Optoelectronic Method for Increasing the Signal-to-Noise Ratio in Mass Spectrometry for Urinary Disulfoton Identification

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Abstract. Mass spectrometry is an optoelectronic method of determining organic substances by comparing their mass spectrum with mass spectra found in system libraries. In the case of biological products, substances of interest, biotic or xenobiotics, may be "hidden" from the background of the analyzed matrix noise, which alters the major aspect of the mass spectrum obtained and faces the impossibility of their identification. A gas chromatograph coupled with mass spectrometer (GC-MS) Varian was used, to develop a selected ion monitoring (SIM) method for increasing the signal-to-noise ratio for identifying the disulfoton in urine samples.

Keywords: GC-MS · SIS · Disulfoton · Urine

1 Introduction

Organophosphorus compounds is a class of substances widely used as a pesticide. These include disulfotone, which is on the list of class I*a* (extremely hazardous) compounds according to the degree of toxicity [1]. Disulfotone (O,O-diethyl-S-2-ethylthioethylphosphorodithioate) known as Di-Syston, Disultex, Dimaz, Solvigran and Solvirex is a systemic insecticide used to combat plant lice (Aphide) and for seed and soil treatment. The mean toxicity value is: DL50 = 2.6–12.5 mg/kg in male rats and for female rats DL50 = 1.9–2.5 mg/kg, orally [2].

The effectiveness of organophosphorus compounds in pest control in agriculture refers to their ability to inhibit acetylcholinesterase (AChE). By inhibiting the enzyme acetylcholinesterase organophosphorus compounds has prevented proper functioning

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of a nerve junction. Recovery of acetylcholinesterases in the blood is localized (0.5–1% per day), severe poisoning remaining below normal over 3 months [3, 4].

Mass spectrometer represents the relative abundance of ions resulting from the ionization process of a family of molecules. The mass spectrum characterizes the unique molecules from which it has been evidenced, which gives it the property to identify with its respective molecules, from an unknown sample. If the concentration of the substance of interest is very low, the system will also ionize the molecules that accompany the sample of interest, the spectra of these molecules overlapping the spectrum of the target molecule. For this reason, methods of increasing system signal/noise ratio must be found [5].

For urine analysis of disulfotone, various detectors for chromatographic gas analysis techniques can be used, such as flame photometric detectors that exhibit good reproducibility [6] or high specificity mass spectrometers [7], presented in Table 1. The detection limit for all samples −1 μg/kg.

Table 1. Method for determination of a disulfoton in a biological sample.

Optoelectronic method	Sample	Method of preparation	Biblio
GC/FPD	feces	Chloroform extraction, oxidation with m- chloroperbenzoic acid	[8]
GC/MS (SIM)	urine blood	Extraction with hexane, concentration, dilution with acetonitrile	[9]
GC/MS (SIM)	urine plasma	Plasma: extraction with ethyl acetate. Urine: pH adjustment of 7.4, centrifugation, extraction with ethyl acetate	[10]
GC/FPD capilar GC/MS	urine blood	Dilution with 2% saline, fractionation by column chromatography, oxidation with potassium permanganate, Fractionation by column chromatography	[11]
GC/FPD capilar	bovine liver	Extraction with methanol-methyl chloride, chromatographic column cleaning concentration	[12]

FPD - flame photometric detector

The objective of this paper is to develop an optoelectronic method for increasing the signal/noise ratio of the mass spectra obtained in determining the very low concentrations of disulfoton present in the matrices of interest. The method is developed on a gas chromatographic column chromatography system coupled with a mass spectrometer.

2 Experimental Set-up

The objective of this paper is to develop an optoelectronic method for the determination of disulfoton by capillary column gas chromatography coupled with mass spectrometry and to show the importance of the application of selected ion monitoring (SIM) method

in the detection of disulfotone in the matrices of interest. Generally, disulfotone, as is the majority of organophosphorus compounds, is analyzed by gas chromatography with mass spectrometer (GC-MS) using the classical method. These analytical techniques, however, have precision problems in detecting disulfotone that may occur due to matrix-related interferences. The mass spectrometric detection method in the ionic monitoring variant contributes to the elimination of these interferences between disulfoton and other chemical compounds in the matrix that remained after separation and did not separate by gas chromatography.

2.1 Material

It was used a system GC-MS/MS Varian, consisting of a Chrompack 3800 gas chromatograph and a Saturn 2000 mass spectrometer. The gas chromatograph is equipped with a Factor Four column of 30 m length and 0.25 mm diameter. The mass spectrometer is an ion trap Paul with electron impact ionization. Electronic ionization is preferred in the analysis of compounds with low polarity and relatively low molecular weight.

