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Research Article

Simultaneous determination of alachlor, bifenox and nitrofen as herbicides in lake, municipal wastewater and soil samples by gas chromatography mass spectrometry

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Abstract

The benefits derived from pesticide usage are a worldwide phenomenon which has helped prevent food shortages and prevented deadly diseases. However, negative effects that have resulted from these very same chemicals has led to regulations and bans on some of them. This study was aimed at simultaneously determining three herbicides in lake, wastewater and soil samples by gas chromatography mass spectrometry. The samples selected are very commonly contaminated by pesticide production and application, and they are important environmental resources that need to be preserved. The figures of merits determined for the analytes were appreciable. None of the analytes were detected in the samples (according to limits of detection) and spiked recovery tests were therefore performed to ascertain the amount of analytes that can be determined when present in the samples. The percent recoveries calculated for lake and wastewater spiked at two concentrations ranged between 90 – 104%. The spiked soil sample was extracted with acetonitrile and a blank soil extract was used to prepare matrix matched calibration standards. The percent recovery obtained using the matrix matching standards ranged between 92% and 99% for the three analytes.

Keywords: Herbicides; GC-MS; Wastewater; Lake water; Soil.

1. Introduction

For many years, pesticides have been used to control, prevent or terminate pests that pose a threat to human beings and other organisms [1, 2]. Pesticide usage has led to the prevention of diseases that were major plagues in the past, and prevention of faming by the production of quality food crops that can also be stored very long periods of time [3]. Pesticides include natural extracts from plants and chemically synthesized compounds that target pests. Mass production of synthetic pesticides begun after dichloro-diphenyl-trichloroethane (DDT) was successfully used to eradicate pests during the 1940s but the risks associated with some chemical class of pesticides (e.g. organochlorines) drew the attention of environmentalists and regulatory authorities [4, 5]. Herbicides are a group of pesticides classified according to their target organism, which are unwanted plants/weeds. Weeds have the tendency to grow over any land or field viable for plant life and herbicides are therefore used to suppress their growth or to destroy them [6]. According to the mode of action, herbicides are classified into contact and translocated herbicides. Contact herbicides only affect the part of the plant where it comes into contact with, while translocated pesticides move through different parts of the plant to disrupt its regular function. Herbicides are usually less toxic when applied in the right way and appropriate concentration [7].

Alachlor is a chloroacetamide herbicide which is widely used in controlling broad leaf weeds mostly on corn farms. It has high water solubility and is therefore a very mobile chemical with the capability to leach down soil into ground water, and also get absorbed by non-target plants [8]. This makes it a very toxic chemical and it is classified as a B-2 carcinogen and a potential endocrine disruptive compound [9]. Alachlor is a regulated pesticide and must therefore be purchased with license and applied according to specifications on the label. Bifenox is a nitroaromatic herbicide which is used in photobleaching unwanted broad leaf weeds on farms, gardens and recreational parks [10]. It has the tendency to enter environmental water systems and residues can be found on consumable food products. Exposure to high concentrations and accumulation in body tissues over time can lead to severe health effects. Bifenox is included in the list of priority substances stated by the European Union's Water Framework Directive [11]. Nitrofen is a diphenyl ether structured herbicide which was widely used for the control of weeds. Its mode of action is inhibition of photosynthesis and respiration through plant organelles (mitochondria). It was however banned about three decades ago due to its potential to cause cancer and movement through the placenta to cause malformation of embryos [12]. Nitrofen as an endocrine disruptor chemical exhibits both estrogenic and androgenic effects, while its accumulation in the fetus causes kidney and lung defects, but mainly cardiac defect [12-14]. Determination of pesticide residues in environmental samples and food products is therefore very important to ensure that humans and other organisms are protected from these negative effects.

The most widely used technique for the determination of pesticides and other organic contaminants is instrumental chromatography [15]. Instrumental chromatography can be broadly classified into liquid chromatography (LC) and gas chromatography (GC) based on the physical state of the mobile phase being used [16]. LC is applicable to a greater percentage of organic compounds in comparison GC due to the latter being suitable for only compounds with high vapor pressures (volatile compounds) [4]. There are several detectors available coupled with GC and these include flame ionization detector (FID), thermal conductivity detector (TCD), electron capture detector (ECD), nitrogen phosphorus detector (NPD) and flame photometric detector (PID) [17]. GC coupled with mass spectrometry (GC-MS) is a very sensitive, accurate and precise technique which can also be used to characterize unknown compounds [18].

The aim of this study was to determine three herbicides (alachlor, bifenox and nitrofen) by GC-MS in lake, wastewater and soil samples with high accuracy and precision.

