

**ANALYTICAL METHOD FOR DETERMINATION OF SOME
PRIORITY SUBSTANCES IN SURFACE AND GROUND WATER
SAMPLES BY LIQUID CHROMATOGRAPHY - MASS
SPECTROMETRY**

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Abstract. Chemical status of water bodies is determined through environmental quality standards, defined for priority pollutants (Directive 2008/105/EC), mostly represented by persistent organic compounds and pesticides. There is a need for precise and accurate analytical methods for reliable assessment of environmental status of surface and ground waters. The aim of this work was to develop an innovative multiresidue analytical method for simultaneous determination of some priority substances in surface and ground waters. Determined target compounds include the pesticides: Acetochlor, Alachlor, Chlorfenvinphos, Chlorpyrifos, Diuron, Isoproturon, Pendimethalin and Trifluralin. Analytical approach was based on a solid-phase extraction (SPE) as a sample pretreatment procedure and a liquid chromatography–mass spectrometry (LC–MS) as an instrumental method. Optimal chemical conditions for quantitative separation and preconcentration of the analytes in a single step extraction procedure are defined. LC–MS method performance is discussed in details. The achieved analytical recovery for all compounds was greater than 86% and repeatability is up to 10%. The established detection limits were ranged between 1 and 10 ng/L and the quantification limits were between 5 and 50 ng/L, respectively. The concentration levels of measured priority substances in main Bulgarian rivers were presented.

Keywords: pesticides, surface water, ground water, multiresidue determination, solid phase extraction (SPE), liquid chromatography mass spectrometry (LC-MS).

1. Introduction

Organic contaminants in the environment are released from different anthropogenic activities such as industrial chemical production, agricultural application and domestic activities (Reemtsma et al., 2006). Many of these contaminants are stable and can persist for a long period of time in the environment. So due to their extensive use and persistence, many of these compounds can be transferred in surface and ground waters and have to be considered as a potential risk for aquatic and soil ecosystems (Barth et al., 2007; Domingo et al., 2007). In this context, strict regulations for the control of contaminants concentration levels in the environment have been established. In the field of water policy, the European Union (EU) adopted the Water Framework Directive (WFD) (2000/60/EC), which is probably the most significant international legislation in the field of water quality. The Directive takes a broad view of water management, setting the prevention of any further deterioration of water bodies and the protection and enhancement of the status of aquatic ecosystems as its key objectives. The overriding objective of the policy is the achievement of “good status” in all water bodies. Its implementation should result in an intensification of monitoring of aquatic ecosystems and increased control of contaminants. Article 16 of the WFD sets out EU strategy against pollution of water by chemical substances. According to the provisions of this article, a list of 33 priority substances, which represent a significant risk to or via the aquatic environment at the EU level, has been established and will have to be re-examined by the European Commission (EC) at least every four years (Decision No 2001/2455/EC). Most of the current list are organic contaminants (hydrocarbons, organochlorine compounds, organic solvents, pesticides, and chlorophenols).

According to the WFD, good chemical status for a water body is obtained when the concentrations of the priority substances in water, sediment or biota are below the Environmental Quality Standards (EQSs): this is expressed as “compliance checking”. In the next stage, the EC has proposed EQSs for water only. They are listed in a daughter Directive (2008/105/EC). In order to reach the “good status” for all European water bodies, Member States have to implement river basin management plans, including water monitoring programs, and take measures when results do not comply with the EQSs. Under this Directive Member States are required to take actions to meet those quality standards by 2015. For this purpose a programme of measures are in place and should become operational by 2012.

Bulgaria, as a Member of the EU, must meet the WFD environmental standards in order to achieve “good chemical status” of its waters. Therefore precise and accurate analytical methods are needed for reliable assessment of environmental status of surface and ground waters. The objective of this work was the development and optimization of an innovative multiresidue analytical method, based on solid phase extraction (SPE) followed by liquid chromatography mass spectrometry (LC–MS) for a simultaneous determination of some of the priority substances in surface and ground waters. The target compounds are eight pesticides from the list of 33 priority substances: Acetochlor, Alachlor, Chlorfenvinphos, Chlorpyrifos, Diuron, Isoproturon, Pendimethalin and Trifluralin. Chemical structures of the selected analyzed pesticides are presented on a Figure 1.

