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Determination of methamidophos by LC-ESI-MS/MS in a fatal poisoning case.

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ABSTRACT

Methamidophos is an organophosphorate pesticide which has been associated with acute fatal intoxications in Colombia. This paper describes an analytical method for the determination of methamidophos in blood and urine which consists in the precipitation of proteins in blood and a solid phase extraction (SPE) in urine. The analysis is done by LC-ESI-MS/MS using methamidophos-d6 as internal standard. The detection limit obtained was 50 ng/mL and this paper reports the results for precision, linearity, accuracy and matrix effect. The method was applied to a fatal intoxication case in which methamidophos was detected both in urine and blood. The concentration in blood was greater than 5,0 µg/mL. The study shows the utility of using LC-ESI-MS/MS in the analysis of methamidophos in biological samples for its application in forensic toxicology.

Keywords: Methamidophos, Liquid chromatography with electrospray ionization and tandem mass spectrometry, forensic toxicology

Introduction

Methamidophos is a tioorganophosphate used as an acaricide and insecticide. It inhibits cholinesterase and is employed in agriculture since 1970 [6]. Methamidophos is used for insect control in alfalfa, potato, cotton and tomato crops.

Methamidophos is highly toxic when ingested orally, through the skin or by way of the respiratory tract [5] [7] and the information related to the distribution and metabolization of methamidophos in human beings is scarce. It is eliminated mainly by way of the urinary system either without

transformation or as its metabolite O,S-Dimethylhydrogen phosphorothioate (O,S-DMPT) [6] [8]. Its main function is to inhibit the acetylcholinesterase enzyme thus preventing the degradation of the acetylcholine which produces a cholinergic hyperactivity which in turn puts the individual in a state of lethal risk.

Methamidophos has been associated with acute fatal intoxications in Colombia [4]. This makes its determination in biological matrices a very important issue in the field of forensic toxicology in the country.

For this study a quick and simple method was developed in order to quantify the methamidophos in the blood and its qualitative identification in urine using LC-ESI-MS/MS. This analytical methodology was applied to a case of fatal intoxication suspected of the presence of pesticides.

Materials and methods

Methamidophos and methamidophos-d6 standards were acquired from Dr Ehrenstorfer GmbH. All the reagents were analytical grade except methanol which was HPLC grade. The methamidophos and methamidophos-d6 stock solutions were prepared at a concentration of 1 mg/mL in methanol. The blanks for urine were acquired from volunteers. The blank for the blood was prepared from a 50:50 concentrated red cell solution and deionized water.

Procedure

Blood: One milliliter of the whole blood sample was pipetted into a test tube and 50 µL of methamidophos-d6 (Internal standard) were added at a concentration of 5,0 µg/mL and then stirred. 1 mL of cold acetonitrile was slowly added drop by drop (at freezer temperature) while in the vortex (It is essential not to stop the stirring process). The test tube was sealed and left in the freezer during 30 minutes. Then it was centrifugated at 2000 rpm and the organic fraction was transferred to a new test tube. The solution was evaporated- while being stirred- at 45ºC to a final volume of 0.5 mL. It was then evaporated under vacuum until fully dry.

Finally the analyte was reconstituted with 100 µL of a methanol/water solution 50:50 (v/v) with 0,1% formic acid. This procedure was applied to each level of the calibration curve.

Urine: 100 µg L of methamidophos-d6 solution was added to a 2 mL-urine sample, it was then stirred and extracted by a solid phase procedure. In this procedure a Hypersep verify CX 200mg/6mL cartridge was prepared by adding 2,0 mL of methanol first followed by 2,0 mL of a pH 6.0 phosphate buffer. Once the urine sample was added to the cartridge it was washed with 2,0 mL deionized water, 0,5 mL acetic acid 0,01M, and 100 µL methanol then it was dried under vacuum during 10 minutes. The dried sample was then eluted with the following series of solvents: 1,5 mL acetone-chloroform 50:50 (v/v), mL acetone /dichloromethane 50:50 (v/v), 1,5 mL ethyl acetate/ammonium hydroxyde 98:2 (v/v) and finally 1,5 mL dichloromethane/isopropanol/ammonium hydroxyde 78:20:2 (v/v/v). The eluate was dried at 45ºC and reconstituted with 100 µL of a methanol/water solution 50:50 (v/v) with 0,1% formic acid.

