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Application of head-space solid-phase microextraction coupled to comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the determination of multiple pesticide residues in tea samples

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ABSTRACT

A new method has been developed to detect 36 pesticides that may contaminate tea samples (green, black and fruit tea). The hyphenation of solid-phase microextraction in head-space mode with a comprehensive two-dimensional gas chromatography coupled with high-speed time-of-flight mass spectrometry (HS-SPME-GC × GC/TOF MS) proved to be a quick alternative to conventional GC/MS methodology which employs solvent-based extraction. The key parameters for controlling HS-SPME performance were optimized, including fiber coating type, temperature and absorption time settings and tea matrix modification by adding water. Quantification was carried out using matrix-matched calibration. The repeatability of measurements, expressed as relative standard deviation (R.S.D.), was less than 24% for all analytes. The limits of quantification ranged from 1 to 28 μg kg⁻¹. The optimized method was applied to analyze real life samples obtained from a retail market. Results generated by the new SPME procedure and those obtained by using a conventional one involving ethyl acetate extraction and high-performance gel permeation chromatography (HPGPC) clean up agreed with each other for positive (containing residue) samples.

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1. Introduction

As pressure from regulatory bodies increases and consumer demand for guaranteed food quality and safety grows, the need for new, hi-tech technologies in pesticide residue analysis has never been greater. It should be noted that all developing innovations have to withstand the test of time and decrease in cost per analysis without diminishing relevant performance characteristics.

While extensive monitoring of pesticide residues in fruit and vegetables has been performed in many countries, a regular control of tea, one of the most popular commodities in the world, is rare. Pesticide preparations, most typically insecticides, which contain active ingredients such as ethion, quinalphos, malathion and dimethoate are applied to tea plants either during cultivation or, occasionally, during storage. In this way, some residue left on dried tea leaves can be transferred to tea infusions and contribute to the dietary

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exposure of a consumer to hazardous chemicals [1]. Similarly, crops used for fruit and herbal tea production can also be a source of pesticide residue intake [2,3].

Most existing analytical methods used to control levels of residues in commodities involve a time and labour-intensive extraction step such as solvent extraction [4], Soxhlet extraction [5] and/or supercritical-fluid extraction (SFE) [6]. The procedures used for purification of crude extracts, e.g. chromatography on a Florisil column, high-performance gel permeation chromatography (HPGPC) and/or solid-phase extraction (SPE) are also quite demanding [4,5]. Moreover, in many cases, these sample-handling steps require large amounts of solvents that may be harmful to humans and the environment.

In the recent years, solventless solid-phase microextraction (SPME) has gained widespread acceptance in analysis of (semi)volatile food components that include contaminants. Sampling of analytes is done either through direct immersion of suitable fiber into a liquid phase, or in head-space, followed by thermal desorption of the extracted analytes in the hot injection port of the gas chromatograph (GC) [7]. Regarding the determinative step, mass spectrometric detectors, most often employing quadrupole or ion-trap (ITD) mass analyzers, are the most popular in SPME-GC analysis of pesticide residues. Generally, SPME offers many advantages over traditional approaches because it is simple, fast and easy to automate. Until now, however, most SPME applications in pesticide residue analysis have been focused on liquid samples, such as drinking water, fruit and vegetable juices or soft drinks [8–14]. The number of methods dealing with solid samples is limited [15,16]. Only one paper dealt with tea samples [17].

The aim of the present study was to investigate the possibility of controlling pesticide residues in various teas by head-space HS-SPME sampling combined with GC and/or comprehensive two-dimensional gas chromatography (GC \times GC) employing a recently introduced high-speed time-of-flight mass spectrometric detector (TOF MS). The objective of the second part of the study was to compare performance characteristics of the new SPME-based approach with a conventional one involving solvent extraction followed by the HPGPC purification step for sample preparation.

2. Experimental

2.1. Reagents

Certified standards of pesticides with purities ranging from 95% to 99% were purchased from Dr. Ehrenstorfer (Augsburg, Germany). For the list of compounds, see Table 2. Ethyl acetate for solvent extraction of pesticide residues was analytical grade (Scharlau, Barcelona, Spain). Methanol was obtained from Merck (Darmstadt, Germany). Deionized water was prepared by a MilliQ system (Millipore, Billerica, MA, USA). Helium 6.0 from Siad (Prague, Czech Republic) was used for GC analyses.

Individual stock solutions of pesticides containing 1000 $\mu\text{g mL}^{-1}$ were prepared in ethyl acetate and stored at -18°C . Working solutions (concentration 1.25–200 $\mu\text{g L}^{-1}$) were prepared by a series of dilutions of the stock solutions

with ethyl acetate and stored at -18°C for a maximum period of 6 months.

2.2. Material

In total, 67 different tea samples, including green, black and fruit teas of different origins (Asia, Africa, Central Europe), were analyzed for pesticides. All the samples were bought at a local market. For optimization and validation purposes, a residue-free matrix (a mixture of black and fruit organic teas) was chosen.

To obtain homogeneous distribution of target pesticides in the spiked matrix, the following procedure was used: 250 g of the previously mentioned blank tea sample was suspended in 250 mL of methanol containing all tested pesticides (each at concentration 50 $\mu\text{g L}^{-1}$). After 2 h, the methanol was evaporated from the suspension at ambient temperature using a vacuum rotary evaporator (Buchi, Flawil, Switzerland). The material was stored at 8°C and no degradation of the target compounds was observed within 2 weeks of the preliminary experiments.