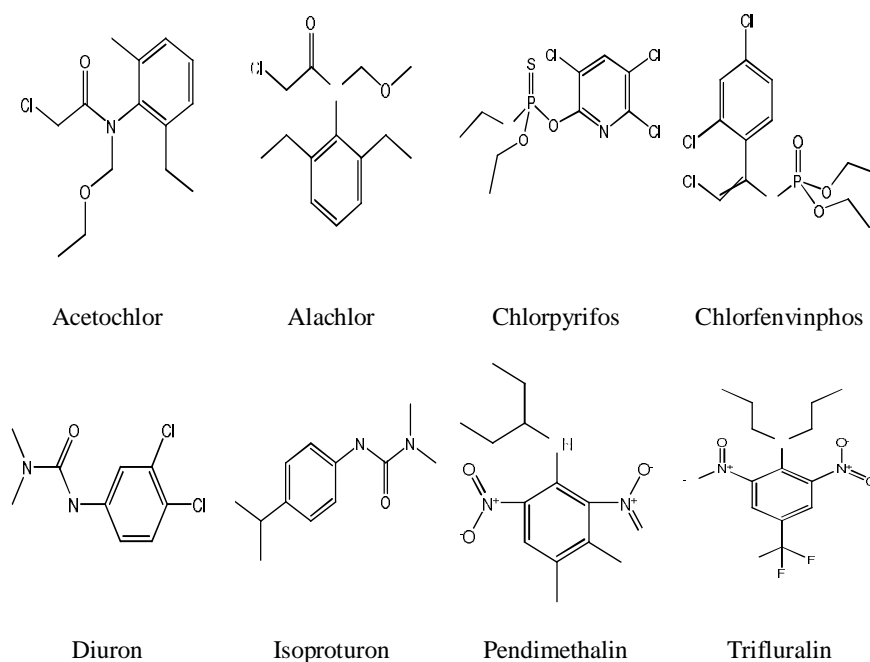


Figure 1. Chemical structures of the analyzed pesticides.

2. Experimental Section

2.1. CHEMICALS AND REAGENTS

Acetochlor (2-Chloro-2'-methyl-6'-ethyl-N-ethoxymethyl-acetanilide) and Pendimethalin (3,4-Xylidine, 2,6-dinitro-N-(1-ethylpropyl)-) were provided by Sigma-Aldrich (St. Louis, MO, USA). Alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide), chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioic acid), chlorfenvinphos (O,O-diethyl-O-1-(2',4'-dichloro-phenyl)-2-chlorovinyl-phosphate), diuron (1,1-Dimethyl-3-(3,4-Dichloro-phenyl)urea), isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea) and trifluralin (2,6-Dinitro-N,N-dipropyl-4-(trifluoromethyl)-benzamine) were provided by Dr. Ehrenstorfer (Augsburg, Germany). Isoproturon-d6 (Dr. Ehrenstorfer, Augsburg, Germany) was used as internal standard. All analytical standards were of high purity (>97%). The solvents methanol, acetonitrile and acetone (HPLC grade) and formic acid (for analysis) were provided from Merck (Darmstadt, Germany). The individual stock standard solutions were prepared in methanol at the concentration of 100 mg/L and stored in the dark at 4 °C. The composite working standard solution was prepared by mixing the appropriate amounts of the individual standard solutions and diluting with methanol to the final concentration range between 5 and 100 ng/mL. Recovery studies were performed in different matrix samples (surface water, ground water and distilled water) by appropriate dilution of composite standard solution, in order to obtain a final concentration for each compound in the samples of 0,05 µg/L. All aqueous solutions did not contain more than 0,1% methanol.

2.2. SAMPLE COLLECTION

All water samples were collected in 1 L glass amber bottles and stored in a dark place with temperature at 4 °C. All samples were transported and extracted within 24 h after the sampling. If it is necessary, the pH value of the samples were adjusted in the range from 5,0 to 7,5 with 0,1 M HCl.