2. Materials and methods

2.1 Instrumentation

Simultaneous determination of the analytes in this study was performed using a gas chromatograph mass spectrometry (Agilent 6890 GC-MS). Separation of analytes before transferring to the mass spectrometer was achieved with a non-polar 30 m long HP-5MS column with 250 µm internal diameter and 0.25 µm film size. The quantifier/qualifier ions used for alachlor, bifenox and nitrofen were 160/188, 341/343 and 283/285 (m/z), respectively. The oven containing the column was programmed to increase from 70 °C – 180 °C and 180 °C – 300 °C at ramp rates of 60 °C/min and 40 °C/min, respectively. Helium served as both carrier and make-up gas at 1.5 mL/min constant flow. The injection port was heated to 250 °C and 1.0 µL of samples and standards were injected in the splitless mode. The MS transfer line temperature was 280 °C and ionization energy was 70 eV.

2.2 Reagents

Alachlor (15972-60-8), bifenox (42576-02-3) and nitrofen (1836-75-5) were purchased from Dr. Erhrenstorfer (Augsburg – Germany) and each standard was dissolved in acetonitrile (Merck – Germany) to prepare approximately 1000 mg/L solutions. Aliquots from the three standard solutions were diluted with acetonitrile to prepare a 100 mg/L mixed standard solution, which was subsequently diluted to prepare working and calibration standard solutions. Analytical grade ethanol and methanol (Merck – Germany) were used for pre- and post-injection washing of the 10 µL GC syringe. Ultrapure deionized water was used to prepare aqueous standard solutions and rinsing of washed glassware.

2.3 Samples

Municipal wastewater was sampled from an open air aeration tank at a treatment facility and kept between 4 – 5 °C. Before using for analysis, the wastewater was filtered through a paper with 125 mm pore size and then through a syringe filter with 0.45 µm pore size. Lake water was sample into clean plastic bottles and stored under room conditions. Soil was sampled from different units of a field and grinded after homogenization. Very fine soil particles were obtained by sieving through a 100 mm pore sized sieve.

3. Results

All instrumental measurements were performed at least three times and the average of these measurements used to either plot calibration graphs or calculate percent recoveries of analytes from spiked samples.

3.1 Separation and determination of analytes

The temperature program specified in Section 2.1 was tested on 50 mg/L mixed standard solution and a well separated chromatogram of the three analytes was obtained as shown in Figure 1. The five largest peaks (*m/z*) of each analyte was taken from the National Institute of Science and Technology (NIST) mass spectrum search program and extracted from the total ion chromatogram to determine their respective retention times. The ChemStation mass spectrum database of compounds was also used to confirm the analytes and their retention times. The retention times for alachlor, nitrofen and bifenox were 4.11 min, 4.89 min and 5.55 min, respectively. Calibration standards were prepared between 0.10 mg/L and 100 mg/L and each calibration point measured three times. The lowest concentration of each analyte (0.20 mg/L for alachlor, 0.50 mg/L for nitrofen and bifenox) was determined at the point where the signal-to-noise ratio (S/N) was equal to or more than 3. The standard deviation (StdDev) calculated from 6 measurements of the lowest calibration concentration was used to determine the limits of detection (LOD) and quantification (LOQ), and the percent relative standard deviation (%RSD) using the expressions below:

LOD: 3 x StdDev/slope
LOQ: 10 x StdDev/slope
%RSD: (StdDev/Average) x 100

Table 1 summarizes the figures of merit calculated for the analytes. The calibration plots were linear (R^2>0.9995) over a broad concentration range and the low %RSDs obtained established good repeatability for replicate instrumental measurements.

