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Rapid micro liquid–liquid extraction method for trace analysis of organic contaminants in drinking water

Andreas Zapf, Regine Heyer, Hans-Jürgen Stan*

Institute of Food Chemistry, Technical University of Berlin, Gustav-Meyer-Allee 25, 13355 Berlin, Germany

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Abstract

The applicability and performance of a micro liquid–liquid extraction method for trace analysis of organic compounds in drinking water is reported. Tap water samples of 400 ml are saturated with sodium chloride and extracted once with 500 μl of toluene. Extracts are analyzed directly without further treatment by gas chromatography using simultaneous electron-capture and nitrogen–phosphorus detection. Recoveries of 82 organic compounds, including organochlorine and organophosphorus insecticides, triazine and acetanilide pesticides, chlorinated anilines and phenols from tap water samples spiked at 50 to 500 ng/l were determined and relative standard deviations were calculated. For 68 compounds the recoveries were higher than 50%. The mean relative standard deviations at spiking levels of 50, 100 and 500 ng/l were 7.9, 6.6 and 5.2%, respectively. The extraction method proved to be rapid, simple and inexpensive. In most cases compounds were reproducibly detected well below the European Union maximum tolerance level for pesticide residues in drinking water of 100 ng/l.

1. Introduction

In recent years, many man-made organic compounds, mainly pesticides, have been found as contaminants in ground and surface water. Maximum tolerance levels were set as in the European Union (EU) guideline for drinking water [1], which establishes a maximum tolerance level for individual pesticides of 100 ng/l. This so-called "zero tolerance" level represents the performance standard of current trace analysis methods as well as being a benchmark for new procedures.

Trace analysis of organic compounds in water is carried out by several methods. The traditional liquid–liquid extraction with large volumes of organic solvent followed by clean up and concentration steps is known as an effective procedure regarding accuracy and sensitivity. However, interferences from solvents used, their toxicological relevance and possible cross-contaminations favoured the development of solid-phase extraction, which is relatively simple in use and needs only small amounts of solvents. But even in this case, interferences from solid-phase materials may occur [2].

As a simple and inexpensive but exact and sensitive method, micro liquid–liquid extraction (mLLE) combined with gas chromatography (GC) has been successfully applied to analysis of organic compounds in water [3–6]. In a field study, we determined alachlor and two degradation products in ground water saturated with sodium chloride by extracting 400-ml samples

* Corresponding author.

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with 500 μl of toluene. The extracts were analyzed without further treatment with GC using nitrogen–phosphorus detection (NPD) and mass-selective detection [7]. A solid-phase extraction procedure first applied was lengthy and not suitable for the analysis of the volatile metabolite 2,6-diethylaniline [7,8]. Since results of the micro liquid–liquid extraction were found to be satisfactory with respect to speed, accuracy and sensitivity, the applicability of this extraction method to the analysis of other environmental contaminants usually dealt with in our laboratory was tested. Substances from important pesticide classes were selected, including organochlorine, organophosphorus, triazine and acetanilide pesticides together with a few degradation products. Chlorinated phenols and anilines which represent common water pollutants from industry as well as metabolites of phenyl urea, acylanilide and carbamate pesticides were also investigated. To obtain an overview of the applicability of mLLE to the investigated contaminant classes, only those substances were chosen which could be determined by GC with electron-capture detection (ECD) and/or NPD without the need of derivatization or mass-selective detection techniques.

2. Procedure

2.1. Materials

Standard substances were of analytical purity, purchased from Promochem (Wesel, Germany), or of Pestanal quality, from Riedel de Haën (Seelze, Germany). Sample vials, screw caps and septa were purchased from Zinsser (Frankfurt, Germany) and 200-μl inserts for the sample vials were obtained from CS-Chromatographie Service (Langerwehe, Germany). Narrow-necked bottles used for extraction were obtained from H. Jürgens & Co. (Bremen, Germany) and PTFE-faced seals were purchased from Schott (Mainz, Germany).