2.3. SAMPLE EXTRACTION

The optimization of the sample preparation method is an important process to achieve the highest enrichment efficiency and the best recovery. The following parameters that may affect the SPE procedure efficiency were optimized: the sorbent type in combination with different elution solvents and the sample volume. The SPE procedure was performed with 24-ports SPE tubes vacuum

manifold equipped with vacuum pump (Alltech, USA). Two different SPE cartridges were compared for extraction of selected pesticides: Bond Elut C18 LRC (C18, 200 mg/10 mL, Agilent Technologies, USA) and Oasis HLB (hydrophilic–lipophilic balance, 200 mg/6 mL; from Waters, Milford, MA, USA) (Table 1), using three different elution solvents: methanol, methanol–dichloromethane (1:1) and methanol–acetone (3:2). For this experiment, 1000 mL of ultrapure water was spiked with the working standard solution in order to achieve a final concentration of 100 ng/mL for each analyte in the final extract. Spiked water samples were loaded at a flow rate of 10 mL/min followed by 15 mL of ultrapure water. The cartridges were then dried under air flow for about one hour and analytes were eluted with 10 (4+4+2) mL of selected elution solvent methanol/acetone (3:2) at a flow rate of 2 mL/min. Extracts were evaporated to 1 mL under N₂ stream and water bath up to 30 °C. The final extracts were filtered through 0,20 µm polytetrafluoroethylene (PTFE) filters and analyzed.

After the selection of the cartridge type the sample volume was optimized. The tested volumes were: 250, 500 and 1000 mL. Ultrapure water was spiked with working standard solution to produce concentration of 100 ng/mL for each pesticide in the final extract. Extracts were prepared in the same way as in the previous experiment. The developed method was validated by performing recovery studies using both ground water and surface water samples spiked with standard solution to produce concentrations of 30 ng/mL in the final extracts. The developed and optimized method was finally applied to the analysis of ground and surface water samples.

TABLE 1. Extraction parameters - recoveries and residual standard deviation (n = 6) of the selected pesticides using two different SPE cartridges in combination with elution solvent methanol/acetone (3:2).

Compound	Bond Elut C18 LRC (C18, 200 mg/10 mL)		Oasis HLB (hydrophilic–lipophilic balance, 200 mg/6 mL)	
	Recovery, %	RSD, %	Recovery, %	RSD, %
Acetochlor	52	5,9	92	6,2
Alachlor	55	6,1	93	7,3
Chlorfenvinphos	78	7,7	89	9,2
Chlorpyrifos	82	13,8	95	6,6
Diuron	61	12,1	112	8,0
Isoproturon	58	10,4	86	12,7
Pendimethalin	42	15,6	89	10,7
Trifluralin	53	9,8	94	8,9

2.4. LC-MS/MS ANALYSIS

The chromatographic analysis was performed on Agilent LC system 1200 Series (Agilent Technologies, USA) equipped with a binary pump, automatic injector and a thermostatted column compartment connected to a Agilent 6300 Series Ion Trap MS equipped with electrospray ionization source (Agilent Technologies, USA). The chromatographic separation was performed with Zorbax Eclipse® XDB-C18 (150 mm×2.1 mm i.d. and 3.5 µm particle size) column from Agilent (Agilent Technologies, USA). The separation was achieved with the gradient elution conditions described in Table 2. Used mobile phase components are 0,05% formic acid solution in ultra-pure water (A) and acetonitrile (B) with a constant flow rate of 0.25 ml/min. The column temperature was set at 30°C and the injection volume was 20 µL. Chromatograms of a standard mixture of the studied compounds and the transitions used for quantification are shown on a Figure 2.

TABLE 2. Gradient elution conditions.

Step	Time (min)	Flow (ml/min)	% A	% B
0	0	0,250	60	40
1	20	0,250	20	80
2	25	0,250	5	95
3	35	0,250	5	95
4	35,1	0,250	60	40
5	38	0,250	60	40

Mass spectra were obtained by electrospray ionization (ESI) technique in the positive ionization mode and Multiple Reaction Monitoring (MRM) with a minimum of two precursor-product-ion transitions for each compound. All MRM transitions for each compound are shown in Table 3. The optimal source working parameters for monitoring all ions were as follow: spray voltage (3.5 kV), the nitrogen flow 40 PSI for the nebulizer gas (N₂), dry gas (N₂) flow (10 L/min) and heated capillary temperature (350 °C).