2.3. Instrumentation

2.3.1. GC/MS instrumentation

The GC \times GC/TOF MS system consisted of a HP 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with a split-splitless injector and Pegasus III time-of-flight mass spectrometer (LECO, St. Joseph, MI, USA) with 10 mL min^{-1} pumping capacity. The detector operated in electron impact ionisation mode (EI). A dual-stage jet modulator and the secondary oven were mounted inside the GC oven. Resistively heated air was used as a medium for hot jets, while cold jets were supplied by gaseous nitrogen cooled by liquid nitrogen.

GC separation was performed using a BPX-5 column (40 m \times 0.18 mm \times 0.18 μm) from SGE Analytical Science (Ringwood, Australia). The oven temperature program was as follows: 70°C for 1 min, $45^\circ\text{C min}^{-1}$ to 260°C , hold for 12 min; helium flow: 1.0 mL min^{-1} ; injection mode: pulsed splitless (50 psi) for 1.0 min; injection temperature: 270°C . The second dimension column SupelcoWax (2.5 m \times 0.1 mm \times 0.1 μm) from Supelco (Bellefonte, PA, USA) was already installed in the secondary oven. The temperature of the secondary oven was held 25°C above the temperature of the main oven; hence no retention in the second dimension occurred. In GC \times GC separation, the first column was operated under the same temperature program as in GC separation. The temperature of the secondary column was held 10°C above the temperature of the main oven. Modulation was carried out using a 15°C temperature offset and a 5 s modulation time (hot pulse 1.5 s).

The following instrumental set-up of MS was used—solvent delay: 5 min (used for liquid injection); acquisition rate: 125 Hz (for GC \times GC) and 10 Hz (for one-dimensional GC, 1D-GC); mass range 45–500 amu; ion source temperature: 220°C ; transfer line temperature: 270°C ; detector voltage: -1850 V .

Total analysis time was 23 min for both GC \times GC/TOF MS and GC/TOF MS methods. ChromaTOF software (LECO) was used to process collected data.

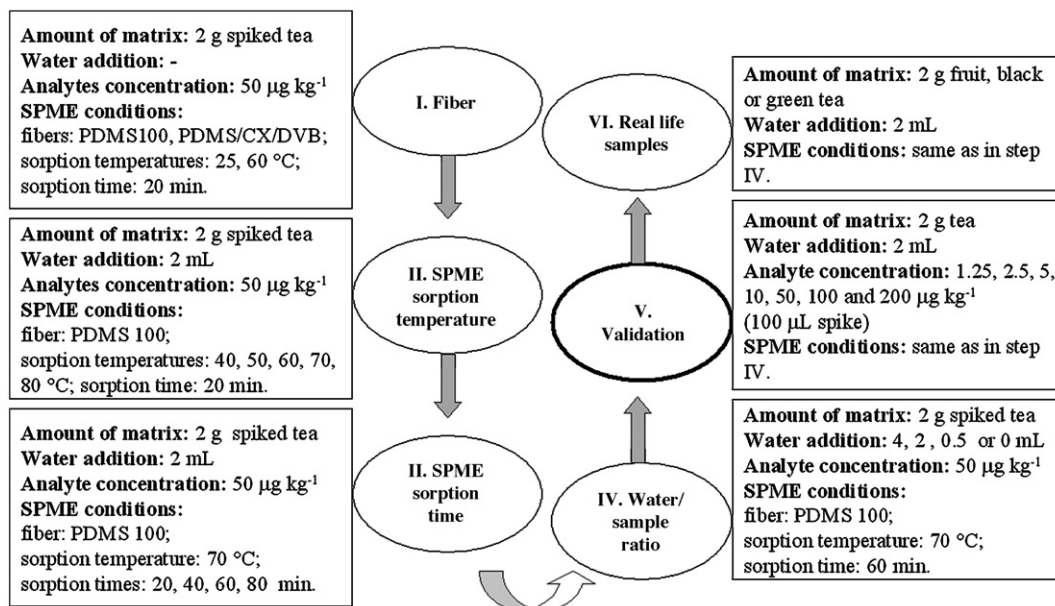


Fig. 1 – An overview of the experiments carried out within the optimization and validation process of the new approach.

2.3.2. HS-SPME instrumentation

Automated HS-SPME extraction and desorption was carried out with the multipurpose sampler MPS2 (Gerstel, Mülheim/Ruhr, Germany).

Two different fiber types were studied: polydimethylsiloxane (PDMS, 100 μm) and polydimethylsiloxane/carboxen/divinylbenzene (PDMS/CX/DVB, 30/50 μm), both supplied by Supelco. Before they were used, fibers were conditioned as recommended by the manufacturer (at 250 °C for 0.5 h in the case of PDMS coating and at 270 °C for 1 h in the case of PDMS/CX/DVB coating) and cleaned each day by heat in the injection port of the chromatographic system (30 min at 250 °C).

2.3.3. HPGPC instrumentation

An automated HPGPC system Aspec XL (Gilson, Middleton, WI, USA) equipped with a PL gel column (600 mm \times 7.5 mm, particle size 10 μm , 50 Å; Polymer Laboratories, Church Stretton, UK) was used for purification of crude tea extracts. Polytetrafluoroethylene (PTFE) filters (5 μm ; National Scientific, Rockwood, TN, USA) were used for the filtration of crude extracts prior to the clean-up step.

Waring Blender (Waring Products, Torrington, CT, USA) was used to homogenize samples. Ethyl acetate extraction of tea leaves was performed using Ultra-Turrax (IKA Werke, Staufen, Germany).

2.4. Analytical procedures

The two procedures described below were used for isolation of target analytes (36 pesticides including isomers, see Table 2) from the tea matrix. The reagent blank samples were prepared under the same conditions as ordinary samples, using all reagents but not using tea leaves.

