Application info 36

AUTOMATED ANALYSIS OF N-METHYL CARBAMATE PESTICIDES IN WATER SAMPLES USING HPLC WITH ON-LINE SOLID PHASE EXTRACTION AND POST-COLUMN REACTION FLUORESCENCE DETECTION.

INTRODUCTION

The world wide increase in the use of pesticides during the last two decades has led to the presence of residues of them, not only on the crops to which they are applied but also, owing to leaching into and run-off from soil, in ground water and surface water. The European Community Directive states that individual pesticides should not exceed the 0.1 µg/l.
A multiresidue method for the determination of N-methylcarbamates and their polar metabolites in surface water and drinking water samples has been developed. The method consists of on-line solid phase extraction, reversed-phase LC separation, postcolumn hydrolysis of the carbamates on a solid-phase catalyst, reaction of the methylamine formed with o-phtalaldehyde reagent and fluorescence detection of the derivatives.
Instead of the post column hydrolyse system with a solid phase catalyst and OPA reagent mixing, a Pickering carbamate system can be linked with the PROSPEKT SPE system.

EXPERIMENTAL

INSTRUMENTATION

An Hewlett-Packard 1050 quaternary pump was used for the chromatographic separation. The OPA-reagent was added postcolumn with an HP 1050 isocratic pump. Detection was performed with a HP 1046A fluorescence detector and data acquisition with an HP ChemStation. Automated on-line solid phase extraction (SPE) was performed by a Spark Holland PROSPEKT system which controlled the MARATHON autosampler and Solvent Delivery Unit (SDU). The water samples are introduced by the MARATHON autosampler (3.0 ml loop). The switching diagram gives a schematic view of the system configuration.

CHROMATOGRAPHIC CONDITIONS

Anal. column	:	Merck LiChroCART, Supersphere RP-8, 250 × 4.0 mm, 4 µm
SPE cartridges	:	Analytichem Bondesil, C18OH 40 µm, 10 x 3.0 mm
Mobile phase	:	for gradient see Table 1
Flow rate	:	0.75 ml/min
Temperature	:	35°C
Post-column	:	S.S., 50 × 4.0 mm I.D. Aminex A-27, 15 µm
Temperature	:	ca.120°C
Flow rate OPA	:	0.1 ml/min
Detection	:	Fluorescence (ex: 340 nm; em: 445 nm)

The OPA reagent was prepared by dissolving 2,0 g of disodium tetraborate in ca. 500 ml purified water, adding 250 mg OPA (dissolved in 2 ml acetonitrile) and making up the volume with water.

SAMPLE PREPARATION

Acidify water sample with acetic acid (pH 3).
For drinking water: add also 100 mg/l sodium thiosulphate to prevent oxidation of carbamates due to hypochlorite.
The MARATHON 3.0 ml loop is filled in the pressure injection mode with 4.5 ml of sample using a peristaltic pump controlling the sample waste line.
On-line SPE was performed using the following sample preparation (flow rate 0.5 ml/min):

1. Activate cartridge with 1 ml methanol.
2. Condition cartridge with 5 ml water.
3. Load cartridge with 3.0 ml water.
4. Wash sample with 1.0 ml water.
5. Elute the sample during 28 minutes with the mobile phase.

Some matrix components show irreversible binding to the packing material, causing clogging and reduced performance in time. Therefore the cartridge has to be replaced after each run.

