Original Research

Screening of Alkanocarboxylic and Phenolic Herbicides in Water Samples by Means of Derivatization-Based Gas Chromatography

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Abstract

This work presents a method of screening of alkanophenoxy carboxylic (2,4-D, 2,4,5-T, MCPA, MCPP) and phenolic (dinoterb, dinoseb, pentachlorophenol) herbicides in water. The successive steps of the method, i.e. liquid-liquid extraction with ethyl acetate and methyl-tert-butyl ether and solid phase extraction using C18-modified silica gel and styrenedivinylbenzene copolymer-packed cartridges as well as an ininjection port derivatization with trimethyl phenylammonium hydroxide, were tested. The conditions of GC-FID and GC-MS analysis were optimized. The method developed was applied to determine selected herbicides in surface waters in the Gdańsk region.

Introduction

Acidic herbicides can be hazardous to the environment even when present at low concentrations, mainly due to high physiological activity and some toxicity. Herbicides 2,4-D and 2,4,5-T exhibit cytotoxicity, can damage DNA structure, hinder peptide synthesis, and are potential carcinogens and mutagens [1]. Due to relatively good water solubility they can be found in different parts of the environment once applied in any environment. The application of chloroorganic pesticides, including some herbicides, has been forbidden due to harmful environmental effects. However, those forbidden herbicides can also be found in some environments. Selected water environments should be regularly monitored and some should be checked occasionally for herbicide residue. In the latter case the method, not necessarily very accurate, which will per-

mit herbicide determination in a fast and simple way or screening would be very attractive.

Due to matrix complexity, low concentration and chemical and physical properties of carboxylic and phenolic herbicide's selective, sensitive and high-resolution techniques, mainly chromatography most often gas chromatography, should be applied [2, 3,4]. A necessary step of analysis is appropriate preparation of aqueous samples before the analysis proper; this is generally based on exchange of aqueous matrix for organic solvent, increase in analytes concentration and their conversion to GC analyzable derivatives.

Simple, convenient and well-recognized techniques of isolation and enrichment of organic components from aqueous samples are liquid-liquid extraction (LLE) and solid phase extraction (SPE) [5, 6, 7, 8].

LLE is simple, universal and does not need sophisticated apparatus and can give high recoveries. However, it is unfriendly to the environment because of the use of high amounts of solvents unless non-harmful solvents are used.

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In SPE only small amounts of organic solvents are used, a severe problem of emulsion formation is omitted, and automation is easy. Due to this, solid phase extraction is increasingly often used [9]. Though different sorbents have been applied for isolation of herbicides from water, the most popular are RP-18 and SDB.

Acidic herbicides are polar, poorly volatilite and partially dissociate in water; they can be analysed by GC after conversion to less polar, sufficiently volatile and thermally stable derivatives [10].

Methylation by means of trimethylphenyl ammonium hydroxide (TMPH) seems to be a convenient approach because the process can be performed on-line in an injection port of GC (Fig. 1). No special extract preparation is needed – a reagent is simply added to an extract and a mixture injected to a heated GC injection port. Such an approach is convenient but it was found to be of poor repeatability, which, however, can be considered sufficient for the purpose of screening. The derivatization proceeds according to the scheme presented in Fig. 1.

The aim of this work was to develop a simple GC procedure for screening acidic herbicides in aqueous samples. Sample preparation was to be based on liquid-liquid extraction or solid phase extraction and ininjector TMPH derivatization.