The samples were collected from patients admitted to the ATI II Department of the Emergency Clinical Hospital Bucharest.

2.2 Method

To obtain the injection matrix, 50 ml of urine was used. These spiked with 100 μl disulfoton solution stock are mixed with 5 ml of phosphate buffer and 5 ml of a mixture of solvents (chloroform, dichlorethane and dichlormethane 1:1:1). For the quality control of the results it adds 300 μl internal standard (midazolam solution stock). The mixture is mixed for 10 min. Take the supernatant and repeat the procedure above. After washing the supernatant, the remaining mixture is centrifuged for 8 min at 2500 rpm. After centrifugation the supernatant is discarded and the remaining mixture is brought to dryness at 80 °C. The residue was diluted with 100 μl of the solvent mixture previously used. This represents the injection matrix.

The operating parameters of the GC: gas carrier He with flow 1.2 ml/min, split ratio 1:10, septum purge 0.5 ml/min, injection temperature 300 °C, oven program start at 140 °C, wait 1 min, increase the temperature to 290 °C with 5 °C ratio, wait 12 min at 290 °C.

The operating parameters of the MS: manifold temperature 80 °C, trap temperature 170 °C, line transfer temperature 260 °C, ionization current 10 μA, mode AGC (Automatic Gain Control), acquisition data 50–450 amu, background mass 45, 1 scan/s, for fool scan.

To increase the signal-to-noise ratio its used selected ion monitoring (SIM) method for disulfoton: 85–92; 140–145; 150–159; 272–278 amu.

The ionization current was 50 μA, background mass 80 amu, 1 scan/s.

3 Experimental Results

Following the application of the GC/MS in full scan and GC/MS selected ion monitoring (SIM) methods with established and optimized parameters to obtain a separation and subsequently a satisfactory disulfoton identification. The analysis method consists in identifying the chromatographic peaks by comparing the mass spectra obtained with the spectra in the mass spectrum spectra library (Nist98, PMW, Wiley6) and the retention time.

Molecules are ionized bombarded with energetic electrons (normally 70 eV) in a low pressure region (less than 10^{-5} torr). The heaviest loaded fragment normally observed under EI conditions is the molecular ion, M +, produced by the loss of an electron from the neutral molecule and generates fragments of the compound. The number of ions created depends on Ionization Time so as long as the ionization time is higher, the more ions are created.

The following results:

Disulfoton ($C_8H_{19}O_2PS_3$) Molecular weight: 274 Retention time: 20.335 min.

Electron ionization (EI +) generates positively charged ions.

In Fig. 1 there is presented the ion chromatograms and mass spectrum for disulfoton obtained in full scan.

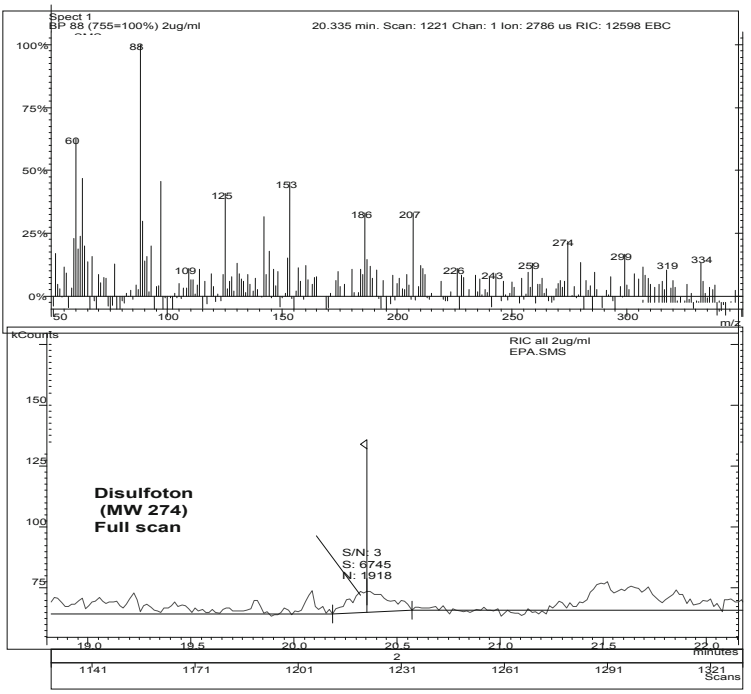

Fig. 1. The ion chromatogram and mass spectrum for disulfoton obtained in full scan.

In Fig. 2 there is presented the ion chromatograms and mass spectrum obtained from same sample for disulfoton obtained in selected ion monitoring (SIM).

