS	MS/MS	MS	MS/MS
Trifluralin	0.9990	0.9992	0.003	0.001	8.5	7.0	80.5	85.1
Benfluralin	0.9992	0.9996	0.001	0.001	6.7	8.1	81.5	91.9
Diazinon	0.9998	0.9997	0.010	0.005	14.5	8.6	81.9	83.7
Chlorpyrifos methyl	0.9995	0.9991	0.002	0.002	5.1	5.4	83.3	95.6
Tolclofos methyl	0.9993	0.9990	0.004	0.003	9.0	2.9	94.6	82.1
Fenchlorphos	0.9997	0.9996	0.003	0.001	6.2	3.7	94.3	93.5
Aldrin	0.9990	0.9992	0.003	0.003	7.4	0.7	85.6	91.6
Butralin	0.9989	0.9993	0.005	0.003	2.7	3.9	87.6	81.8
Bromophos methyl	0.9991	0.9996	0.003	0.001	11.9	2.9	88.7	91.7
Isopropalin	0.9990	0.9997	0.002	0.002	15.4	11.0	95.1	93.3
Pendimethalin	0.9994	0.9993	0.003	0.003	5.5	6.9	92.3	98.8
Prothiofos	0.9995	0.9992	0.003	0.003	9.7	7.0	84.0	91.0

(butralin) to 15.4% (isopropalin) in GC-MS, and 0.7% (aldrin) to 11.0% (isoproaplin) in GC-MS/MS (Table 2).

The limits of detection were calculated by comparing the signal-to-noise ratio (S/N) of the lowest concentration to the limit S/N=3.

Recoveries

To check the validity of the method, we used fortified samples of flue-cured tobacco prepared by adding a known volume of the mixed agrochemical standard solution to a 0.25 g portion of the ground tobacco. The tobacco samples were subjected to the overall treatment described above in the SPME procedure. The recoveries obtained from the analysis of the fortified tobacco samples ranged from 80.5% to 98.8 % for each agrochemical at tested concentration, indicating high accuracy of this method (Table 2).

CONCLUSIONS

This study clearly shows that a combination of SPME with a single quadrupole mass spectrometer (GC-MS) and a triple quadrupole mass spectrometer (GC-MS/MS) can be used to accurately determine 12 selected agrochemicals in tobacco, even though these compounds come from very different chemical classes. Through this method, we could achieve significant improvements in selectivity and sensitivity, and identify and quantify low traces of 12 agrochemicals conveniently.

The PDMS fiber was shown to be the most efficient at extracting the 12 selected agrochemicals. The operation parameters were optimized: 80℃ adsorption temperature, 30 min of adsorption time, 270℃ desorption temperature and 1 min of desorption time.

The two GC-MS systems used in this study provided comparable results in terms of sensitivity. The performances of the two MS instruments gave good linearity, low LOD and high precision. The triple quadrupole MS-MS instrument gave better precision than the quadrupole MS system, but generally the relative standard deviations of both instruments were acceptable. The LOD fully satisfied the requirements of the CORESTA GRL. Recoveries of the 12 selected agrochemicals in tobacco were more than 80% and reproducibility was found to be better than 10% of the RSD.

In conclusion, SPME with the mass spectrometric analytical system seems to be a fast, simple and solvent-free method for qualifying and quantifying agrochemical residues in tobacco samples.

REFERENCES

Castro, J., Perez, R.A., Sanchez-Brunete, C., Tadeo, J.L. (2001) Analysis of pesticides volatilized from plants and soil by headspace solid-phase microextraction and gas chromatography. *Chromatographia Supplement*, 53: 361-365.

Dugay, J, Miege, C., Hennion, M-C. (1998) Effect of the various parameters governing solid-phase microextraction for the trace-determination of pesticides in water. *J Chromatography A*, 795: 27-42.

Goncalves, C., Alpendurada M.F. (2002) Comparison of three different poly (dimethylsiloxane) - divinylbenzene fibres for the analysis of pesticide multiresidues in water samples: structure and efficiency. *J Chromatography A*, 963: 19-26.

Haib, J., Hofer, I. and Renaud, J.M.(2003) Analysis of multiple pesticide residues in tobacco using pressurized liquid extraction, automated solid-phase extraction clean-up and gas chromatography - tandem mass spectrometry. *J. Chromatography A*, 1020: 173 - 187.

Mueller, L., Ward, M.R. (1999), Tobacco, Production, Chemistry and Technology, Davis, D.L., Nielsen, M.T.(Eds.), Blackwell, London,

pp. 250 - 264.

Natnagelo, M., Tabaxzzi, S., Ranelli, R., Benfenati, E. (1999) Analysis of some pesticides in water samples using solid-phase microextraction-gas chromatography with different mass spectrometric techniques. *J. Chromatography A*, 859: 193-201.

Shen, J., Xu, X., Cai, J., Shao, X. (2006) Determination of pyrethroid residues in tobacco by means of solid phase microextration and GC/MS with the aid of ultrasonic assisted extraction using water as extracting solvent. *Analytical Sciences*, 22: 241-244.

Tahboub, Y.R., Zaater, M.F. and Al-Talla, Z.A. (2005) Determination of the limits of identification and quantitation of selected organochlorine and organophosphorous pesticide residues in surface water by full-scan gas chromatography/mass spectrometry. *J. Chromatography A*, 1098: 150 - 155.

Vitali, M., Guidotti, M., Giovinazzo, R., Cedrone, O. (1998) Determination of pesticide residues in wine by SPME and GC/MS for consumer risk assessment. *Food Additives and Contaminants*, 15: 280-287.