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Short communication

Determination of pesticides in red wines with on-line coupled microporous membrane liquid–liquid extraction-gas chromatography

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Abstract

Microporous membrane liquid–liquid extraction (MMLLE) was coupled on-line with gas chromatography for the determination of pesticides in wine. The MMLLE-GC provided to be efficient and selective and the method was linear, repeatable and sensitive. The limits of detection ranged from 0.05 to 2.3 μg/l and the limits of quantification were 0.2–7.5 μg/l for all the analytes using FID as detector. With MS detection LODs in the range 0.03–0.4 and LOQs of 0.3–3.5 μg/l were achieved. The method was applied to the determination of pesticides in several red wines of different origin.

Keywords: Wine; Microporous membrane liquid–liquid extraction; Pesticides

1. Introduction

Pesticides are used on agricultural commodities such as grapes and wine grapes and as a result, a part of pesticides left on the grapes at harvest, particularly late-season fungicides, can be carried into the wine [1,2]. For pesticides in wine no uniform limits have been established yet, except for procymidone for which the European Union has established maximum residue limit (MRL) of 0.5 mg/kg [3]. There is, however, a worldwide trend towards setting specific, lower MRLs for pesticides in wine, which would range from 0.01 to 2 mg/kg for different pesticides [4,5].

Routine methods used in pesticide residue analysis are often time and solvent consuming due to the steps involved in sample preparation before chromatographic analysis. Generally, pesticide analyses are carried out by gas chromatography (GC) or liquid chromatography (LC) [5–25]. Major techniques for extraction and concentration of pesticides prior to the chromatographic separation in wine are liquid–liquid extraction (LLE) and solid-phase extraction (SPE) [2,6–13,16,20–24]. Other procedures, including solid-phase microextraction (SPME) and microporous membrane liquid–liquid extraction (MMLLE) have also been used [7,14–16,25–29].

Simplification and increasing automation of sample preparation steps are one of the modern trends in analytical chemistry. On-line combination of sample pretreatment and chromatographic analysis is attractive in this aspect, as the whole analysis can be carried out in a closed system, which can be easily automated. In this way, many of the problems associated with the traditional sample preparation approaches could be avoided. For liquid samples, the on-line methods developed involve RPLC, SPE, LLE and various membrane-based sample enrichment methods as sample pretreatment before the on-line analysis by LC or GC [24,30,25,31–35]. Among the various extraction techniques suitable for on-line coupling, SPE and membrane-based techniques are the most attractive also for routine analysis. Although the instrumentation for SPE-GC is rather complicated, automated systems have been developed. The main advantage of SPE-GC is the large sample capacity, and therefore, an efficient enrichment of the analytes. However, careful drying of the adsorbent is required before elution of the extract, as even small amounts of water are problematic in on-line combination with GC. The benefit of membrane-based extraction, particularly MMLLE, is that it allows to perform classical LLE in an automated way, and the extract can be directly transferred

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to a chromatographic instrument. The instrumentation is fairly simple and easy to use and the tuning of the selectivity of extraction is easy; by choosing of suitable solvent or solvent-mixture the selectivity can be optimized. The instrumentation required for on-line coupling of membrane-based methods with chromatography is simpler than, e.g. SPE-GC [25].

In our previous study, an off-line MMLLE-GC was developed for the analysis of pesticides in wine [26]. The aim of this study was to develop the system further to allow the whole analysis to be performed in a closed on-line system. Different quantitative parameters such as extraction efficiency, linearity, repeatability and limits of quantification were studied. The method was applied to the determination of pesticides in red wines of different origin.

2. Experimental

2.1. Reagents and solvents

All the solvents were of high-performance LC quality. Cyclohexane and toluene were from Lab Scan Analytical Sciences (Dublin, Ireland). Water was distilled and deionized. Pesticide standards included lindane, vinclozolin, quinalphos, procymidone, endosulfan sulfate and tetradifon and a pesticide standard mixture containing 20 pesticides (Pesticide Mix 16, AE-00030) and they were purchased from Accustandard (New Haven, USA). Two internal standards, diphenylamine (extraction standard) from Merck (Darmstadt, Germany) and 1,1′-binaphthyl (GC-MS standard) from Acros Organics (New Jersey, USA) were employed. Stock solutions (1 mg/ml) of pesticides were in isooctane or toluene and were diluted via isopropanol to water. A 10 μg/l solution of pesticides was prepared in deionized water.