Stock solutions of all compounds were prepared in toluene or methanol. Standards and samples were finally dissolved in toluene. All solvents were Pestanal products from Riedel de Haën. Sodium chloride, trisodium citrate dihydrate and citric acid were purchased from Merck (Darmstadt, Germany).

As a buffering mixture trisodium citrate dihydrate–citric acid monohydrate (40:1, w/w) (pH 6.5–7.0) was used.

2.2. Instrumentation

Shaker

A KL2 shaker from Edmund Bühler (Bodelshausen, Germany) was used.

GC–ECD and GC–NPD

An HP 5890 gas chromatograph with electron-capture and nitrogen–phosphorus detectors, an HP 7673 autosampler and a split–splitless injector for capillary columns was employed. Nelson analytical software 2600, V 4.1 was used for data acquisition.

A fused-silica column (25 m × 0.32 mm I.D.), coated with SE-54 material with a film thickness of 0.17 μm, was used with helium as carrier gas. The effluent was split into equal parts by means of a Y press fit connector. The temperatures of the injection port, the electron-capture detector and the nitrogen–phosphorus detector were set at 210, 300 and 280°C, respectively. The column temperature program was started with 1 min at 100°C, increased at 30°C/min to 150°C, held for 2 min, then increased at 3°C/min to 205°C and at 10°C/min to 260°C, held at 260°C for 20 min. A 2-μl volume of sample was injected with the autosampler using hot splitless injection with the split closed for 1 min.

2.3. Micro liquid–liquid extraction

A 400-ml tap water sample in a 500-ml narrow-necked bottle was saturated with 150 g NaCl and buffered to pH 6.5–7.0 by addition of 6 g of the buffering mixture. The water sample was spiked with analyte mixtures in 100 μl methanol to achieve concentrations of 50, 100 and 500 ng/l. After addition of 500 μl toluene, the bottle was sealed and shaken for 20 min at 420 rpm. Screw caps with PTFE-faced silicone rubber

seals were used. After phase separation, the solvent layer was brought up to the bottleneck by addition of a saturated NaCl solution using a Pasteur pipette connected to a separating funnel as demonstrated in Fig. 1. About 150 μl of the toluene phase were transferred into sample vials of 200 μl volume by means of another Pasteur pipette. 2 μl were injected into the GC–ECD/NPD system.

2.4. Calibration

Extraction rates were determined by external standard calibration. Concentrations of standard solutions were 25, 50, 100, 250 and 500 pg/μl in toluene. Calibration curves were calculated by linear least-squares regression using peak areas.

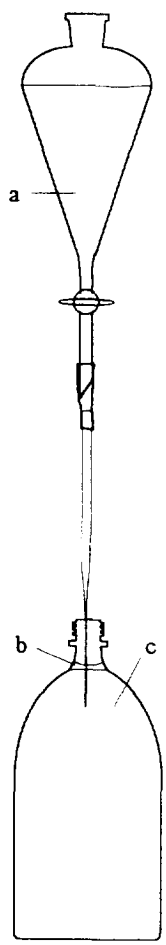

Fig. 1. Simple equipment to bring the toluene phase up to the bottle neck. a = Separating funnel filled with saturated NaCl solution; b = solvent layer; c = sample.

3. Results and discussion

Recovery experiments with a variety of pesticides and other environmental pollutants from spiked tap water samples were carried out at concentration levels of 50, 100 and 500 ng/l. At each concentration level, five analyses were performed.

Illustrating the results compiled in Table 1, chromatograms obtained from extracts of samples containing 100 ng/l of each compound are shown in Figs. 2–5. This concentration level represents the EU guideline maximum tolerance level for pesticides in drinking water.