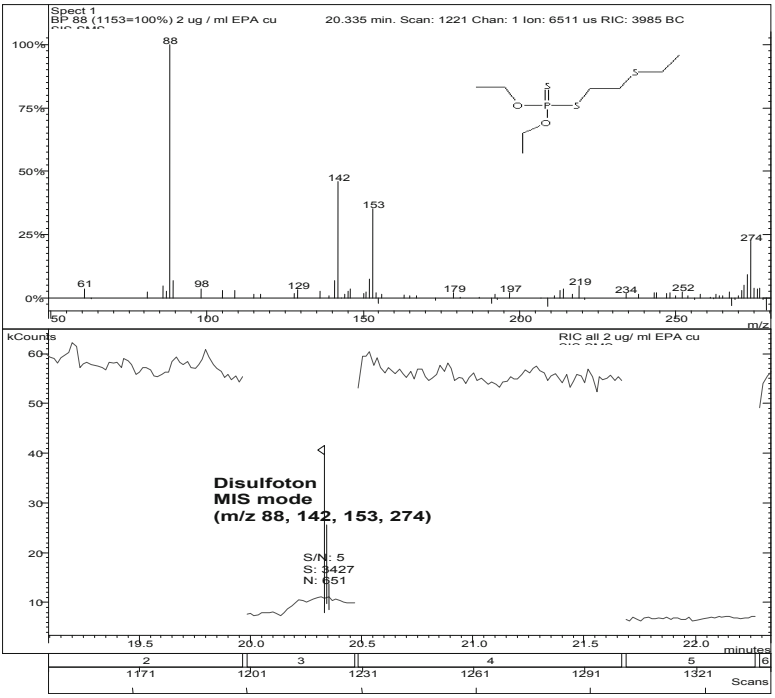

Fig. 2. The ion chromatogram and mass spectrum for disulfoton obtained in selected ion monitoring (SIM).

4 Conclusions

Peaks that have formed following the analysis of the sample by the selected ion monitoring (SIM) method, increasing the signal-to-noise ratio of disulfoton in the urine sample.

Increasing the signal/noise ratio also results in an increased sensitivity of the method for determining the disulfoton in the urine samples.

References

1. WHO IPCS: The WHO Recommended Classification of Pesticides by Hazard. IPCS (International Programme on Chemical Safety). ISBN 978 92 4 154796 3 (2009)
2. Wagner, S.L.: The acute health hazards of pesticides. In: Witt, J.M. (ed.) Chemistry, Biochemistry, and Toxicology of Pesticides. Oregon State University Cooperative Extension Service, Corvallis, OR (1989)

3. Voicu, V., Macovei, R.A., Miclea, L.: Clinical toxicology guide, 2nd edn., Brumar, Timişoara (2012)
4. Macovei, R., Dănescu, I., Ionică, M., Caragea, G.: The pattern of acute pesticide poisoning admitted in ICU II Toxicology Emergency Clinical Hospital Bucharest between 1997–2007. Clin. Toxicol. **47**(5), 507 (2009). meeting abstract 285
5. Ionică, M.: Chromatography of gases and liquids coupled with mass spectrometry. Training course. Military-Medical Scientific Research Centre Bucharest (2016)
6. Holstege, D.M., Scharberg, D.L., Richardson, E.R., et al.: Multiresidue screen for organophosphorus insecticides using gel permeation chromatography-silica gel cleanup. J. Assoc. Off. Anal. Chem. **741**, 394–399 (1991)
7. Kawasaki, S., Ueda, H., Itoh, H., et al.: Screening of organophosphorus pesticides using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J. Chromatogr. **595**, 193–202 (1992)
8. Bowman, M.C., Beroza, M.: Rapid GLC method for determining residues for fenthion, disulfoton and phorate in corn, milk, grass and feces. J. Assoc. Off. Anal. Chem. **52**, 1231–1239 (1969)
9. Hattori, H., Suzuki, U., Yasuoka, T., et al.: Identification and quantitation of disulfoton in urine and blood of a cadaver by gas chromatography/mass spectrometry. Nippon Hoigaku Zasshi **36**, 411–413 (1982)
10. Singh, A.K., Hewetson, D.W., Jordon, K.C., et al.: Analysis of organophosphorus insecticides in biological samples by selective ion monitoring gas chromatography-mass spectrometry. J. Chromatogr. **369**, 83–96 (1986)
11. Yashiki, M., Kojima, T., Ohtani, M., et al.: Determination of disulfoton and its metabolites in the body fluids of a Di-Syston intoxication case. Forensic Sci. Int. **48**, 145–154 (1990)
12. Holstege, D.M., Scharberg, D.L., Richardson, E.R., et al.: Multiresidue screen for organophosphorus insecticides using gel permeation chromatography-silica gel cleanup. J. Assoc. Off. Anal. Chem. **74**, 1394–1399 (1991)