2.2. Samples

Standard solutions were made in methanol. Further dilutions were made either in 95/5 water: methanol (v/v) or in diluted pesticide-free red wine.

Thirteen red wines of different origin were analyzed for pesticides. Organic wine was used as blank matrix, after confirmation that it did not contain any pesticides. Wine samples were diluted 1:3 with deionized water and internal standard (diphenylamine) was added, and the sample was filtered through 0.45 μm filters (Gelman Sciences, Ann Arbor, USA). Dilution of the samples was done because high concentrations of ethanol might affect the extraction.

2.3. Apparatus

The on-line MMLLE-GC apparatus is presented in more detail previously [25,26]. Shortly, the MMLLE unit was connected to a loop installed in a six-port valve of the Dualchrom 3000 Series on-line HPLC-HRGC apparatus (CE Instruments, Milan, Italy). The MMLLE unit consisted of two blocks of Teflon and PEEK with grooves of 11 μl volume in both blocks. A porous polypropylene membrane (Celgard 2400, Hoechst Celanese, Charlotte; NC, USA) was used (thickness 25.4 μm, pore dimensions 0.05 μm × 0.125 μm, porosity 0.4). The membrane was wetted with acceptor solvent by pumping the solvent through the acceptor channel and it was changed after every 100 extractions.

The GC was a Fisons Instruments Dualchrom 3000 Series containing a Phoenix 30CU pump. In the GC a 10 m × 0.53 mm i.d. DPTMDS (1,2-diphenyl-1,1,3,3-tetramethyldisilazane) deactivated retention gap (BGB Analytik AG, Zürich, Switzerland) was connected to a 20 m × 0.25 mm i.d. analytical column (HP-5) of 0.25 μm phase thickness (Agilent Technologies, USA) and to a solvent va-

Table 1
Repeatability of the MMLLE-GC system (as relative standard deviations, c = 25 μg/l, n = 4), the enrichment factor of MMLLE, linearity (in the range 7.5–100 μg/l) using either FID or MS (extracted ion trace) and limits of quantification (μg/l)

Compound	Repeatability (R.S.D.%)	MMLLE E_e	R (FID)	R (MS)	LOQ, FID (μg/l)	LOQ, MS (μg/l)
Aldicarb	9	8	0.9945	0.9935	5.0	2.0
Lindane	1	10	0.9912	0.9975	5.0	2.1
Simazine	18	2	0.9952	0.9983	7.5	1.5
Atrazine	7	12	0.9951	0.9978	2.5	1.5
Terbuthylazine	13	4	0.9966	0.9912	7.5	1.3
Metoxuron	20	3	0.9974	0.9916	1.4	1.0
Metobromuron	22	1	0.9720	0.9934	6.3	1.0
Vinclozolin	7	11	0.9900	0.9866	1.6	1.0
Isoproturon	1	14	0.5036	0.9992	1.8	3.5
Chlortoluron	14	8	0.8841	0.9719	4.3	1.0
Cyanazin	15	10	0.9775	0.9842	0.2	1.0
Endosulfan I	16	4	0.9964	0.9773	7.5	0.3
Quinalphos	13	4	0.9960	0.9737	1.7	0.3
Procymidone	2	6	0.9962	0.9779	7.4	0.5
Endosulfan II	13	17	0.9916	0.9779	4.8	0.5
Endosulfan sulfate	23	8	0.9841	0.9732	2.8	0.5
Tetradifon	18	5	0.9971	0.9797	2.0	0.5

por exit (SVE) via a glass pressfit Y-piece. The detection was made either by a flame ionization detector (FID) at 300 °C or by a quadrupole MS (Automass Solo, Thermoquest, Argenteuil Cedex, France). The carrier gas was helium at 150 kPa. The oven was programmed from 85 °C (8 min) to 150 °C (2 min) at 40 °C/min and then to 300 °C (10 min) at 5 °C/min. In MS, the electron ionization at 70 eV was applied and fragmented ions were monitored with total ion current (TIC) from 50 to 500 amu.