Experimental

The analysis of phenoxy acid herbicides (MCPP - 2-(4-chloro-2-methylphenoxy)-propanoic acid; MCPA – (4-chloro-2-methylphenoxy)-acetic acid; 2,4-D – (2,4-dichlorophenoxy)-acetic acid; 2,4,5-T – (2,4,5-trichlorophenoxy)-acetic acid) and phenolic herbicides (dinoterb – 2-(1,1-dimethylethyl)-4,6-dinitro phenol; dinoseb – 2-(1-methylpropyl)-4,6-dinitro phenol, pentachlorofenol) in water was based on analyte isolation by means of solid phase extraction (sorbents - RP C18; SDB; elution solvents - ethyl acetate and methyl-*tert*-butyl ether) or liquid-liquid extraction with the same solvents; derivatization with trimethylphenylammonium hydroxide in injection port of gas chromatograph; and final determination by gas chromatography with flame ionization detection and mass spectrometric detection.
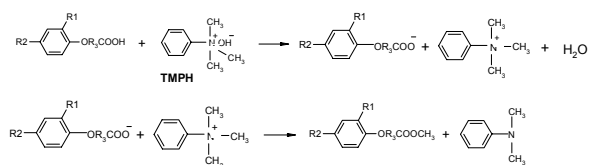
HERBICIDE	R1	R2	R3
2,4-D	Cl-	Cl-	-CH2-
MCPA	CH3-	Cl-	-CH2-
MCPB	CH3-	Cl-	-(CH2)3-

Fig. 1. Pyrolytic alkylation of acidic herbicides.

Reagents and Solutions

The solvents used were reagent grade: ethyl acetate and methyl-*tert*-butyl ether (MTBE) (Merck, Germany). Phenoxy acids (2,4-D; 2,4,5-T; MCPP; MCPA), methyl esters (2,4-D; 2,4,5-T; MCPP; MCPA) and phenols (pentachlorophenol, dinoterb, dinoseb) were from Riedel-de-Haën, Germany.

Working standard solutions of analytes were prepared by dissolving weighed amounts of reagents in methanol (stock standard solutions) and successive dilution with MTBE or ethyl acetate. Aqueous model samples (concentrations of 40 and 80 µg/l for phenoxy acid herbicides, and 80, 160 µg/l for phenols) were obtained by spiking distilled water with stock standard solutions. Before extraction, the model samples were acidified with sulphuric acid to pH=2; and sodium sulphate was added in quantities of 10, 20 and 30 g per 250 ml sample (LLE) and 10, 20 g per 250 ml in the case of SPE application.

Derivatization reagent was trimethylphenylammonium hydroxide (TMPH) – 0.2 M solution in methanol (SUPELCO, USA).

Sampling

The water was sampled in November 2002 at four different sampling sites in the region of Gdańsk. The samples were collected directly into sterile 500 ml glass bottles using routine methods of environmental sample collection. The time between sampling and analysis was less than 6 hours. The samples were treated with H_2SO_4 (up to pH 2).

Liquid Liquid Extraction

Aqueous model samples or real surface water samples of a volume of 250 ml (treated as described above) were extracted twice with 5 ml ethyl acetate or methyl-*tert*-butyl ether (MTBE) by manual shaking for 20 min. Both portions were combined and the extract volume reduced to 1 ml by gentle evaporation under a stream of nitrogen.

Solid Phase Extraction

The SPE cartridge (RP-18 – 500 mg and LiChrolut EN - 200 mg, MERCK) was conditioned by successive percolation of MTBE or ethyl acetate (1x3ml), methanol (2x3ml) and then deionized water (3ml) just before analysis. Attention was paid not to allow sorbent bed drying during column conditioning and sample percolation. Model and real samples (250 ml) were percolated through the cartridge at a controlled rate of about 2 ml/min. Then distilled water (3 ml) was passed and sorbent bed dried under a gentle stream of air. The analytes were eluted with MTBE or ethyl acetate (5x2 ml), extracts combined and evaporated to 1 ml under a gentle stream of nitrogen.

Derivatisation

TMPH methanol solution was added to extracts and a mixture injected to a heated GC injector. The 20-fold excess of the derivatizing reagent was applied [11].

Final Analysis

The final determination was carried out using a gas chromatograph equipped with a flame ionisation detector (FID) or with mass spectrometric detector (MSD). The GC runs parameters are given in Table 1.