2.4.1. HS-SPME procedure

The implementation of HS-SPME is shown in Fig. 1. Samples in 10 mL sealed glass vials were placed in the MPS-2 autosampler for HS-SPME extraction under conditions described in the respective figure. Extracted compounds were thermally desorbed from the fiber after its insertion into the hot (270 °C) splitless GC injection port. Desorption time was 2 min. For the target analytes quantification, a matrix-matched external standard calibration at 7 concentration levels (1.25, 5, 10, 25, 50, 100 and 200 $\mu\text{g kg}^{-1}$) was used.

2.4.2. Isolation and HPGPC purification

25 g of a homogenized tea sample was weighed in a glass beaker. After adding 100 mL of ethyl acetate and 10 g of anhydrous sodium sulphate, the sample was extracted for 2 min using an Ultra-Turrax homogenizer at 10,000 rpm. The suspension was filtered under vacuum through a layer of anhydrous sodium sulphate, and both the beaker and the filtrate cake were rinsed with 3 \times 25 mL of ethyl acetate. Combined filtrates were evaporated using a vacuum rotary evaporator (temperature maximum 40 °C, pressure 220 mbar) to a volume of approximately 25 mL. The concentrated crude extract was transferred into a volumetric flask. The final volume was filled with cyclohexane to 50 mL (final sample concentration 0.5 g mL^{-1}).

An aliquot part of this crude extract was filtered through a PTFE filter and then loaded into an automated HPGPC system. The following conditions were used for sample clean-up: mobile phase ethyl acetate-cyclohexane (1:1, v:v), flow rate 1 mL min^{-1} , injection volume 2 mL, collected "pesticide" fraction 14.5–31.0 mL. The purified pesticide fraction was evaporated using a vacuum rotary evaporator (temperature maximum 40 °C, pressure 220 mbar) until it was nearly dry. The residual solvents were removed with a gentle stream of nitrogen. After 1 mL of ethyl acetate was added, the purified

Table 1 – Effect of ratio between water and tea sample on R.S.D. (%) and absolute abundance (peak area) of selected analytes (spiked level $50 \mu\text{g kg}^{-1}$, $n = 5$)

Pesticides	Water/sample ratio (w/w) ^a							
	0		0.25		1		2	
	R.S.D.	Peak area	R.S.D.	Peak area	R.S.D.	Peak area	R.S.D.	Peak area
Chlorpropham	5	6,200	6.8	5,890	0.9	7,520	2.3	6,340
HCB	2.9	20,120	2.1	22,100	1.1	24,500	1.8	20,100
Chlorpyrifos-methyl	11	11,400	10.9	12,400	12.9	14,600	10.2	12,050
Heptachlor	15	13,200	14.7	16,810	8.3	17,590	8.3	15,570
Fenthion	14.1	9,510	18.1	9,920	9.9	11,170	10.3	8,610
Diazinon	8.7	17,800	11.7	18,070	9.0	19,930	11.8	17,780

^a Water/sample ratio of 0, 0.25, 1 and 2 corresponded to: 0 mL, 0.5 mL, 2 mL and 4 mL of water added, respectively, to 2 g tea sample.

sample extract was ready for GC × GC/TOF MS analysis. Sample concentration in the final extract was 1 g mL^{-1} (injection volume was $1 \mu\text{L}$). Quantification of pesticides was carried out using a matrix-matched external standard calibration at the same concentration range ($1.25\text{--}200 \mu\text{g kg}^{-1}$) as used in the HS-SPME procedure.

3. Results and discussion

3.1. SPME optimization

As was described by Huang et al. in another report [18], and also based on our experience, determination of pesticide residues in tea (*Camellia sinensis*) is a rather difficult task due to the complexity of the matrix. In addition to caffeine (an alkaloid that may constitute up to 2% of a tea leaf's dry weight), many other natural components can be co-isolated by conventional solvent extraction. By this account, contrary to other plant matrices such as fruit and/or vegetables, sample processing prior to a determinative step has to involve multi-step purification. Under these conditions, it was a challenge to replace the conventional approach with solvent-free SPME sampling. During the preliminary experiments with spiked dry tea samples, we tested head-space extraction efficiency using two types of fibers: PDMS and PDMS/CX/DVB coatings. Unfortunately, the repeatability of measurements was poor for some of the target pesticides and obtaining reliable data was impossible. In the following experiments, we therefore decided to examine an aqueous suspension of spiked tea samples. We expected that the addition of water would facilitate the release of analytes from the matrix and accelerate their SPME sampling from the head-space as it has in other similar studies [3,19,20]. The arrangement of the large set of HS-SPME optimization experiments conducted within this part of study is shown in Fig. 1 and characterized in the paragraphs below.

When comparing model experiments with fixed extraction time (20 min) carried out using PDMS/CX/DVB and PDMS fiber at test temperatures 25 and 60°C , the PDMS fiber provided both higher responses of analytes (1.4–1.9 times) and improved repeatability (7–28% expressed as relative standard deviation, R.S.D., $n = 5$). In the case of PDMS/CX/DVB fiber, RDS was in the range of 11–42%. Using a PDMS fiber coating and changing sampling temperatures from 40 to 80°C in the next step, we observed an increase in extraction

efficiency for all pesticides (up to 70°C), obviously due to enhanced diffusion of these analytes from aqueous suspension into the head-space [21]. Further increase of temperature resulted in a relative drop of signals of several analytes, such as mevinphos, monocrotophos, malathion, parathion-methyl, chlorfenvinphos, propham, chlorpropham, carbaryl, diazinon and hexachorobenzene (HCB). The responses obtained under these SPME conditions were in the range of 41–75% compared to those obtained at 70°C . Worth noticing is that, with HCB being the only exception, these pesticides represent medium polar substances with water solubility in the range of $10^1\text{--}10^2 \text{ mg L}^{-1}$, i.e. compounds better soluble than the other analytes. The following several phenomena (possibly in combination) may be responsible for this effect: (i) exothermic nature of the adsorption process starts to be limiting above a certain critical temperature, (ii) increasing water vapor pressure impairs the extraction process at elevated temperatures, (iii) better solubility of target residues in water at higher temperature (resulting in a decrease of their distribution coefficients) accompanied by their reduced transfer into the head-space. These arguments, however, do not explain the behaviour of HCB. The most probable cause of the drop in HCB recovery at higher experimental temperature could be associated with its losses by volatilization.