Best peak sizes were obtained from analytes exhibiting high ECD response factors. All chlorinated pesticides investigated (Fig. 2), a number of organophosphorus pesticides (Fig. 3), the chlorophenols with the exception of the relatively low chlorinated 2,4-dichlorophenol (Fig. 5), the higher chlorinated anilines and 4-chloro-2-nitroaniline produced peak sizes indicating that these compounds should be easily detectable at concentrations well below the 100 ng/l level shown in the chromatograms.

NPD signals of all organophosphorus pesticides except the last eluting compound dialifos and most of the nitrogen-containing pesticides were found to be high enough for reliable screening at the 100 ng/l level (Figs. 3 and 4). According to their low nitrogen content, the investigated anilines exhibit low NPD response factors. However, peak heights were clearly above a signal-to-noise ratio of 3 which is commonly set as the detection limit, thus enabling their detection at the 100 ng/l concentration level in screening analyses.

Many of the analytes investigated show sufficient response to both detectors used enabling their identification by the presence of peaks in both detector traces at the same retention time and with the expected response ratio. These compounds include the majority of the organo-

Table 1
Recovery of various organic compounds from tap water samples spiked with 50, 100 and 500 ng/l

Compound	50 ng/l		100 ng/l		500 ng/l		Det.
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	
Chlorinated insecticides							
Aldrin	46	8.0	52	11	61	7.6	ECD
Chlorfenson	61	13	67	6.8	80	13	ECD
o,p-DDD	66	3.5	71	1.8	71	3.4	ECD
o,p-DDE	53	17	62	3.4	71	3.6	ECD
p,p-DDT	68	3.7	69	2.0	76	4.7	ECD
Dichlobenil	70	6.1	70	3.5	65	4.5	NPD
Dieldrin	66	4.1	68	2.1	70	6.3	ECD
α-HCH	70	1.4	66	3.3	68	3.5	ECD
β-HCH	50	8.0	61	3.6	67	2.4	ECD
Heptachlor	70	10	69	9.5	73	5.1	ECD
Heptachlor epoxide, *trans*	61	6.8	66	2.9	70	3.3	ECD
Hexachlorobenzene	35	12	52	12	64	7.6	ECD
Lindane	65	4.3	62	3.2	65	2.4	ECD
Methoxychlor	80	14	81	4.0	81	3.7	ECD
Mirex	49	18	64	2.0	66	3.4	ECD
Pentachlorobenzene	+		41	14	56	11	ECD
Quintozene	60	4.2	62	3.6	64	4.7	ECD
Triazine and acetanilide herbicides							
Ametryn	76	3.9	73	3.2	74	2.9	NPD
Atraton	70	4.3	62	2.4	61	3.6	NPD
Atrazine	67	2.1	64	5.3	64	3.4	NPD
Desethylatrazine	n.d.		n.d.		n.d.		NPD
Desisopropylatrazine	n.d.		n.d.		n.d.		NPD
Desmetryn	105	19	79	14	74	2.9	NPD
Metazachlor	79	1.5	75	2.1	71	3.4	NPD
Methoprotryn	93	4.0	84	2.7	82	3.3	NPD
Metolachlor	73	10.2	68	4.3	69	2.3	NPD
Metribuzin	53	6.2	41	19	41	3.5	NPD
Prometon	76	5.1	72	3.1	73	3.4	NPD
Prometryn	74	2.6	72	2.6	72	2.9	NPD
Propazine	72	4.9	72	6.5	72	3.2	NPD
Sebuthylazine	71	4.2	73	8.5	69	3.4	NPD
Simazine	49	3.5	35	4.0	30	4.1	NPD
Terbutryn	76	3.0	73	2.0	72	2.9	NPD
Terbutylazine	76	12	72	5.5	70	3.5	NPD
Triadimefon	89	3.8	83	1.8	77	2.5	NPD
Organophosphorus insecticides							
Acephate	n.d.		n.d.		n.d.		NPD
Azinphos-ethyl	112	8.7	114	13	116	8.3	ECD
Bromophos-ethyl	69	5.3	66	10	76	7.7	NPD
Bromophos-methyl	70	6.4	64	10	74	8.4	NPD
Chlorpyrifos-methyl	89	4.7	77	8.7	82	9.6	NPD
Chlorthion	90	2.7	84	3.3	75	3.2	NPD
Demeton-S-methyl	85	3.1	63	3.3	44	5.0	NPD
Demeton-S-methyl sulfone	81	9.8	71	9.7	77	13	NPD
Dialifos	64	9.6	64	11	74	6.8	ECD