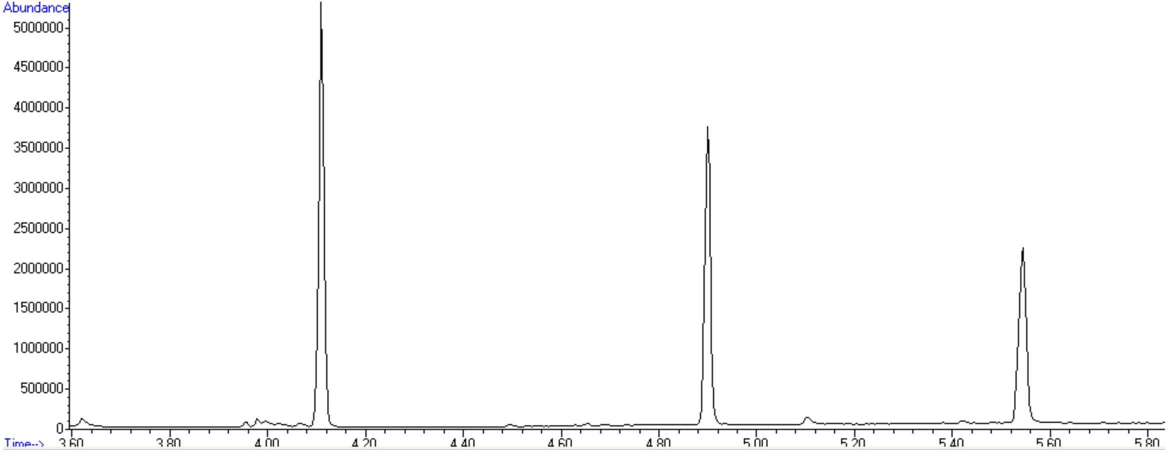

***Figure 1**. Total ion chromatogram of 50 µg/mL mixed standard solution.*

***Table 1**. Analytical figures of merit for alachlor, nitrofen and bifenox by GC-MS.*

Analyte	**Dynamic Range, mg/L**	**R^2**	**%RSD**	**LOD, mg/L**	**LOQ, mg/L**
Alachlor	0.20 – 50	0.9998	8.6	0.05	0.17
Bifenox	0.50 – 50	0.9996	6.1	0.18	0.59
Nitrofen	0.50 – 50	0.9997	2.9	0.15	0.50

3.2 Lake and wastewater recovery

Most farm lands are located next to lakes as a constant source of irrigation, and this makes contamination due to pesticide application common. Wastewater could also contain pesticides and other environmental contaminants which require removal before the treated water can be released into the environment. The purpose of the recovery study was to determine the amount of these analyte that can be determined when present in wastewater and lake water. Each sample was directly filled into vials and analyzed according to instrumental parameters in section 2.1 but none of the analytes was detected in the blank measurements. The samples were then spiked to 2.0 mg/L and 10 mg/L final concentrations and analyzed alongside calibration standards prepared in acetonitrile. Integrated peak areas of the standard solutions at the same concentration as the spiked samples were more than twice the samples' peak area values, resulting in less than 50% recovery. The calibration standards were therefore prepared in deionized water and the peak areas obtained were similar to the spiked samples, giving percent recoveries close to 100% as shown in Table 2.

***Table 2**. Percent recoveries for lake and wastewater spiked at 2.0 mg/L and 10 mg/L (N=3).*

Analyte	**Lake water**		**Wastewater**	
	2.0 mg/L	10 mg/L	2.0 mg/L	10 mg/L
Alachlor	101.1 ± 4.9%	96.9 ± 2.6%	95.4 ± 6.5%	93.4 ± 1.7%
Bifenox	90.6 ± 3.3%	104.9 ± 4.9%	98.3 ± 8.2%	102.5 ± 5.4%
Nitrofen	93.0 ± 3.9%	103.9 ± 4.2%	90.2 ± 7.1%	104.0 ± 4.5%

N = 3 to calculate %RSD.

3.3 Soil recovery

Unlike the water samples which were directly analyzed, the soil sample had to be extracted before sending to the instrument. Since the analytes showed good solubility in acetonitrile (standard solution solvent), it was used as extractant for the spiked soil sample. The soil sample was sieved as written in section 2.3 to ensure that the finest particles gave a large surface area for extraction. The soil sample was dried at about 70 °C for 2.0 h before spiking with the standards. Two 0.50 g samples were spiked to a final concentration of 2.0 mg/L and extracted with 4.0 mL acetonitrile on a mechanical shaker for 10 min. The supernatants were filtered through 0.45 µm syringe filters into vials for GC-MS injections together with a blank soil extract and calibration standards. The percent recovery recorded for the analytes were appreciable, but the blank soil extract was used to prepare calibration standards with the aim of matching the soil matrix. The percent recovery obtained with the matrix matched standards showed an improvement over the regular standard solutions as given in Table 3.

Table 3. Percent recoveries for soil sample spiked at 2.0 mg/L.

Analyte	Conventional Standards	Matrix Matched Standards
Alachlor	87.0 ± 5.2%	92.7 ± 0.9%
Bifenox	91.9 ± 7.7%	98.8 ± 2.2%
Nitrofen	89.4 ± 1.7%	96.6 ± 5.3%

4. Conclusions

This study presents a direct GC-MS analysis method for the determination of alachlor, bifenox and nitrofen in environmental samples. The analytes were well separated from each other using an optimum temperature program and the figures of merit determined for each analyte were appreciable. Suitability of the method to real samples was tested on lake, wastewater and soil samples which tend to have complex matrices. The water samples were directly spiked and analyzed, yielding close to 100% recovery results. The soil sample was spiked and extracted with acetonitrile, and a blank soil extract was used to prepare matrix matching standards to improve the accuracy of the results.