Retention times and characteristic ions for SIM mode MS operation were determined by analysis of standard solutions in scan mode. They are given in Table 2. Each sample was injected thrice. Quantitative analysis was based on the external standard method.

Results and Discussion

Derivatization

Comparing the results obtained with different tetraalkyl ammonium salts and TMSH one can conclude that TMPH should rather be selected for derivatization of phenoxy acid and phenolic herbicides in organic solvent extracts in GC injector. The yields of derivatization of the phenoxy acids studied with TMPH do not differ much from acid to acid: they are on the level of 80% for MCPP, ca. 70% for 2,4-D and MCPA, and 60% for 2,4,5-T. The yield appeared to be optimal for 20-fold reagent excess.

MTBE appeared not to be a good derivatization medium - storage of reaction mixture results in non-repeatable data – recoveries can drop even 4 times on long storage. When DCM was a reaction medium a drop in yield was not significant even after 12-h storage.

Table 1. The working conditions of a gas chromatograph.

Element of analytical system	GC-MS	GC-FID
Gas chromatograph	HEWLETT PACKARD 5890 SERIES II	
Autosampler	HEWLETT PACKARD HP 6890 Injector	
Column (length x inside diameter x film thickness)	30 m x 0.25 mm x 0.25 µm phase: Rtx-5MS, 5% diphenyl- 95% dimethyl polysiloxane	
Injector temperature	250ºC	
Injection system	splitless	
Detector	mass spectrometer (MSD) HEWLETT PACKARD 5972	flame ionisation detector (FID) HEWLETT PACKARD 5890
Carrier gas	helium, 1 ml/min	
Oven temperature program	80ºC → 6ºC/min → 200ºC → 30ºC/min → 280ºC → (5 min)	90ºC (1 min) → 6ºC/min → 280ºC (5 min)

Table 2. Retention times and mass ions selected for analysis of methyl derivatives of phenoxy acid and phenolic herbicides and an internal standard.

Methyl derivative of	GC-MS		GC-FID
	Retention time [min]	Ion mass	Retention time [min]
MCPP	12.8	169, 228	13.59
MCPA	13.1	155, 214	13.9
2,4-D	16.2	175, 199	15.2
2,4,5-T	17.2	233, 235, 268	17.9
pentachlorophenol	14.5	165, 237, 265	17.1
dinoterb	18.3	209, 239	18.1
dinoseb	18.5	195, 225	18.3
dodecanoic acid	16.47	74, 87	21.3

Phenolic herbicides can also be analyzed by GC in acidic form. However, methylation gives more symmetrical and higher peaks and, hence, much lower detection limits (ca. 4 times lower) when FID is used [11].

Liquid-Liquid Extraction (LLE)

Due to the relatively high polarity of analytes of interest, polar solvents should give higher extraction yields. Methyl-*tert*-butyl ether and ethyl acetate were selected because they are less toxic than typical chloroorganic solvents of high extraction power. Aqueous samples were acidified with sulphuric acid to a value below pK_a of the studied acidic herbicides (pH ca. 2).

In order to lower solubility of analytes in aqueous phase and further increase extraction yield, sodium sulphate was added. The recoveries of the phenoxy acids studied for different sodium sulphate content are presented in Figure 2. At the repeatability obtained (not high due to the derivatization process) the change in sodium sulphate content in the range of 4-12% did not show statistically significant effects on the extraction yield on a significance level of $\alpha = 0.05$.