Table 1 *(Continued)*

Compound	50 ng/l		100 ng/l		500 ng/l		Det.
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	
Diazinon	76	7.9	64	12	70	9.4	NPD
Chlorfenvinphos	104	6.7	98	12	107	6.2	ECD
Dichlofenthion	73	4.7	70	5.0	62	4.5	NPD
Dichlorvos	47	2.9	38	1.6	34	4.5	ECD
Dicrotophos	97	2.4	72	6.6	56	4.1	NPD
Ditalimfos	92	18	65	6.1	69	5.5	NPD
Ethoprophos	62	5.8	63	8.9	68	4.3	ECD
Etrimfos	73	11	58	8.4	71	11	NPD
Isofenphos	69	9.4	78	5.8	76	4.2	ECD
Malathion	83	4.9	74	11	82	12	NPD
Methamidophos	n.d.		n.d.		n.d.		ECD/NPD
Parathion-ethyl	55	10	72	12	77	4.9	ECD
Parathion-methyl	65	10	85	18	83	5.4	ECD
Phosalone	104	7.7	105	14	111	8.7	ECD
Tetrachlorvinphos	126	2.5	112	11	111	7.8	ECD
Tolclofos-methyl	78	10	72	4.8	70	3.2	ECD
Anilines							
2-Chloroaniline	46	6.0	40	4.5	39	5.0	NPD
3-Chloroaniline	47	9.9	36	3.4	31	4.4	NPD
4-Chloro-2-nitroaniline	42	6.0	46	2.5	51	5.7	ECD
2,3-Dichloroaniline	77	7.8	65	3.5	64	4.9	NPD
2,4-Dichloroaniline	44	21	60	9.0	66	6.6	NPD
2,6-Dichloroaniline	96	8.9	76	11	68	4.9	NPD
3,4-Dichloroaniline	66	15	65	12	66	7.8	NPD
3,5-Dichloroaniline	82	11	68	7.7	69	4.1	NPD
2,6-Diethylaniline	59	12	73	4.0	70	2.8	NPD
2,6-Dimethylaniline	52	3.1	47	4.7	46	5.8	NPD
2,4-Dinitroaniline	n.d.		n.d.		n.d.		ECD/NPD
3-Nitroaniline	n.d.		n.d.		n.d.		ECD/NPD
2,3,4,5-Tetrachloroaniline	68	2.2	69	3.1	63	2.8	ECD
2,3,5,6-Tetrachloroaniline	56	6.5	67	12	75	4.0	NPD
3,4,5-Trichloroaniline	74	10	71	3.8	76	6.1	ECD
Phenols							
2,4-Dichlorophenol	59	32	55	10	54	7.3	ECD
2-Nitrophenol	n.d.		n.d.		n.d.		ECD/NPD
Pentachlorophenol	85	5.4	75	5.3	66	6.2	ECD
2,3,4,5-Tetrachlorophenol	92	8.6	88	4.8	90	3.6	ECD
2,3,5-Trichlorophenol	72	10	71	5.0	74	4.1	ECD
2,3,6-Trichlorophenol	71	8.9	70	4.6	75	4.3	ECD
2,4,5-Trichlorophenol	70	11	71	4.8	77	4.4	ECD

Mean recoveries and relative standard deviations (R.S.D.s) given in percent. Det. = Detection method used for quantification; n.d. = not detected after extraction; + = detected, but below limit of determination.

phosphorus pesticides investigated, the acetanilide herbicides metolachlor and metazachlor, the triazine herbicide metribuzin and the azol fungicide triadimefon as can be seen in Figs. 3 and 4.