Within the third step of the optimization process, the extraction times were varied, with four values in the range of 20–80 min. The 60 min extraction was chosen as a compromise between the sensitivity and practical method throughput.

Poor repeatability of measurements (up to 46% when expressed as R.S.D., $n = 5$) was obtained when spiked samples of dry tea leaves were examined. Therefore, to promote and standardize the transfer of pesticides from the tea matrix to the gas phase, water in different quantities was added to the sample. The data summarized in Table 1 shows that the best result (low limits of detection, LODs and variability of analytes measurement) was achieved at a 1:1 (w:w) water to sample ratio. A decrease in HS-SPME extraction yield with the more water that is added can possibly be explained by an increased barrier between the sample and gaseous phase [21,22].

3.2. Comparison of HS-SPME coupled with GC and GC × GC separation

In the current study, the SPME extracts were analyzed using conventional GC and GC × GC, both coupled with a high-speed

Table 2 – Comparison of detectability of the 36 target pesticides in HS-SPME GC/TOF MS and HS-SPME GC × GC/TOF MS system under optimal conditions (spiked level 50 µg kg⁻¹, n = 5)

Pesticides	Quantification (bold) and Identification ions	Enhancement factor ^a	MS match factor (similarity/reverse) ^b	
			GC	GC × GC
Mevinphos	109, 127, 192	7	721/967	944/947
Propham	93, 137, 179	1.8	678/730	748/802
Methacrifos	180, 208, 240	2.1	–	952/956
Chlorpropham	127, 171, 213	1.8	916/928	931/942
Monocrotophos	97, 127, 192	2.2	831/884	849/849
HCB	282, 284, 286	11	857/888	911/914
Alpha-HCH	181, 217, 219	9.4	807/886	935/940
Beta-HCH	109, 183, 219	6.9	735/875	758/889
Gamma-HCH	181, 183, 219	10.2	798/874	851/898
Etrimfos	153, 181, 292	4.4	806/817	899/905
Chlorpyriphos-methyl	125, 286, 288	3.1	833/838	861/875
Vinclozolin	198, 212, 285	10	–	875/820
Parathion-methyl	109, 125, 263	14.7	–	920/920
Tolclofos-methyl	125, 265, 267	1.7	–	720/703
Carbaryl	115, 116, 144	9.6	921/934	949/949
Heptachlor	237, 272, 372	1.2	–	913/801
Pirimiphos-methyl	276, 290, 305	12	–	906/909
Fenitrothion	125, 260, 277	2.5	872/823	996/903
Malathion	125, 127, 173	4.5	860/888	955/995
Chlorpyriphos	199, 258, 314	1.4	757/851	840/869
Fenthion	125, 169, 278	2.3	764/785	816/853
Parathion	139, 261, 291	4.2	678/620	893/850
Chlorfenvinphos	267, 269, 323	2.3	821/862	853/882
Alpha-endosulfan	195, 241, 339	2.4	768/860	885/890
Dieldrin	79, 149, 263	7	–	927/942
Aldrin	109, 263, 265	15.2	876/864	905/906
<i>p,p</i> -DDD	165, 199, 212	3.5	–	892/897
<i>p,p</i> -DDE	316, 318, 246	7.1	–	897/915
<i>o,p</i> -DDE	316, 318, 246	3.4	877/919	917/933
<i>o,p</i> -DDT	165, 235, 237	4	859/870	867/908
<i>p,p</i> -DDT	165, 235, 237	3.9	778/846	899/908
Endrin	263, 261, 317	8	872/895	901/915
Ethion	153, 231, 384	3.6	771/878	855/876
Bifenthrin	165, 166, 181	5.1	762/799	763/792
Quinalphos	146, 156, 157	1.5	854/879	742/863
Diazinon	152, 179, 304	6.4	–	881/898

^a Enhancement factor is defined as ratio between S/N of the pesticide peak in GC × GC and GC.

^b Quality of mass spectral information given by the ChromaTOF software.

TOF MS detector. The aim was to compare detectability of analytes in these systems. The results achieved in both systems are summarized in Table 2. When employing one-dimensional GC/TOF MS, the confirmation based on “MS match” factors of deconvoluted mass spectra with NIST library could not be achieved (the value of this parameter higher than 600 was set as minimum requirement) for 9 out of 36 pesticides (spike at 50 µg kg⁻¹ level) due to many coelutions. Even in the case of those few pesticides that were automatically identified with a high enough spectral match, the limits of quantification (LOQs) were higher than the EU maximum residue limits (MRLs) [23]. As shown in Table 2, both significant improvement in quality of mass spectra and distinct increase of S/N ratios (i.e. decrease of LOQs) was achieved by the application of GC × GC separation. Under conditions of enhanced separation power, the confirmation was fairly more reliable. All analytes were identified (at the concentration level 50 µg kg⁻¹) and 79% of “MS match” factors were above 850. Similar ben-

efits, in terms of improved identification potential, were also reported in the studies using this comprehensive separation technique for analysis of pesticides in food crop samples after a conventional solvent extraction [24]. The detectability enhancement effect, expressed as the ratio of the signals from GC × GC system and those from 1D-GC system, ranged in our study from 1.2 (heptachlor) to 15.2 (aldrin). In Fig. 2, improved performance parameters attained by GC × GC/TOF MS are documented for parathion as an example. In this particular case, the signal-to-noise ratio increased 4.2 times (from 20 to 84) and the “similarity”/“reverse” factors increased from 678/620 (values in 1D-GC) to 893/850. Besides the other positive outcomes, by adding the second column (SupelcoWax) to the GC system, the complete separation of critical pair chlorpyriphos-parathion was also achieved. It should be noted that to collect a very narrow parathion peak in GC × GC system (150 ms compared to 3 s in one-dimensional GC), the detector acquisition rate had to be increased 12.5 times (to 125 Hz) to obtain enough