In the case of MTBE as an extracting solvent, dispersion of total recoveries was greater most likely because of the non-repeatability of the derivatization step: 50-75% for analyte concentrations of 40 µg/l and 65-80% for 80 µg/l.
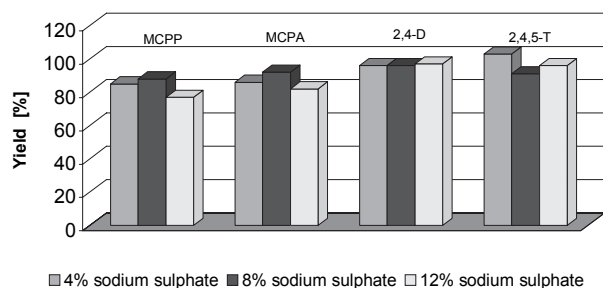

Fig. 2. Total recoveries of phenoxy acid herbicides in the combined process of extraction and derivatisation at different concentrations of sodium sulphate. Concentration of each analyte - 80 µg/l.
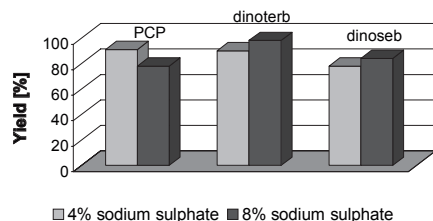

Fig. 3. Extraction yields of selected phenolic herbicides in aqueous samples at different sodium sulphate concentrations using SPE with SDB as a sorbent and ethyl acetate as an eluent (concentration of each analyte - 80 µg/l), GC-FID in final analysis.

Extraction yields for phenolic herbicides were quite high when ethyl acetate was used as an extractant; 95% for analyte concentrations of 160 µg/l and 4% Na_2SO_4 as a salting agent.

Solid Phase Extraction

In the case of SPE the sorbents used were silica gel modified with RP18 and co-polymer of steryne and divinylbenzene (SDB). Identical to LLE, samples were acidified to pH ca. 2 and sodium sulphate was added.

According to literature evidence, 10-20% salt addition should be optimal [9]. We have not observed statistically significant differences in the range of 4-12% Na_2SO_4 concentration. Exemplary data for phenolic herbicides for 4 and 8% are given in Fig. 3. Higher concentrations of sodium sulphate clogged the sorbent bed, so a small amount was added (4% sodium sulphate).

It was found that recovery depends on sorbent, Na_2SO_4 content and eluent. For phenolic herbicides at 80 µg/l concentration the highest yields were obtained when SDB packed cartridges and ethyl acetate as eluent were used; they were 90%, 95% and 80% for PCP, dinoterb and dinoseb, respectively (Fig. 3). When RP18 sorbent and MTBE eluent were used, extraction yields were lower.

In the case of phenoxy acid herbicide extraction yields at 80 µg/l concentration of analytes were not influenced to a noticeable degree by the sorbent cartridge used. They were on the level of 65-85% for RP18 and 63-93% for SDB in the case of both eluents.

Recoveries of both groups of herbicides in the LLE process dropped with a decrease in analyte concentration.

On the basis of the studies performed, the procedure was proposed for simultaneous determination of phenolic and phenoxy acid herbicides with the use of either LLE or SPE and in-injection port derivatization and GC separation and quantification. Copolymer of styrene and divinylbenzene was proposed as a sorbent for SPE and ethyl acetate as an eluent for SPE and extractant for LLE. For derivatization, TMPH solution was used.

Detection Limits

Phenolic herbicides can be determined by gas chromatography in original and derivatized forms. Gas chromatographic peaks of ethers are more symmetrical and FID response higher; hence, detection limits are better. Phenoxy acid herbicides can be only gas chromatographed after conversion to more volatile derivatives. When FID is used as a detector, detection limits for PCP, dinoterb and dinoseb are on the level of 40-50 mg/l in an extract. After conversion to methyl derivatives, detection limits were lowered to 1 mg/l for PCP, and ca. 2.5 mg/l for dinoterb and dinoseb. In the case of methyl esters of phenoxy acids, detection limits were 0.25 mg/l for MCPP, 0.5 mg/l for MCPA; 2,4-D; 2,4,5-T in the extract. Taking into account the fact of analyte enrichment, detection limits can be estimated on the high level of

Fig. 4. Map of sampling sites.