Contaminants of tap water responding to ECD

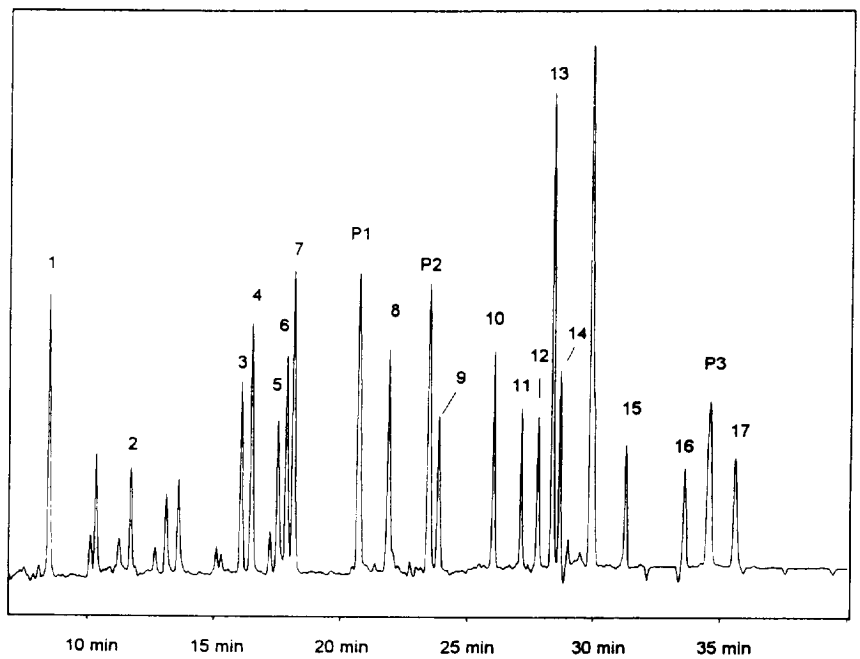

Fig. 2. GC–ECD of a mLLE extract of a tap water sample spiked with chlorinated insecticides (100 ng/l of each compound). Peaks: 1 = dichlobenil; 2 = pentachlorobenzene; 3 = α-HCH; 4 = hexachlorobenzene; 5 = β-HCH; 6 = lindane; 7 = quintozene; 8 = heptachlor; 9 = aldrin; 10 = heptachlor epoxide (*trans*-); 11 = *o,p*-DDE; 12 = chlorfenson; 13 = dieldrin; 14 = *o,p*-DDD; 15 = *p,p*-DDT; 16 = methoxychlor; 17 = mirex; P1 = dibutylphthalate; P2 = diisobutylphthalate; P3 = di-2-ethylhexylphthalate.

and NPD were found in all extracts. Three peaks in the ECD trace were identified as phthalate plasticizers, namely dibutyl, diisobutyl and di-2-ethylhexyl phthalates (P1, P2, P3). One large peak in the NPD trace was found to be tributyl phosphate (T). The identity of these contaminants was confirmed by GC–MS.

3.1. Recovery at three concentration levels

mLLE as described works with an extreme ratio of extracting solvent and extracted liquid of 1:800 and relies on one single extraction step. Only analytes with a very favourable partition ratio can be expected to be extracted sufficiently. On the other hand the simplicity of the procedure could compensate for lower recoveries if these are reproducible. To achieve high reproducibility, extraction was carried out using a mechanical mixing device. Samples were citrate buffered and saturated with NaCl, this ensuring a reproducible near-neutral pH and constant high ionic strength of the aqueous phase. NaCl saturation of the sample proved to be necessary not only to produce a salting out effect shifting the partition ratio of most analytes in favour of the organic solvent, but to minimize solubility of toluene in water and allow sufficient recovery of the small solvent amount applied. Solvent recoveries were estimated by removing the solvent layer completely by means of a scaled 0.5-ml pipette. A recovery of 325 to 350 μl or 65–70% was found as the range of 10 experiments with a mean of 68%. This demonstrates the ruggedness of the method.