The sample was extracted for 40 min with the flow-rate of 0.2 ml/min (donor feed). After extraction, the donor flow was stopped and the acceptor phase was eluted with toluene to a loop in the GC transfer valve. The sample was injected from the loop to the GC for analysis using a flow rate of 0.2 ml/min for 1 min 10 s to ensure the transfer of the whole extract and to flush the sample loop with fresh solvent. SVE was kept open 35 s after the transfer was completed. After the transfer, the donor and acceptor channels were flushed with water and toluene for 10 min, respectively. The next extraction could be started while the GC analysis was progressing.

3. Results and discussion

Development of the MMLLE-GC method was based on our previous off-line study, in which MMLLE using cyclohexane as extraction solvent was used prior the GC determination of six pesticides in wine [26]. In addition, in a further study, on-line coupled MMLLE-GC-FID was developed for the determination of organic pollutants, including PAHs and pesticides, in aqueous samples [25]. In this study, the construction of the on-line system was slightly different than what was used in the study of organic pollutants in aqueous samples [25]. The main difference was the membrane extraction unit. To minimize the coextraction of matrix components from wine, a small MMLLE unit was chosen. For the extraction, both cyclohexane and toluene were studied as extraction solvents for 18 pesticides spiked in red wine, as these solvents have been found suitable for LLE of pesticides [25,26]. Best results were obtained with toluene, and accordingly this solvent was chosen for further studies.

The extraction time was studied in the range of 10–50 min with a flow rate of 0.2 ml/min, which was found to be the best flow rate in the previous study [26]. Increasing the extraction time from 10 to 40 min enhanced the recovery of the analytes on average four fold. After 40 min extraction, leveling out occurred, and the recoveries of some analytes (quinalphos and tetradifon) even decreased slightly (11–22%) with 50 min extraction time, probably due to back-extraction to aqueous phase. A similar trend has been observed also in other studies with MMLLE [25,26,28]. Thus, extraction time of 40 min was chosen.

The performance of the MMLLE system can best be evaluated by means of the enrichment factor (E_e), the calculated ratio of the analyte concentration in the acceptor solvent and in the sample. Under optimized conditions, the enrichment