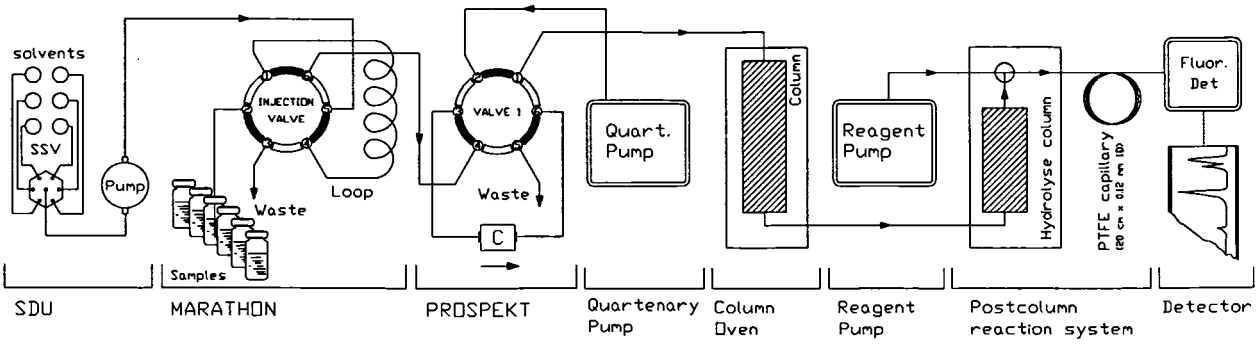

Figure 1: System switching diagram

Spark

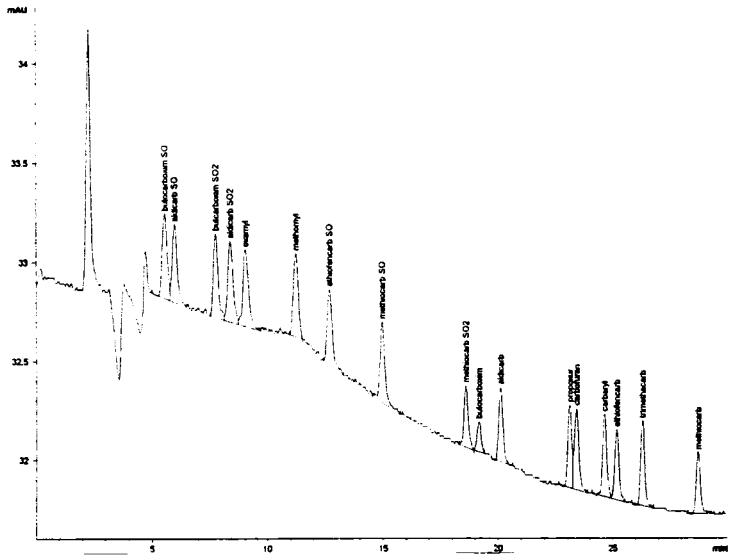

Figure 2: HPLC chromatogram of a 3.0 ml drinking water sample, fortified with 17 N-methyl-carbamates and metabolites at the 0.1 µg/l level, after on-line trace enrichment.

Table 1: Ternary gradient

Solvent A:	Acetonitrile-water (v/v)	20:80 v/v
Solvent B:	Methanol-water (v/v)	20:80 v/v
Solvent C:	Acetonitrile-water (v/v)	60:40 v/v

- Add to each solvent Sodium acetate until a concentration of ca. 0.5 g/l.

Time	%A	%B	%C
initial	75%	25%	0%
5 min	75%	25%	0%
25 min	0%	0%	100%
30 min	0%	0%	100%
31 min	75%	25%	0%
40 min	75%	25%	0%

RESULTS

Table 2

Average percent recoveries and relative standard deviations (n = 7) of N-methylcarbamates and metabolites from drinking water, fortified at the 0.1 µg/l level, after on-line trace enrichment.

Carbamate and metabolite	PROSPEKT On-line trace enrichment of 3.0 ml of drinking water	
	Recovery	RSD
Butocarboxim SO	95.0 %	7.1 %
Aldicarb SO	96.4 %	7.5 %
Butocarboxim SO	90.9 %	7.3 %
Aldicarb SO	87.3 %	6.4 %
Oxamyl	97.0 %	7.5 %
Methomyl	91.6 %	5.4 %
Ethiofencarb SO	102.0 %	6.7 %
Methiocarb SO	102.0 %	5.7 %
Methiocarb SO2	97.3 %	6.3 %
Butocarboxim	80.1 %	9.6 %
Aldicarb	98.3 %	4.8 %
Propoxur	103.1 %	4.2 %
Carbofuran	101.9 %	5.0 %
Carbaryl	102.1 %	3.6 %
Ethiofencarb	100.1 %	2.5 %
Trimethacarb	n.d.	n.d.
Methiocarb	89.1 %	3.8 %

N.B.: Landrin (Trimethacarb) was used as an internal standard 0.1 µg/l. This N-methylcarbamate was never used as a pesticide and is therefore suitable as internal standard. (n.d. = not determined)

CONCLUSIONS

A fully automated PROSPEKT on-line SPE-HPLC method for all carbamates and polar metabolites has been developed, which is suitable for various types of water. The detection limit for each component is 0.01 - 0.03 µg/l. The sample throughput is 24 samples per 24 hours unattended.
The automated cartridge exchange renews the SPE module after each analysis.

TROUBLESHOOTING

Possible causes of malfunctioning of the application are:

- Crystallisation of borax buffer in T-piece.
- Pulsation in the 0.1 ml/min OPA pump, which might be caused by air bubbles.
- Life time of the Aminex column. Average lifetime is between 6 and 9 months. The column will be quickly destroyed if one stops the flow rate without having previously decreased the column temperature.
- The use of not sufficiently pure HPLC water. The authors prescribe the use of Elgastatt or Milli Q purified water.
- The tenability of the OPA reagent which is limited to a few days.
- The optimal temperature for the Aminex column is obtained when the peak height of Methomyl is as high as the peak height of Oxamyl.
- With some fluorescence detectors a back pressure regulator may be required to prevent boiling of the mobile phase.
- Standard solutions should be stored in a freezer.

REFERENCE

G.S.J. Haak, H. Kerkdijk,
Spark Holland B.V.,
P.O. Box 388, 7800 AJ Emmen,
The Netherlands.

Dr. A. de Kok, M. Hiemstra,
J.Chromatogr., submitted for publication,
Regional Inspectorate for Health Protection Food Inspection Service, Department of pesticide analysis, Alkmaar, The Netherlands.