The results of the recovery experiments of 82 target compounds at three concentration levels, namely at 50, 100 and 500 ng/l are compiled in Table 1. At each concentration level, five determinations were carried out from which the standard deviations were calculated. Out of the 82 target compounds, 68 were recovered in the range of 50 to 115% with a single extraction step. From the pesticides, only the two polar

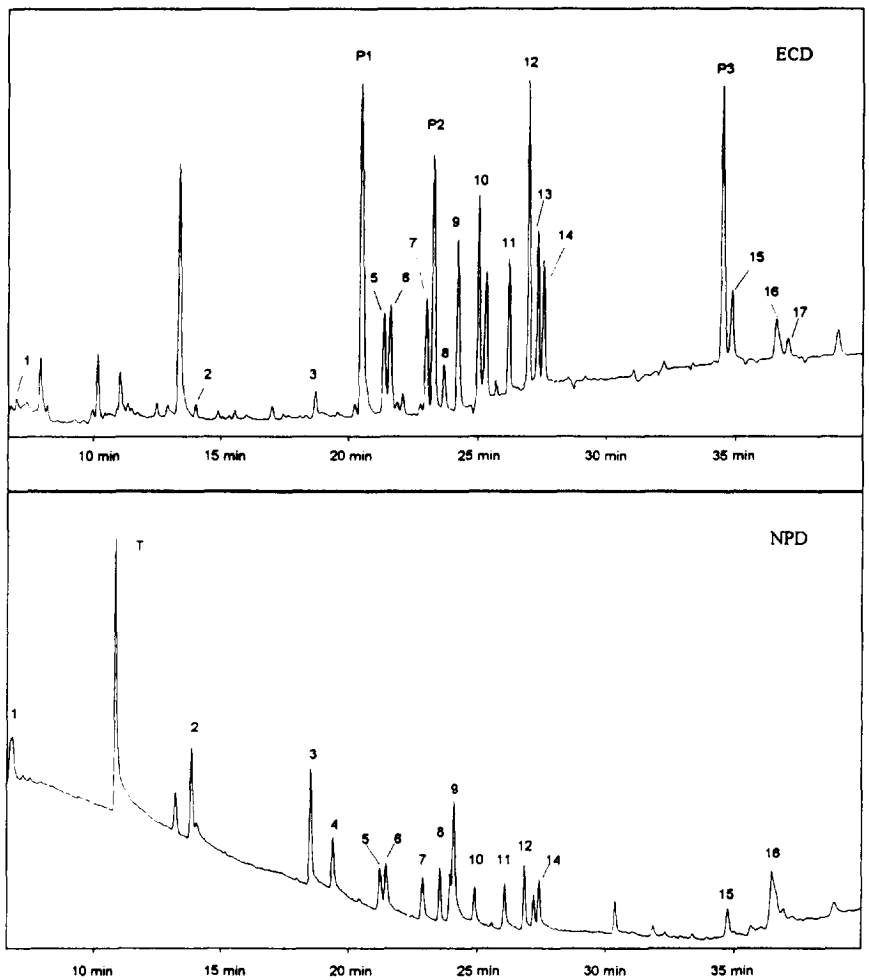

Fig. 3. GC–ECD and GC–NPD of a mLLE extract of a tap water sample spiked with organophosphorus pesticides (100 ng/l of each compound). Peaks: 1 = dichlorvos; 2 = ethoprophos; 3 = diazinon; 4 = etrimfos; 5 = parathion-methyl; 6 = tolclofos-methyl; 7 = demeton-S-methyl sulfone; 8 = malathion; 9 = parathion-ethyl; 10 = bromophos-methyl; 11 = chlorfenvinphos; 12 = bromophos-ethyl; 13 = tetrachlorvinphos; 14 = ditalimfos; 15 = phosalone; 16 = azinphos-ethyl; 17 = dialifos; T = tributylphosphate; P1, P2, P3 as in Fig. 2.