Liquid chromatography-mass spectrometry conditions

The LC-MS system used consisted of a Thermo Electron Corporation model Thermo Surveyor. The chromatographic separation was performed on a HPLC Hypersil Gold PFP column (50 mm x 2,1 mm; 5 µm). The column temperature was 40ºC. The mobile phase was a gradient of a mixture of methanol with 0.1% formic acid and a solution of ammonium formate at a constant flow (200 µL/

min) programmed as follows: Initial 2% methanol for 1 min., then 2-98% methanol for 9-13 min. and finally, decreased to 98-2% methanol for 14-16 min.

All the mobile phase solvents were previously vacuum filtered using a membrane with a pore size of 0,22 µm. The injection volume was 10 µl.

MS Detection

Detection was performed on a **LCQ Advantage Max** ion trap mass spectrometry in tandem in product ion scan mode. The detector was equipped with an electrospray ionization source (ESI) in positive mode. Conditions were optimized for methamidophos and methamidophos-d6 by injecting them apart using a syringe pump in "T" (table 1).

This system permits the mixing of the pesticide previously prepared at a concentration of 2,0 µg/mL with the mobile phase at a 50:50 proportion. The optimized conditions of the MS were as follows:

capillar voltage 9,00 V, source voltage 5,00 kV, capillar temperature 160ºC, lens voltage 5 V, sheath gas flow rate 55 units and auxiliary gas flow rate 15 units.

Table 1.

Compound	Transition (m/z)	CE (%)	Polarity	RT
Methamidophos	141,9→111,9 141,9→93,9	30	positive	2,72
Methamidophos-d6 (Internal Standar)	148,1→114,9 148,1→96,9	30	Positive	2,59

Tab. 1 *shows the values for the ions (m/z), collision energy (CE), polarity and retention time, all of them optimized for the identification of each compound.*

Case history

A 17 years old boy was found dead in his home.

The authorities suspected an exogenous intoxication with a pesticide. Samples of urine, blood and gastric contents were taken during the autopsy. The gastric content was analyzed by GC/MS yielding positive results for methamidophos.

Results

Figure 1 shows the chromatogram and Figures 2 and 3 the mass spectra for methamidophos and its internal standard in the blood sample. The LOD determined by successive dilution of the compound in the matrix was 50 ng/mL for methamidophos and methamidophos-d6 in blood and urine.