20 µg/l for phenolic herbicides in non derivatized form. These are too high to detect these pollutants at permissible levels, even in surface waters. However, when they are derivatized the detection limits are lowered to values which are close to permissible levels for surface water and the procedure can be used for screening purposes. For alkanophenoxy acid herbicide detection limits are of the order of 1-2 µg/l, which makes the procedure applicable for their screening in surface waters. Much lower detection limits can be obtained when MS is used as a detector in SIM mode. Using 250 ml water samples and reducing the final volume of extract to 1 ml detection limits of ca 20 ng/l for phenoxy acids and ca 40 ng/l for phenolic herbicides were reached.

According to the directive of the Minister of Health issued on 4th September 2000, the maximum permissible concentration of 2,4-D in drinking water is 0.1 µg/l. In surface waters the value is 8.0 µg/l [12]. Therefore, if this herbicide is extracted from 250 ml surface water and the final volume of concentrate is 1 ml then convenient and common GC-FID system can be used for monitoring 2,4-D and related herbicides in such environmental samples.
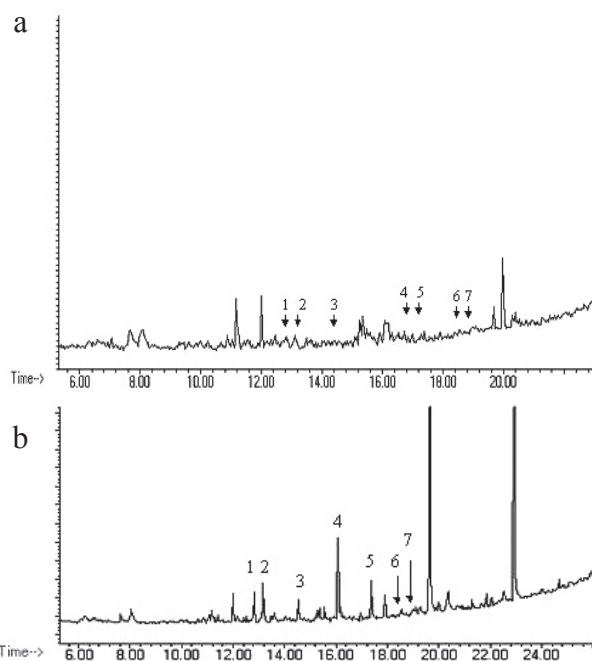

Fig. 5. Example of GC-MS chromatogram of a) blank sample (deionized water-Millipore),   b) sea water sampled at Gdynia Orłowo; herbicides: 1-MCPP; 2-MCPA; 3- PCP; 4-2,4-D; 5-2,4,5-T; 6-dinoterb; 7-dinoseb.

When GC-MS is applied for analysis of the concentrates, the sample preparation procedure described can be also used for screening 2,4-D and other herbicides from the group in drinking water.

Real Samples

The approach based on isolation of herbicides from water by means of SPE (SDB-packed cartridges), derivatization with TMPH in a heated GC injector and final analysis by GC-MS-SIM, was used to detect and quantify acidic herbicides in sea and riverine water. Samples were

Table 3. Acidic herbicide pollution of some surface waters in Gdańsk Province determined by GC-MS (Poland). Samples were taken in November 2002.

Sampling Site / Herbicide	Brzeźno	Gdynia Orłowo	Vistula estuary	Kiezmark
	Analyte content [µg/l]			
MCPP	0.12	0.13	0.09	0.05
MCPA	0.05	0.16	0.11	0.02
2,4-D	< 0.02	0.16	0.17	< 0.02
PCP	< 0.02	0.10	< 0.02	< 0.02
2,4,5-T	0.07	0.22	0.14	0.088
dinoterb	< 0.04	< 0.04	< 0.04	< 0.04
dinoseb	< 0.04	< 0.04	< 0.04	< 0.04

taken from the Gulf of Gdańsk close to Gdańsk and Gdynia, and from the Vistula at Kiezmark (ca. 12 km from the river mouth) and from the Vistula estuary (Fig. 4).