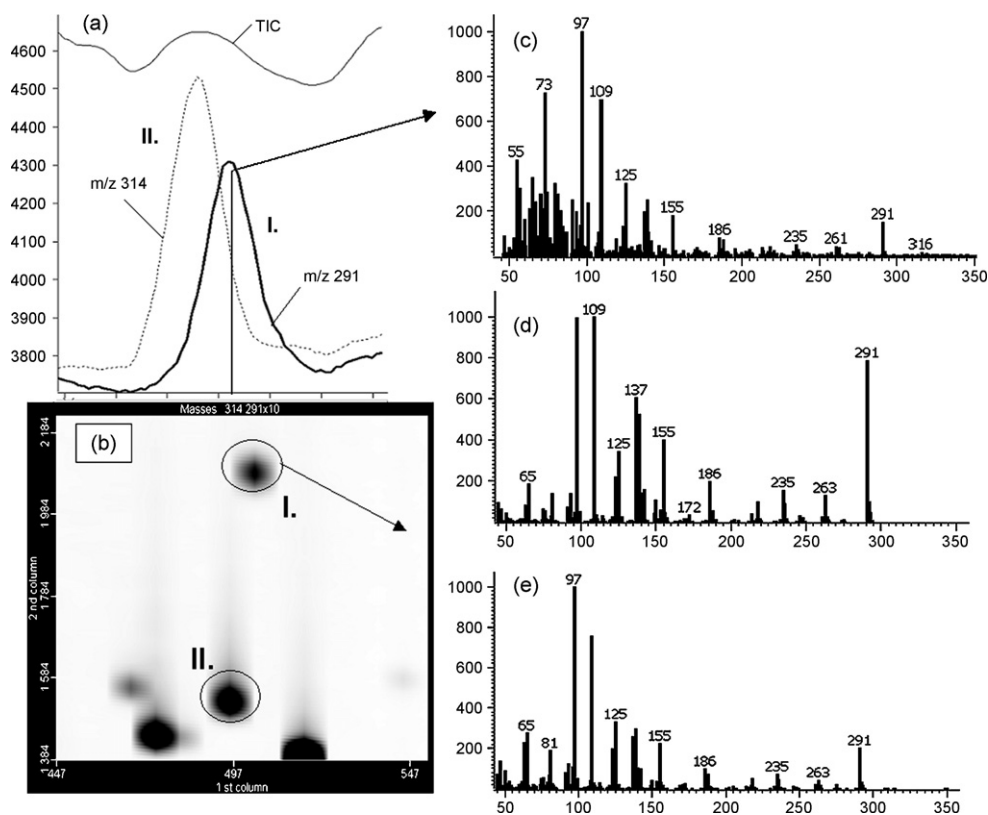


Fig. 2 – A comparison of detection capabilities in HS-SPME GC/TOF MS and HS-SPME GC \times GC/TOF MS (I) parathion and (II) chlorpyrifos in the spiked tea sample (concentration level $50 \mu\text{g kg}^{-1}$). (a) Zoomed in part of GC-TOF MS chromatogram. (b) Zoomed in part of GC \times GC/TOF MS contour plot. (c) Mass spectrum of parathion measured by GC/TOF MS. (d) NIST library spectrum of parathion (#134623). (e) Mass spectrum of parathion measured by GC \times GC/TOF MS.

data points (15–20) [25]. Such values cannot be applied with common scanning detectors employing quadrupole or ITD.

3.3. Comparison of HS-SPME sampling with conventional solvent extraction method

To assess the performance of the overall analytical procedure, spiked tea samples were processed (five replicates) by both the conventional method employing ethyl acetate extraction followed by HPGPC and by optimized HS-SPME. As shown in Table 3, the linearity within the tested range was poorer

when using the latter approach. As much as 86% of the target analytes had regression coefficients lower than 0.995. Better values were calculated when considering the narrower range of $10\text{--}200 \mu\text{g kg}^{-1}$. In this case, the linearity was not fully optimal for 21% analytes. Regarding the HS-SPME method precision, the R.S.D.s at the test level $50 \mu\text{g kg}^{-1}$ were in the range of 2 (HCB) to 24% (alpha-endosulfan). On average, their median value was approximately 1.7 times higher than in the conventional method.

The LOQs were estimated as having the lowest content of analyte in the sample, which can be quantitatively determined