Analytical instrument control and data acquisition were performed with ChemStation and Ion Trap controlling software both provided from Agilent (Agilent Technologies, USA).

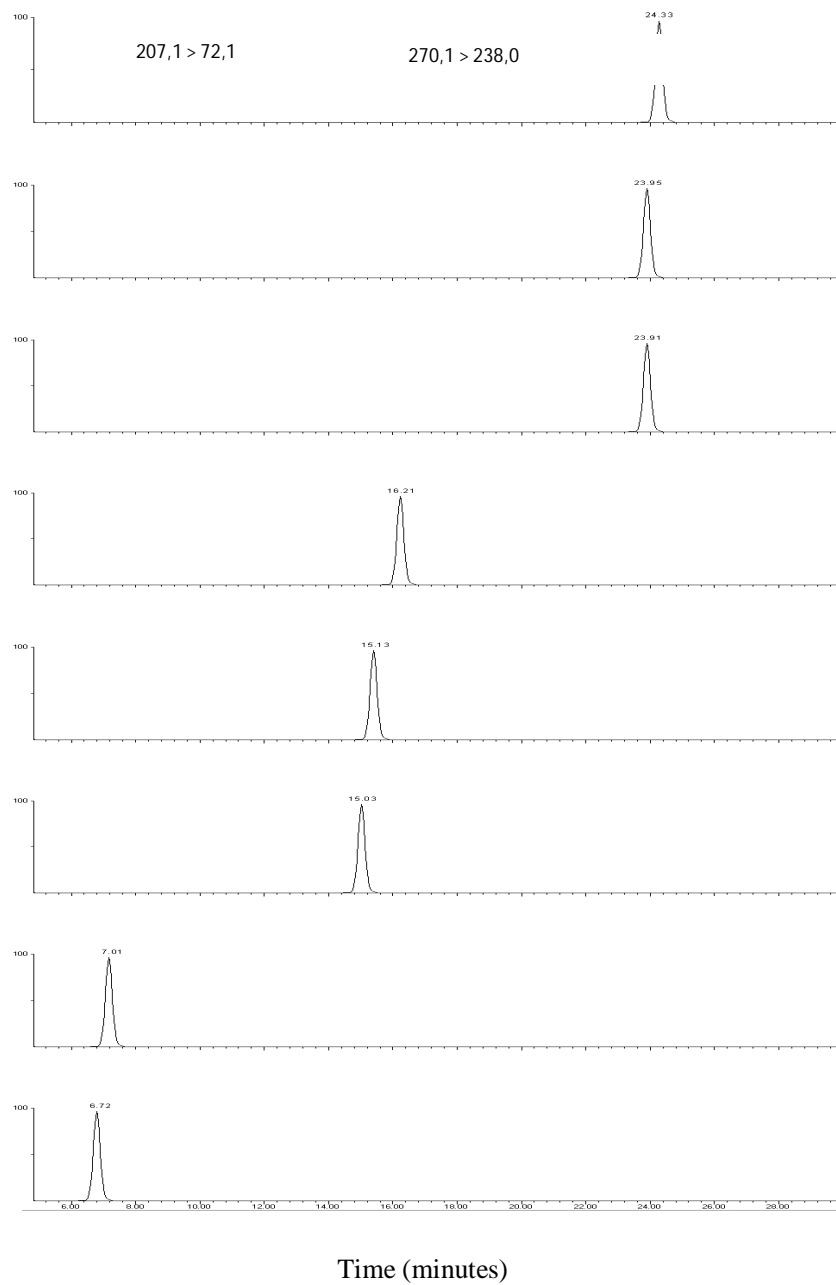


Figure 2. Chromatograms of a standard mixture of studied compounds and the transitions used for quantification.

TABLE 3. MRM transitions of the compounds (in bold are the transitions which have been used for quantification analysis).