References

[1] C.D. Selali, K. Yeşim, K. Sena, Ö. Çağla, B.A. Emin, B. Sezgin, Multivariate optimization of dispersive liquid–liquid microextraction for the determination of paclobutrazol and triflumizole in water by GC–MS, Journal of Separation Science, 40 (2017) 4541-4548.

[2] E.A. Songa, J.O. Okonkwo, Recent approaches to improving selectivity and sensitivity of enzyme-based biosensors for organophosphorus pesticides: A review, Talanta, 155 (2016) 289-304.

[3] J. Cooper, H. Dobson, The benefits of pesticides to mankind and the environment, Crop Protection, 26 (2007) 1337-1348.

[4] A.E. Bulgurcuoğlu, B. Yılmaz, D.S. Chormey, S. Bakırdere, Simultaneous determination of estrone and selected pesticides in water medium by GC-MS after multivariate optimization of microextraction strategy, Environmental Monitoring and Assessment, 190 (2018) 252.

[5] R.L. Zimdahl, Chapter 7 - DDT: An Insecticide, Six Chemicals That Changed Agriculture, Academic Press, San Diego, 2015, pp. 115-133.

[6] P.K. Gupta, Chapter 39 - Herbicides and fungicides, Reproductive and Developmental Toxicology, Academic Press, San Diego, 2011, pp. 503-521.

[7] A.M. Au, PESTICIDES AND HERBICIDES | Types, Uses, and Determination of Herbicides A2 - Caballero, Benjamin, Encyclopedia of Food Sciences and Nutrition (Second Edition), Academic Press, Oxford, 2003, pp. 4483-4487.

[8] L. Durães Sette, L.A. Mendonça Alves da Costa, A.J. Marsaioli, G.P. Manfio, Biodegradation of alachlor by soil streptomycetes, Applied Microbiology and Biotechnology, 64 (2004) 712-717.

[9] M. Dehghani, S. Nasseri, Z. Zamanian, Biodegradation of alachlor in liquid and soil cultures under variable carbon and nitrogen sources by bacterial consortium isolated from corn field soil, Iranian Journal of Environmental Health Science & Engineering, 10 (2013) 21-21.

[10] Y. Jeon, J. Kim, S. Cho, T.H. Kim, Bifenox: methyl 5-(2,4-di-chloro-phen-oxy)-2-nitro-benzoate, Acta Crystallographica Section E: Structure Reports Online, 69 (2013) o1206-o1206.

[11] K. Vorkamp, R. Bossi, K. Bester, U.E. Bollmann, S. Boutrup, New priority substances of the European Water Framework Directive: Biocides, pesticides and brominated flame retardants in the aquatic environment of Denmark, Science of The Total Environment, 470-471 (2014) 459-468.

[12] S.M. Smith, 6.23 - Environmentally Induced Heart Malformations A2 - McQueen, Charlene A, Comprehensive Toxicology (Second Edition), Elsevier, Oxford, 2010, pp. 429-446.

[13] W. Mnif, A.I.H. Hassine, A. Bouaziz, A. Bartegi, O. Thomas, B. Roig, Effect of Endocrine Disruptor Pesticides: A Review, International Journal of Environmental Research and Public Health, 8 (2011) 2265-2303.

[14] R. Keijzer, J. Liu, J. Deimling, D. Tibboel, M. Post, Dual-Hit Hypothesis Explains Pulmonary Hypoplasia in the Nitrofen Model of Congenital Diaphragmatic Hernia, The American Journal of Pathology, 156 (2000) 1299-1306.

[15] A. Stachniuk, E. Fornal, Liquid Chromatography-Mass Spectrometry in the Analysis of Pesticide Residues in Food, Food Analytical Methods, 9 (2016) 1654-1665.

[16] O. Coskun, Separation techniques: Chromatography, Northern Clinics of Istanbul, 3 (2016) 156-160.

[17] E. Forgács, T. Cserháti, CHROMATOGRAPHY | Principles A2 - Caballero, Benjamin, Encyclopedia of Food Sciences and Nutrition (Second Edition), Academic Press, Oxford, 2003, pp. 1259-1267.

[18] X. Cao, P.A. Limbach, Mass Spectrometry: Nucleic Acids and Nucleotides Studied Using MS☆ A2 - Lindon, John C, in: G.E. Tranter, D.W. Koppenaal (Eds.) Encyclopedia of Spectroscopy and Spectrometry (Third Edition), Academic Press, Oxford, 2017, pp. 764-771.