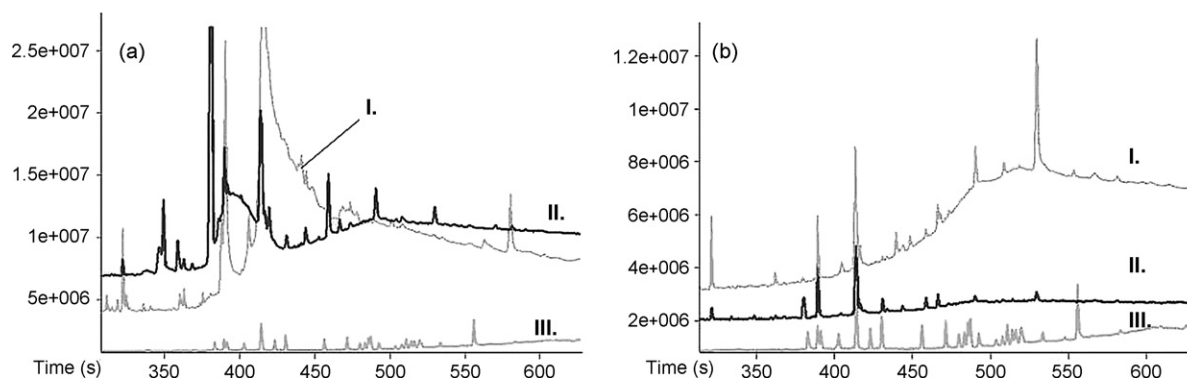


Fig. 3 – Comparison of conventional (a) and SPME-based (b) approach in analysis of pesticide residues in tea samples. GC/TOF MS chromatograms of blank (non-spiked) black (I) and fruit (II) teas; pesticide standard solution ($200 \mu\text{g kg}^{-1}$) (III).

Table 3 – Selected performance characteristics of the HS-SPME GC × GC/TOF-MS and the conventional method (ethyl acetate extraction, HPGPC) applied to pesticide residue analysis in tea samples

Pesticides	EU MRL ($\mu\text{g kg}^{-1}$)	HS-SPME GC × GC/TOF-MS				Ethyl acetate extraction, HPGPC GC × GC/TOF-MS			
		Linearity (r^2)	Linearity range ($\mu\text{g kg}^{-1}$)	LOQs ($\mu\text{g kg}^{-1}$)	R.S.D.	Linearity (r^2)	Linearity range ($\mu\text{g kg}^{-1}$)	LOQs ($\mu\text{g kg}^{-1}$)	R.S.D.
Mevinphos	–	0.9840	5–200	2	8	0.9950	5–200	5	5
Propham	100	0.9961	10–200	6	6	0.9992	5–200	5	6
Methacrifos	100	0.9889	5–200	4	4	0.9993	10–200	7	8
Chlorpropham	–	0.9960	10–200	9	8	0.9990	10–200	8	7
Monocrotophos	100	0.9850	25–200	22	10	0.9981	25–200	14	8
HCB	20	0.9971	5–200	2	2	0.9987	1.25–200	1	3
Alpha-HCH		0.9811	10–200	8	16	0.9897	5–200	2	9
Beta-HCH	20 ^a	0.9890	1.25–200	1	8	0.9989	10–200	10	5
Gamma-HCH	50	0.9812	10–200	6	11	0.9986	5–200	4	6
Etrimfos	–	0.9823	10–200	10	7	0.9923	5–200	3	4
Chlorpyrifos-methyl	100	0.9849	5–200	5	13	0.9992	5–200	4	4
Vinclozolin	100	0.9859	25–200	12	7	0.9990	5–200	2	7
Parathion-methyl	–	0.9849	5–200	5	12	0.9987	5–200	2	6
Tolclofos-methyl	–	0.9951	5–200	3	11	0.9999	5–200	2	5
Carbaryl	–	0.9918	25–200	12	18	0.9998	10–200	10	13
Heptachlor	20	0.9870	10–200	8	8	0.9983	1.25–200	1	5
Pirimiphos-methyl	50	0.9927	25–200	23	7	0.9997	10–200	8	8
Fenitrothion	500	0.9931	10–200	8	13	0.9988	10–200	7	10
Malathion	500	0.9838	50–200	26	13	0.9977	10–200	8	11
Chlorpyrifos	100	0.9870	5–200	3	6	0.9995	5–200	4	4
Fenthion	–	0.9850	25–200	20	10	0.9994	25–200	13	10
Parathion	100	0.9899	25–200	11	3	0.9990	5–200	5	5
Chlorfenvinphos	–	0.9846	25–200	24	21	0.9986	25–200	14	18
Alpha-endosulfan	30,000	0.9812	50–200	27	24	0.9992	10–200	6	8
Dieldrin		0.9880	25–200	14	11	0.9991	5–200	5	10
Aldrin	20 ^b	0.9906	25–200	9	12	0.9989	5–200	3	5
p,p-DDD		0.9879	25–200	24	19	0.9999	5–200	3	4
p,p-DDE		0.9928	25–200	20	17	0.9998	5–200	2	5
o,p-DDE	200 ^c	0.9835	25–200	18	11	0.9975	5–200	2	11
o,p-DDT		0.9917	25–200	18	19	0.9979	1.25–200	1	9
p,p-DDT		0.9945	25–200	16	9	0.9995	5–200	2	7
Endrin	10	0.9928	10–200	9	9	0.9998	5–200	5	8
Ethion	2,000	0.9880	10–200	7	16	0.9990	5–200	5	5
Bifenthrin	5,000	0.9869	50–200	28	22	0.9891	5–200	3	5
Quinalphos	100	0.9931	25–200	18	8	0.9999	10–200	9	4
Diazinon	50	0.9910	25–200	16	13	0.9990	5–200	5	9

Linearity ranges are shown together with appropriate regression coefficients, LOQ estimated at S/N=12 and repeatability at concentration level $50 \mu\text{g kg}^{-1}$ ($n=5$).

^a Sum of isomers except the gamma isomer.

^b Aldrin and dieldrin, combined expressed as dieldrin.

^c Sum of p,p-DDT, o,p-DDT, p,p-DDE and p,p-DDD expressed as DDT.