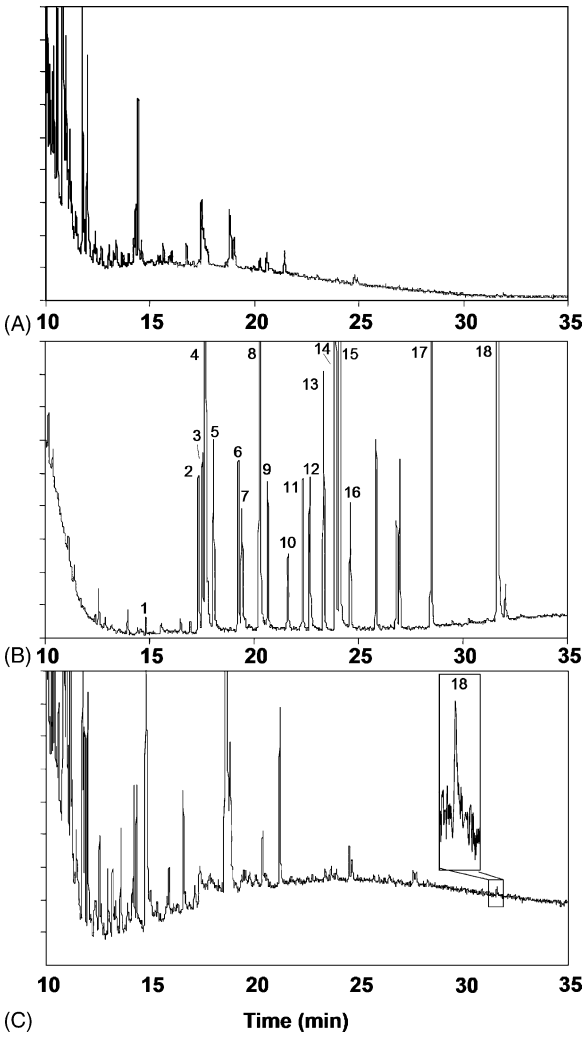

Fig. 1. MMLLE-GC-FID determination of (A) a blank wine, (B) a MMLLE extract of a spiked red wine sample (c = 0.05 mg/l) and (C) a MMLLE extract of an Italian red wine containing tetradifon. Peak identification: 1, aldicarb; 2, diphenylamine (ISTD); 3, simazine; 4, atrazine; 5, lindane; 6, terbuthylazine; 7, metoxuron; 8, metobromuron; 9, vinclozolin; 10, isoproturon; 11, chlortoluron; 12, metazachlor; 13, quinalphos; 14, procymidone; 15, endosulfan I; 16, endosulfan II; 17, endosulfan sulfate; 18, tetradifon.

factors were determined (Table 1) and they were in the range 1.2 to 17.2. Even for the analytes with the lowest enrichment factors, the repeatability of the extraction was good, as can be seen in Table 1. Furthermore, the extract was very clean, and only a few extra peaks from the wine matrix could be observed in the chromatogram (Fig. 1A). To study the possible memory effects, a blank extraction was made after extraction of a spiked sample. The blank extract gave a clean background and no traces of the pesticides were detected. The stability of the membrane was also good and the same membrane could be used for several weeks (on average 100 samples).

After the extraction conditions were chosen, the injection conditions to GC were optimized and the linearity, sensitivity and repeatability of the method were determined (Table 1). The linearity of the method was excellent for most analytes.

For two analytes, namely isoproturon and chlortoluron, the linearity was unsatisfactory. This was due to coelution of matrix components with these two compounds, and by using FID for the detection, the coelution disturbed the analysis of these analytes. With MS detection the linearity was good also for these analytes (see Table 1). The sensitivity of the method was also very good. The limits of quantification were below 7.5 μg/l for all the analytes, using FID as the detector. With MS detection and using total ion current the sensitivity ranged from 0.3 to 3.5 μg/l. The repeatability of the method was on average 14% for the peak areas (c = 25 μg/l).

Thirteen red wines from different origin were analyzed for pesticides. Tetradifon was found in one Italian wine (Fig. 1C). The concentration of tetradifon with FID as detector was 11 ± 2 μg/l and with MS 10 ± 2 μg/l, using extracted ion trace in quantitation (m/z 356).

Compared to the previous off-line MMLLE method, in which only six pesticides were analyzed from wine, the developed on-line system gave on average 2–13 times better sensitivity. Moreover, the LOQs obtained with the developed MMLLE-GC-MS method were comparable or better than those obtained with other sample pretreatment methods, such as LLE, SPE or SPME [6–16]. In comparison with LLE and SPE, the main advantage of the MMLLE-GC system was that the whole analysis could be performed in a closed on-line system without manual sample pretreatment, and even though the recoveries in MMLLE are lower than in LLE or SPE, the sensitivity was on the same level, event when using TIC mode in MS. Using SPME for sample pretreatment in wine analysis the LOQs for pesticides have been ranging from 0.1 to 109 μg/l [14–16] being typically higher than LOQs obtained in this study. The recoveries in SPME and MMLLE are in the same level, however, in SPME the equilibrium is typically not reached in a reasonable extraction time (>60 min) for these analytes, thus the method is more susceptible to errors than is the MMLLE [14–16].

4. Conclusions

The MMLLE-GC provided efficient, selective and repeatable on-line analysis of pesticides in wines. Due to the selective extraction, the extract was clean, which enabled on-line coupling with GC. The construction and operation of the apparatus are straightforward: the MMLLE can be easily connected to GC and the sample can be extracted while the previous sample is being analyzed. The LOQs obtained with this method meet the requirements for MLRs suggested for the pesticides in wine.

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