The content of herbicides ranged from below a detection limit of 20 ng/l to 220 ng/l (Table 3), which is rather low. Generally, the concentartions for the Vistula estuary are higher than for the Vistula at Kiezmark. This suggests that these herbicides can be still applied over the land along the shoreline.

An example of chromatogram of sea water sampled at Gdynia Orłowo is presented in Fig.5.

Conclusion

Methylation of phenoxy acid and phenolic herbicides in an injection port of a gas chromatograph gives a possibility of their gas chromatographic determination. The derivatizing reagent, TMPH can be directly added to a concentrate of these herbicides in an organic solvent. However, the process is characterized by poor repeatibility. The herbicides can be efficiently extracted by means of LLE or SPE. Combining any of these methods of analyte herbicide isolation and GC analysis with on-line ininjector derivatization gives a simple and convenient procedure to screen different waters for acidic herbicides. When the very popular and widely available GC flame ionization detector is used, detection limits are sufficiently low to detect these herbicides at a concentration permissible by Polish legislation for surface waters. Much lower detection limits of the order of 20 ng/l for phenoxy acid herbicides and ca. 40 ng/l for phenolic herbicides can be reached using GC-MS (SIM). In this case, the approach can also be used to screen drinking water for these pollutants, whereby their permissible concentration is 0.1 µg/l.

References

1. CUONG N.V., BACHMANN T.T., SCHMID R.D. Development of a dipstick immunoassay for quantitative determination of 2,4-dichlorophenoxyacetic acid in water, fruit and urine samples. Fresenius J Anal. Chem. **364**, 584, **1999.**

2. RIMMER D.A., JOHNSON P.D., BROWN R.H. Determination of phenoxy acid herbicides in vegetation, utilising high-resolution gel permeation chromatographic clean-up and methylation with trimethylsilyldiazomethane prior to gas chromatographic analysis with mass-selective detection. J. Chromatogr. A. **755**, 245, **1996.**

3. ROMPA M., KREMER E., ZYGMUNT B. Derivatisation in chromatographic determination of acidic herbicides in aqueous environmental samples. Anal. Bioanal. Chem., **377**, 590, **2003**.

4. MACUTKIEWICZ E., ROMPA M., ZYGMUNT B. Sample preparation and chromatographic analysis of acidic herbicides in soils and sediments. Crit. Rev. Anal. Chem. **33**, 1, **2003.**

5. NAMIEŚNIK J., GÓRECKI T. Preparation of environmental samples for the determination of trace constituents. Pol.J. Environ. Stud. **10**, 77, **2001**.

6. NAMIEŚNIK J. Trace analysis – challenges and problems. Crit Rev. Anal. Chem. **32**, 271, **2002**.

7. NAMIEŚNIK J., ZYGMUNT B. Selected concentration techniques for gas chromatographic analysis of environmental samples. Chromatographia **56**, S9, **2002**.

8. RAWA-ADKONIS M., WOLSKA L., Modern techniques of extracton of organic analytes from environmental matrices. Crit. Rev. Anal. Chem. **33**, 199, **2003**.

9. WELLS M.J.M., YU L. Z. Solid-phase extraction of acidic herbicides. J. Chromatogr. A. **885**, 237, **2000.**

10. KREMER E., ROMPA M., SOWIŃSKI P., WARDENCKI W., ZYGMUNT B., Gas chromatographic determination of selected environmental pollutants based on derivatisation (In Polish), in: New horizonts and challanges in environmental analysis and monitoring, (Ed. J. Namieśnik, W. Chrzanowski, P. Szpinek), Gdansk University of Technology, Gdańsk pp 493-525, **2003**.

11. ROMPA M., KREMER E., ZYGMUNT B., Derivatisation of acidic herbicides with selected tetraalkyl ammonium and trimethyl sulphonium hydroxides for their GC analysis. Annal. Lett. (In print).

12. Directive of Health Minister (In Polish), 4 Sept., 2000. Dz. U. Nr 82, poz. 937. Warsaw. URM **2000**.