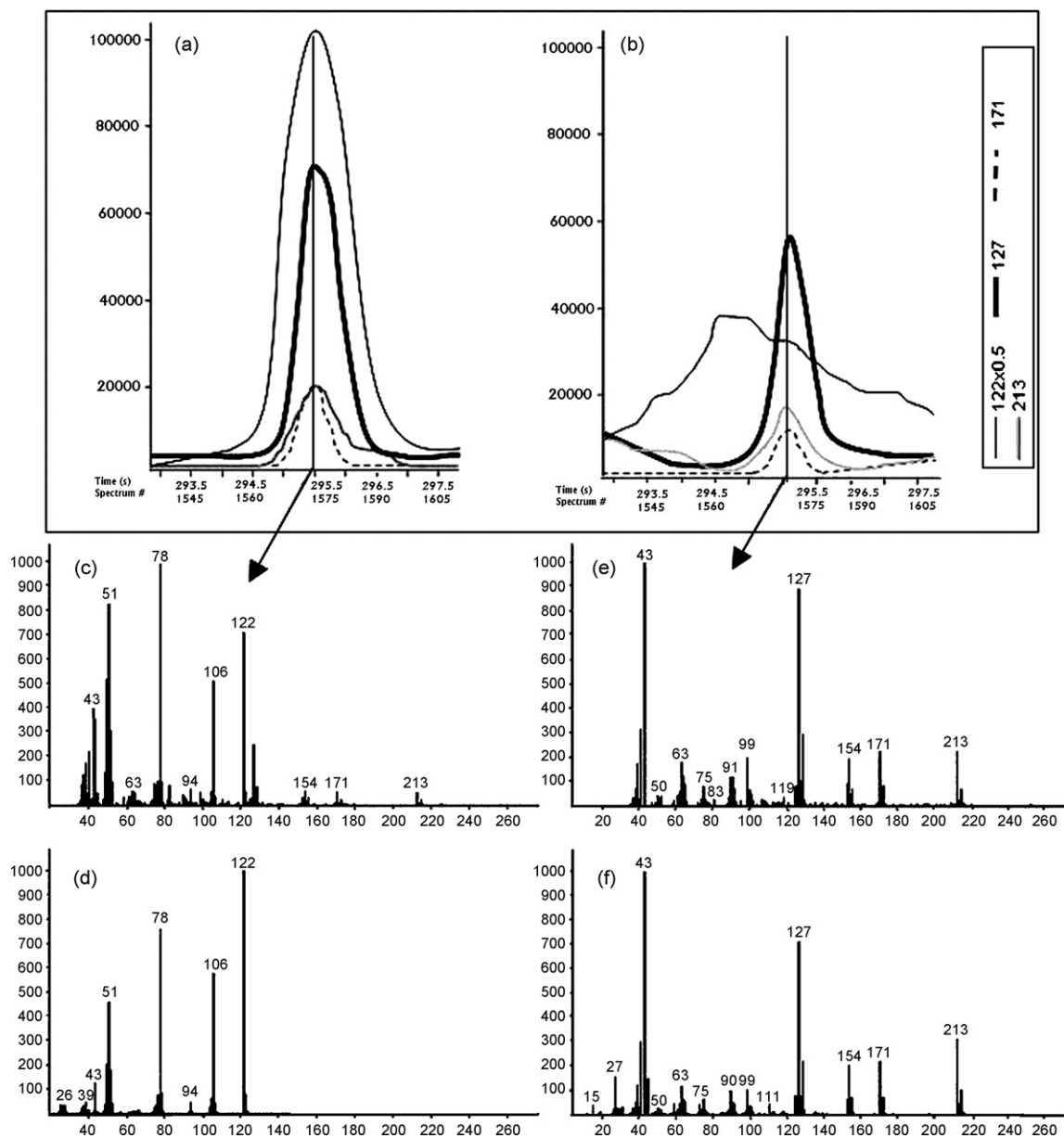


Fig. 4 – Potential of HS-SPME sampling vs. conventional sample preparation approach in detection of target analyte chlorpropham (retention time 295.5 s, GC/TOF MS analysis). (a) Ethyl acetate extract of spiked fruit tea ($50 \mu\text{g kg}^{-1}$). (b) Spiked fruit tea ($50 \mu\text{g kg}^{-1}$) sampled by HS-SPME. (c) Mass spectrum of matrix interference (nicotinamide). The deconvolution algorithm fails to identify chlorpropham. (d) NIST library spectrum of nicotinamide. (e) Mass spectrum of chlorpropham. (f) NIST library spectrum chlorpropham.

with the specified accuracy. According to the Document No. SANCO/10232/2006 [26], R.S.D. values not above 22% and 32% are required in the concentration ranges $0.1\text{--}0.01 \text{ mg kg}^{-1}$ and $0.01\text{--}0.001 \text{ mg kg}^{-1}$, respectively. In this context, the data in Table 3 demonstrates the potential of the HS-SPME method to detect and quantify most of the target pesticides in tea samples at low ppb levels (typically below $20 \mu\text{g kg}^{-1}$, lower than most MRLs required by EU regulations [23] for this particular matrix). The only exception was dieldrin (MRL = $20 \mu\text{g kg}^{-1}$) and endrine (MRL = $10 \mu\text{g kg}^{-1}$). Though they can be detected at this level, the requirements stated in the Commission Directive 97/57/EC [27] for $\text{LOQ} > 0.5 \times \text{MRL}$ are not met in this case.

The superiority of the HS-SPME procedure over conventional extraction strategies is clearly documented in Fig. 3. When analysing solvent extracts of spiked tea, we failed to detect four target pesticides (mevinphos, propham, carbaryl, malathion) in one-dimensional GC/TOF MS system, mainly due to high chemical noise (concentration level $50 \mu\text{g kg}^{-1}$). In spite of purification of the crude ethyl acetate extract by HPGPC, many interfering matrix components remained in the sample, including non-volatiles building up deposits in the injector and front part of the separation capillary. Most of these compounds are distinguished when using HS-SPME; hence, relatively simpler chromatograms are obtained.