organophosphates with good water solubility, acephate and methamidophos, could not be recovered. The same holds true for the two nitroanilines, 2-nitrophenol and the two desalkyl triazines which both are known to give low recoveries in many extraction procedures. Lower recoveries than 50% were observed with demeton-S-methyl, dichlorvos, metribuzine and simazine as well as some anilines. All recoveries for the target compounds were calculated with the mean recovery of the solvent of 68% mentioned above.

Summarizing, most of the pesticides and pollutants under investigation were found to be recovered to an extent that makes their detection in drinking water samples possible at the 100 ng/l concentration level and below applying GC with simultaneous parallel detection by ECD and NPD for screening analysis. In addition, the injected amount of target analytes of 100 pg or

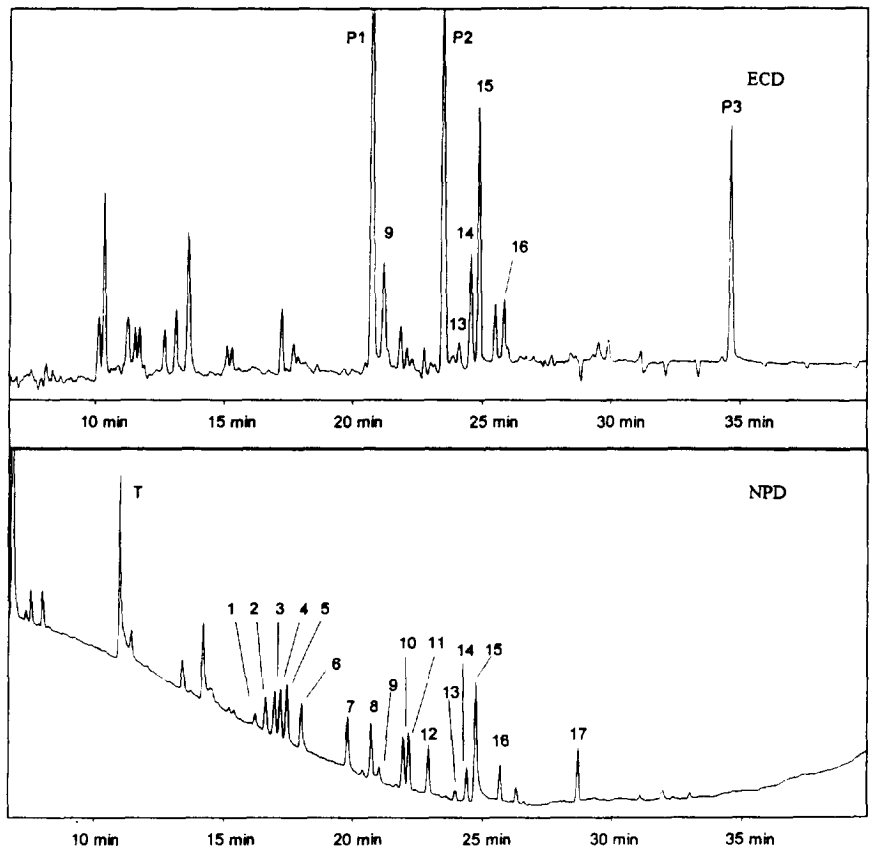

Fig. 4. GC–ECD and GC–NPD of a mLLE extract of a tap water sample spiked with triazines and other nitrogen-containing pesticides (100 ng/l of each compound). Peaks: 1 = simazine; 2 = atraton; 3 = prometon; 4 = atrazine; 5 = propazin; 6 = terbuthylazin; 7 = sebuthylazin; 8 = desmetryn; 9 = metribuzin; 10 = ametryn; 11 = prometryn; 12 = terbutryn; 13 = metolachlor; 14 = triadimefon; 15 = chlorthion; 16 = metazachlor; 17 = methoprotryn; T as in Fig. 3; P1, P2, P3 as in Fig. 2.

more is sufficient for confirmatory analysis by GC–MS in the selected ion monitoring (SIM) mode.