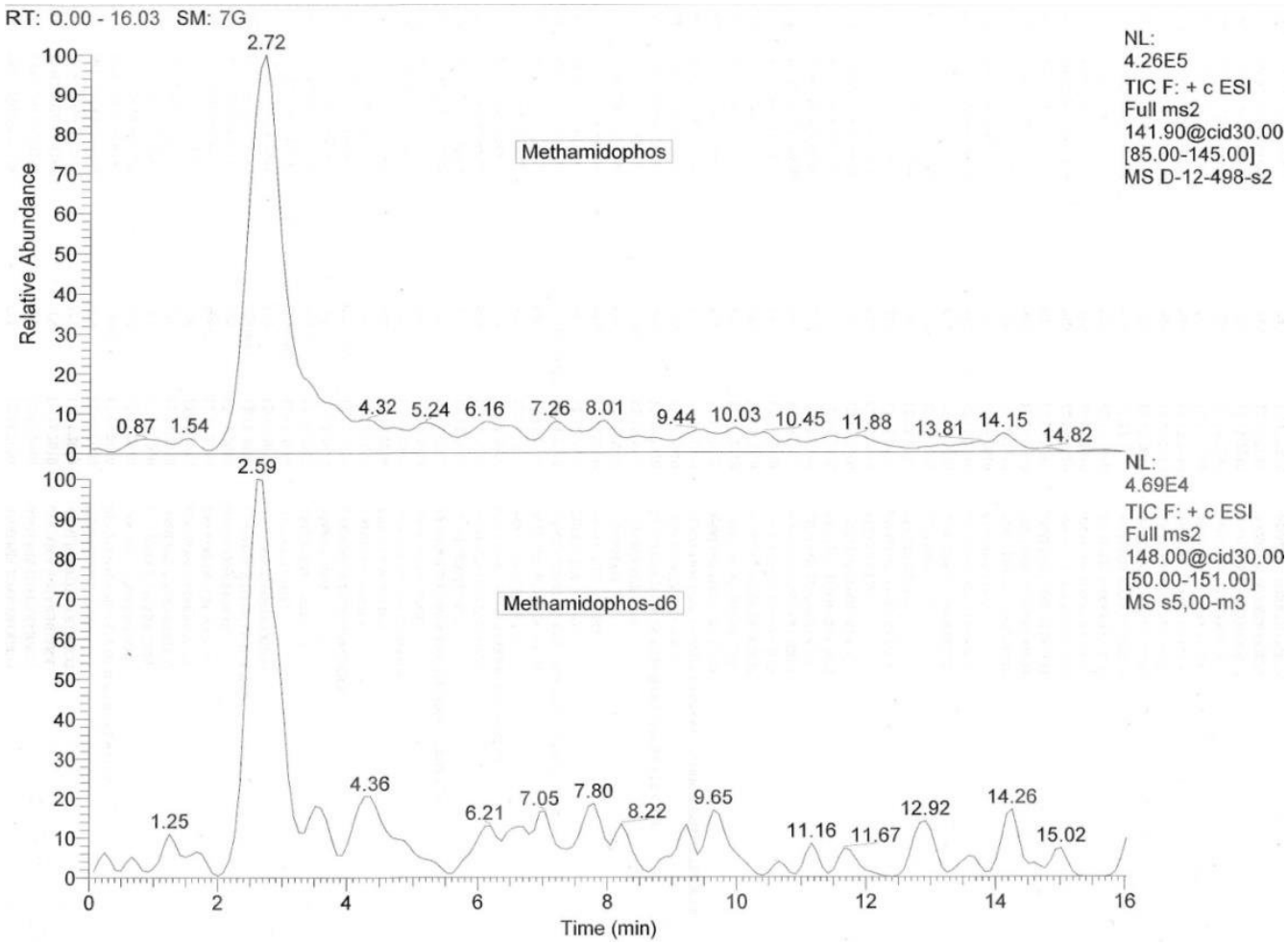

Fig. 1. *Chromatogram of methamidophos and methamidophos-d6 in blood*

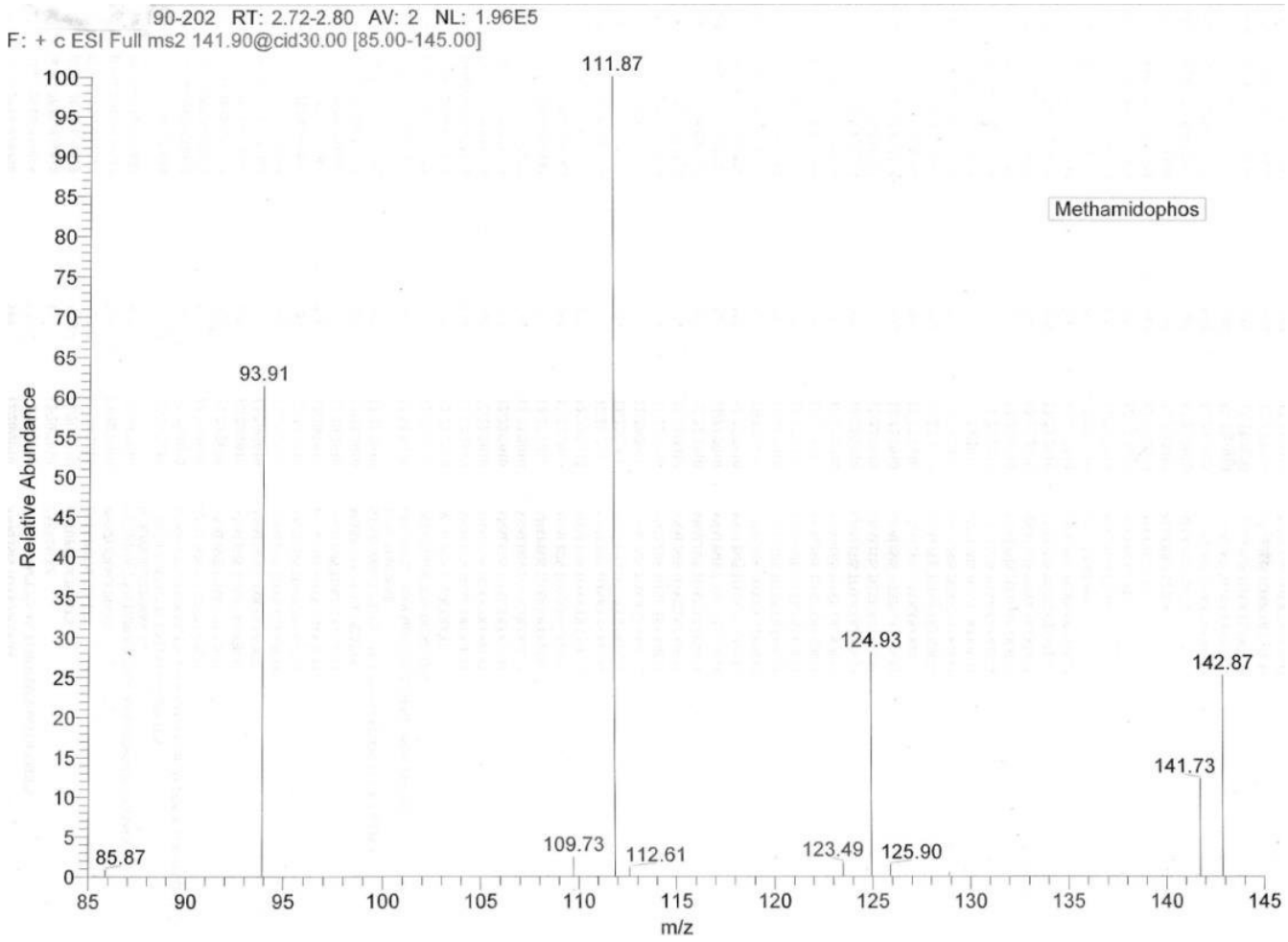

Fig. 2. *Mass spectra of methamidophos.*

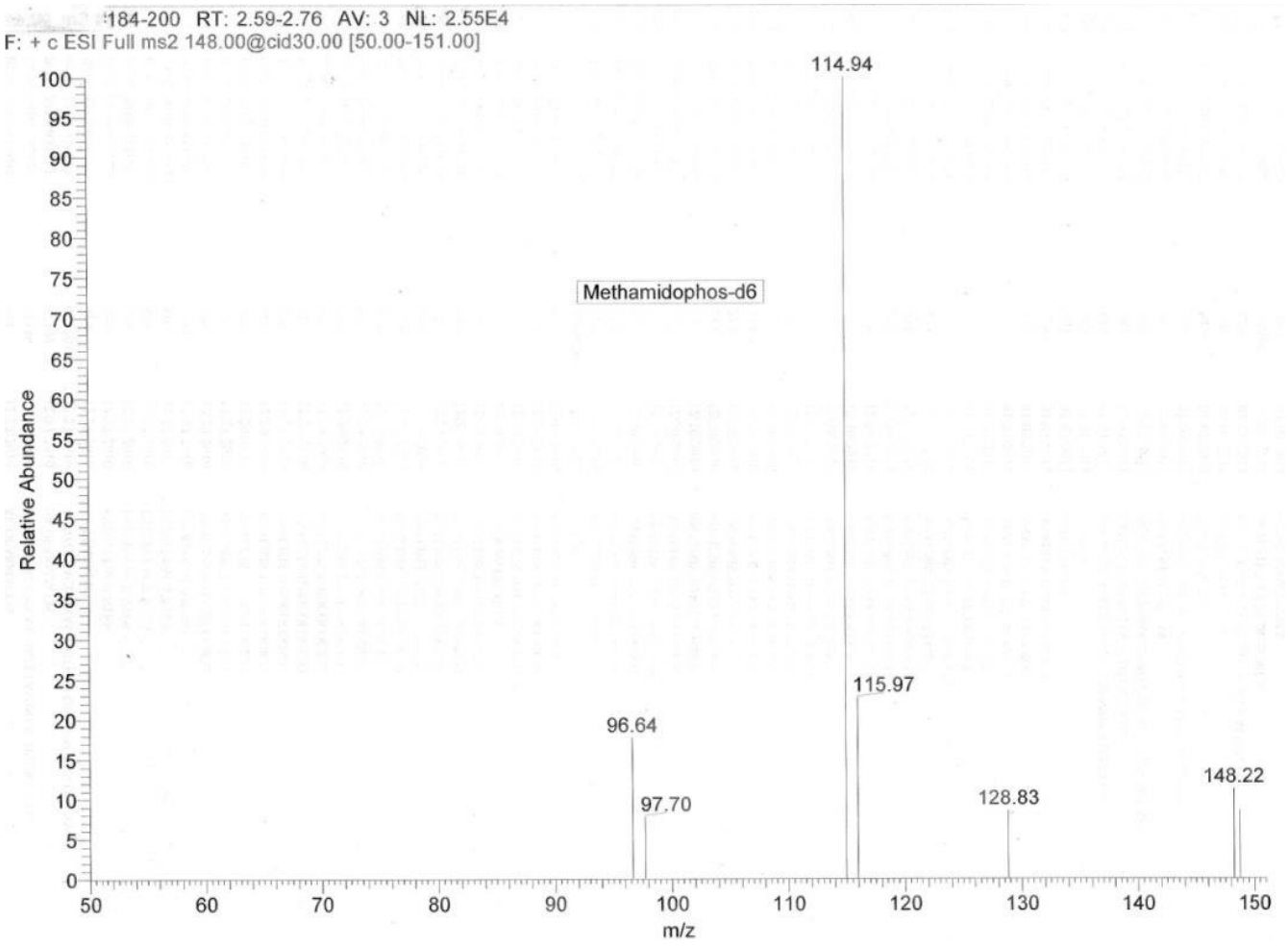

Fig. 3. *Mass spectra of methamidophos-d6.*

The lineal range for the calibration curve was between 0,10 and 5,00 µg/mL with a correlation coefficient of 0,9901. Table 2 shows the data for precision (CV) -evaluated as repeatability and intermediate precision (same day, two analysts and same analyst, two days)- accuracy (relative main error, RME) and recovery percentages evaluated at levels 0,10, 0,25, 2,50 and 5,00 µg/mL

Table 2.

Analite	**Concentration (µg/mL)**	**Repetibility (n=5)**	**Accuracy (n=5)**	**Intermediate precision Analyst (n=10)**	**Accuracy (n=10)**	**Intermediate precisión Analysis day (n=10)**	**Accurac (n=10)**
		C.V.	M.R.E.	C.V.	M.R.E.	C.V.	M.R.E.