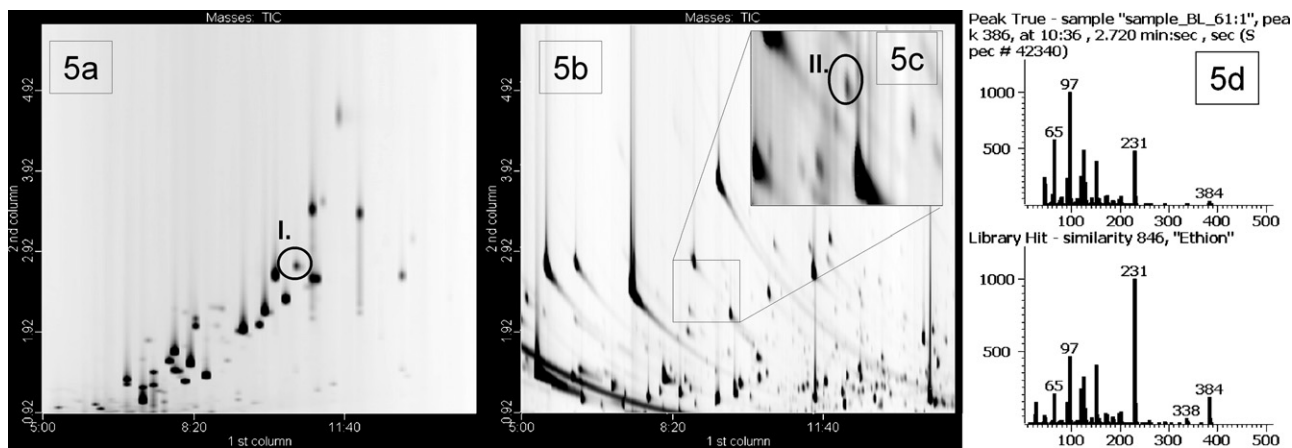


Fig. 5 – Identification of ethion in black tea sample (#61) by HS-SPME GC \times GC/TOF MS. (a) Solvent standard ($50 \mu\text{g kg}^{-1}$) contour plot with marked ethion (I). (b) Black tea sample contour plot (overview). (c) Zoomed in part of contour plot (black tea) with marked ethion (II). (d) Mass spectrum of ethion measured by GC \times GC/TOF MS in black tea sample. (e) NIST library spectrum of ethion.

In Fig. 4, an example of improved determination of chlorpyrifos in spiked fruit tea (concentration level $50 \mu\text{g kg}^{-1}$) is documented. While at an acquisition rate of 10 Hz the deconvolution of this pesticide was not achieved, a high “similarity match” for this analyte was obtained when using HS-SPME for sampling because of close coelution with abundant nicotine (matrix coextract). Under these conditions, better spectral resolution or enhanced chromatographic separation (as discussed in the previous chapter) are other conceivable options to solve such a problem [28]. It should be noted that neither the tandem nor high resolution TOF mass analyzer (HRTOF MS) are completely compatible with GC \times GC separation because of limited data acquisition speed. As only recently introduced, the HRTOF MS instrument can be employed for detection in GC \times GC [29], but broader peaks (0.2–1 s in the second dimension) are obtained by setting a less steep temperature programme to get a sufficient number of points per chromatographic peak [30].

3.4. Monitoring real life samples

The ability of the HS-SPME GC \times GC/TOF MS to determine pesticide residues in real life samples was tested by analyzing black, green and fruit teas obtained at a local market. It should be noted that the trace level target analysis as well as analysis of unknown sample components could be facilitated when working with TOF mass spectrometers due to their excellent confirmation power [31,32]. Of 67 examined samples, only two were positive, one containing chlorpyrifos ($n=5$, $6 \mu\text{g kg}^{-1}$, R.S.D. = 9%) and the other ethion ($n=5$, $14 \mu\text{g kg}^{-1}$, R.S.D. = 18%), both at levels below MRL. Residues were detected using a non-target search enabled by ChromaTOF software. As an example, the identification of ethion in black tea is documented in Fig. 5. To assess the accuracy of quantification of incurred residues, a conventional, accredited method employing ethyl acetate extraction followed by HPGPC was used for analysis of positive samples. The content of particular pesticide residues was estimated

($n=5$) for chlorpyrifos $5 \mu\text{g kg}^{-1}$ (R.S.D. = 9%) and for ethion $18 \mu\text{g kg}^{-1}$ (R.S.D. = 11%). These data document a relatively strong agreement between the results obtained by the new method with those generated by the conventional approach. Furthermore, the overall cost, labour intensity and time effectiveness (in spite of long extraction time) is fairly better for the HS-SPME procedure. The total time required for preparation and GC \times GC/TOF MS analysis (without data processing) of the set of samples consisting of 6 teas and 7 point calibration curve based on matrix matched standards was approximately 16 h for the method applying HS-SPME, approximately 1.6 times shorter than for the conventional method.

4. Conclusions

HS-SPME coupled with GC \times GC/TOF MS, when thoroughly optimized, can be considered a fast and easy alternative for screening semivolatile pesticide residues potentially occurring in black, green or fruit tea. In terms of sensitivity, the developed method showed detection limits at low $\mu\text{g kg}^{-1}$ levels. For 34 of the 36 tested pesticides, the developed method allowed control of the EU MRLs. The main bottleneck of SPME-based analysis was a narrow linearity range. In spite of that, especially for the purpose of negative sample exclusion within a routine control of large batches, this new procedure may replace the time and labour-intensive solvent-extraction based approach.

It has to be emphasized that satisfactory performance parameters could only be achieved when using GC \times GC for separation. 1D-GC did not allow the confirmation of some pesticides even at the MRL level.

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