The reproducibility of an analytical procedure usually is characterized by standard deviations. In the analytical method described, these standard deviations reflect not only the variation of the extraction process but also the reproducibility of the GC separation and the detector response. Many target compounds can be determined with both detectors, the peak areas with the lower standard deviations are reported together with the detector trace used in Table 1. Most of the standard deviations reported in the table are clear below 10% at all three concentration levels. The mean of all standard deviations of all target compounds was calculated at the spike level of 50 ng/l with 7.9%, 100 ng/l with 6.6% and 500 ng/l with 5.2%.

Summarizing the recovery data the mLLE method described can be considered as a simple and reliable method for the screening of drinking water samples for a variety of pesticide residues at the concentration level necessary to meet the requirement of the drinking water guidelines of the EU. The reliability of the results will certainly be enhanced by combination with more selective determination methods such as capillary GC with atomic emission detection or mass spectrometric detection. When analyzing a limited

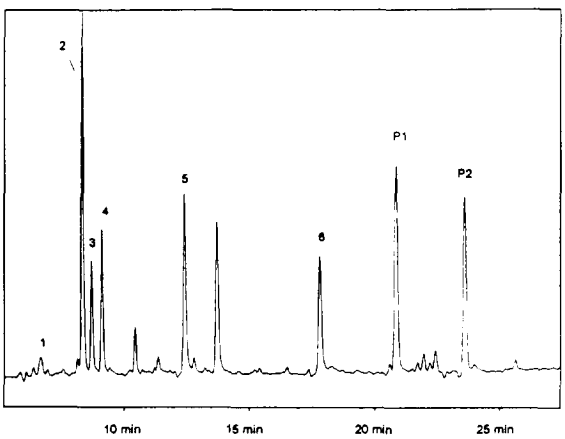

Fig. 5. GC–ECD of a mLLE extract of a tap water sample spiked with chlorinated phenols (100 ng/l of each compound). Peaks: 1 = 2,4-dichlorophenol; 2 = 2,3,5-trichlorophenol; 3 = 2,4,5-trichlorophenol; 4 = 2,3,6-trichlorophenol; 5 = 2,3,4,5-tetrachlorophenol; 6 = pentachlorophenol; P1, P2 as in Fig. 2.

number of related target compounds, for example triazine herbicides, the addition of a suitable surrogate standard will further add to the reliability of quantitative results. Furthermore, such a surrogate standard is an important indicator of the performance of the whole method, when applied to numerous different matrices.

4. Conclusions and outlook

The method presented is found to be suitable for the analysis of a wide range of organic trace compounds in drinking water. It can be easily performed in any GC laboratory with basic equipment and low manpower. Interferences from large solvent volumes or solid phase extraction materials are avoided. Results obtained with a special application of this method to ground water in a field study on leaching of alachlor and its metabolites [7] indicate that the method should be suitable also for this matrix. During this study, one analyst could process up to 50 ground water samples per day. A modification of the method was successfully applied in another field study on leaching of the phenoxycarbonic acid mecoprop and its metabolite 2-methyl-4-chlorophenol. The analytes were extracted from acidified ground water samples and determined by GC after pentafluorobenzylation [9]. An extension of the latter procedure to the determination of further acidic herbicides has been completed [10].

Modifications e.g. of pH or solvent composition may improve results for still poorly extracted compounds and make the procedure applicable to further substance classes.

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