Compound	RT, min	MRM transition
Acetochlor	15,1	270,0 → 224,0 (1,0)
		270,0 → 148,1 (1,0)
Alachlor	15,0	270,0 → 238,0 (1,0)
		270,0 → 162,2 (1,0)
Chlorfenvinphos	16,2	359,0 → 155,0 (1,0)
		359,0 → 127,0 (1,0)
Chlorpyrifos	23,9	350,0 → 198,0 (1,0)
		350,0 → 153,0 (1,0)
Diuron	7,0	233,0 → 72,0 (0,6)
		233,0 → 165,0 (0,6)
Isoproturon	6,7	207,0 → 72,0 (0,9)
		207,0 → 165,0 (0,9)
Pendimethalin	23,9	282,0 → 212,0 (1,0)
		282,0 → 194,0 (1,0)
Trifluralin	24,3	336,0 → 236,0 (1,0)
		336,0 → 185,0 (1,0)
d6-Isoproturon internal standard	6,5	213,0 → 78,0 (0,8)

2.5. METHOD VALIDATION

Extraction recoveries of target compounds were determined using both ground water and surface water samples spiked with 30 ng/L. Prior to analysis, samples used as blanks were proven to be free from the pesticides considered.

The precision of the method was determined by repeated intraday and interday analyses (analyses of six replicated spiked samples in 1 day, and in 3 successive days, respectively), and expressed as the relative standard deviation (RSD).

Limits of detection (LODs) and quantification (LOQs) were determined from ground water and surface water samples spiked at the concentration levels of each compound giving a signal-to-noise ratio (S/N) of 3 and 10, respectively.

For estimation of the linear dynamic range, five point calibration curve was constructed in a calibration range between 10 – 100 ng/mL. Each calibration standard was analyzed using the described method. For each compound was plotted the correlation between the measured values of the signal for the bold MRM transition (Table 3) versus the concentration in the solution by using a

least-square regression analysis. Correlation coefficients greater than 0,99 where observed (Table 4).

TABLE 4. Analytical parameters of the LC–MS/MS method.

Compound	Correlation coefficient, r^2	LOD, ng/L	LOQ, ng/L	RSD, %	R, %
Acetochlor	0,9968	1	5	5,2	92
Alachlor	0,9995	5	10	3,5	93
Chlorfenvinphos	0,9989	5	10	5,3	96
Chlorpyrifos	0,9992	5	10	4,7	103
Diuron	0,9991	6	40	3,1	112
Isoproturon	0,9987	6	50	5,8	89
Pendimethalin	0,9984	10	50	6,6	86
Trifluralin	0,9997	5	10	4,1	94

3. Application to Real Samples

The developed multiresidue analytical methodology was applied in a study carried out in Bulgaria in order to accomplish the Water Framework Directive (2000/60/EC) concerning the survey of the Directive target contaminants in surface water. Twenty samples were analyzed for the eight pesticides listed above. The samples of surface water were collected from several main rivers in Bulgaria. The obtained results are summarized in the next table (Table 5).

TABLE 5. Concentration levels of analyzed pesticides in the water samples.

Compound	Concentration level
Acetochlor	< 5 ÷ 70 ng/L
Alachlor	< 10 ÷ 50 ng/L
Chlorfenvinphos	< 10 ÷ 50 ng/L
Chlorpyrifos	< 10 ng/L
Diuron	< 40 ÷ 90 ng/L
Isoproturon	< 50 ng/L
Pendimethalin	< 50 ÷ 900 ng/L
Trifluralin	< 10 ÷ 80 ng/L

4. Conclusion

With the growing attention to contaminants in the environment, there is a need for fast and sensitive analytical method that can screen for a wide variety of

compounds simultaneously. The analytical methodology described above provides a sensitive method for the determination of some of the priority organic substances, which are pesticides, included in the WFD 2000/60/EC - Acetochlor, Alachlor, Chlorfenvinphos, Chlorpyrifos, Diuron, Isoproturon, Pendimethalin and Trifluralin. The SPE followed by LC-MS/MS gives the sensitivity and selectivity necessary to the detection of these compounds at environmental relevant concentration in the nanogram per liter range. The development of a single extraction procedure for all samples and all analytes, sensitive and robust irrespective of concentration levels and water physico-chemical properties is a real analytical challenge and a real advance in environmental analysis. This analytical method will allow the monitoring launched by the EU on water quality, and will make possible to analyze temporal and/or spatial distributions of described pesticides in the aquatic environment.

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