Methamidophos	LOQ = 0,10	12,17	-9,9E-03	9,84	-2,5E-03	13,20	3,6E-03
	0,25	7,43	4,4E-03	5,63	-0,2E-03	6,72	-25,3E-0
	2,50	1,91	0,3E-03	2,02	1,1E-03	1,63	-7,9E-0
	ULOQ = 5,00	4,61	-49,4E-03	4,04	-25,1E-03	3,46	-34,6E-0

Tab. 2. *Data for precision (CV), accuracy (relative main error, RME) and recovery percentages.*

The matrix effect evaluated by the blood post-extraction addition experiment, n=6 at 0,25 µg/ml, was 14,75 % with a CV of 9,6 while that for a concentration of 5,0 µg/mL was -14,15% with a CV of 13,9.

The above methodology was applied to a real forensic case in which the methamidophos concentration in blood for the victim was found to be above the upper quantification limit. It was reported as being higher than 5,0 µg/mL.

Methamidophos was found in the urine.

Discussion and conclusions

This study established a method for the determination of methamidophos in blood and urine by LC-ESI-MS/MS. The method's recovery percentages were higher than 90% and repeatability, intermediate precision, accuracy, matrix effect and linearity values comply with the acceptation criteria established by bioanalytical guidelines [1]

LC-ESI-MS/MS is a sensible technique for the detection of methamidophos in biological samples as compared with results by GC/MS previously reported [2] [3]. The method shows high selectivity and allows for the unequivocal determination of methamidophos in biological samples, a fundamental condition for the forensic toxicology laboratories.

Even though methamidophos was found in blood at a concentration higher than 5.0 µg/mL quantifying it accurately was not possible due to the small sample size available, nevertheless the analysis of the urine sample confirmed the presence of the pesticide in the victim. Both results support the hypothesis that the cause of the death was poisoning with methamidophos.

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