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Medical applications of the GC/MS method in the acute intoxication with dimethoate – clinical case

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Abstract: *Mass spectrometry is a chemical analytical 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, like organophosphorus compounds, must be separated and identified for rapid and good medical measures (antidotism procedures) in acute intoxication case. A gas chromatograph coupled with a Varian mass spectrometer (GC-MS), was used to develop the application. The proposed objective is presenting the medical applicability in acute organophosphorus compounds intoxication management of the GC/MS method (gas chromatography coupled with mass spectrometry) as a separation and identification method for these compounds and their metabolites in urine samples.*

Keywords: *dimethoate, GC/MS, urine, acute intoxication*

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INTRODUCTION

Acute intoxications represent a worldwide problem that tends to gain more amplitude each year.

Each intoxication presents certain characteristics which stem from the degree of socio-economic development of each country.

The organophosphorus compounds are mainly used to fight pests, as an alternative to chlorinated hydrocarbons, which persist much longer in the environment. Yet these substances are very toxic for humans too.[6] Because of this, measures were taken both to limit their utilization and to control the contamination of the environment.

The most efficient, but also most toxic substances utilized as pesticides are cholinesterase inhibitors (through reversible or irreversible mechanism). For both mammals and insects, the major effect of these substances is the inhibiting of acetylcholinesterase through the phosphorylation of the esterase site. The signs of symptoms which characterize the acute intoxication are caused by the inhibition of this enzyme and the accumulation of acetylcholine. Some of these substances possess a direct cholinergic activity.[4]

The absorption of organophosphorus compounds can be realized through three methods: inhalation, digestive and through the skin. One of their main characteristics is the fixation at hair level, where they enter through the skin, thus representing a permanent source of intoxication. Through the inhalation way, the intoxication is the most rapid. Through direct action on

the bulbar respiratory center (muscarinic phenomenon) and the paralysis of respiratory musculature (nicotinic phenomenon), respiratory arrest occurs. Organophosphorus compounds are rapidly absorbed through the skin or digestively in the thin intestine. They bind well to the plasmatic proteins. High concentrations can be attained in the body within hours.[7]

Organophosphorus compounds represent a group of compounds with liposolubility or hydrosolubility, with high distribution volume. They are rapidly distributed in the liver, lungs, kidneys, heart and brain but do not accumulate.

This type of organophosphorus compounds is liposoluble, accumulating in the lexophile tissues of the organism, being a source for metabolic conversion to very toxic compounds. Thus, the intermediate syndrome can be explained (it appears between the 5^{th} and 18^{th} day from the intoxication). The toxicity of these compounds is manifested through the direct inhibition of cholinesterase, probably through direct poly enzymatic inhibition.[7]

The metabolizing of organophosphorus com-pounds takes place rapidly in the body, and as such they do not accumulate. Compounds such as parathion, malathion, phenthion, chlorpyrifos, normally inactive, upon entering the body they transform at liver microsomal level, through oxidation, in highly active compounds (paraoxon, malaoxon).

This transformation takes place under the action of paraoxonase. Their degradation takes place through hydrolytic and oxidative ways, through liver and kidney enzymes. The erythrocyte-origin cholineste-rase remain blocked for the remainder of the red blood cell's life. Their regeneration takes place slowly (0.5-1% per day), remaining below normal level for over 3 months in severe intoxications.[8]

Organophosphorus compounds are eliminated through urine as such or as metabolites.

In the current global situation, it is very probable that these substances will be used in wars, conflicts, terrorist attacks. In such scenarios, they are used as extremely toxic agents and thus continuously represent severe threats to humans, animals and the fauna.[2]

The dimethoate, patented and introduced in the 50s, is a acetylcholinesterase inhibiting organo-phosphorus compound, it is not volatile, it is water soluble and it is not mobile in soil, where it degrades with a half-life of approximately 2-4 days, depending on the conditions. Dimethoate and omethoate urine levels reflect recent exposure.

Once having entered the body, organo-phosphorus compounds are metabolized to dialkylphosphates, which are eliminated through urine. Their metabolizing takes place rapidly in the body and as such they don't accumulate.

Compounds such as parathion, malathion, phenthion, chlorpyrifos, normally inactive, enter the body and transform at liver microsomal level, through oxidation, into highly active compounds (paraoxon, malaoxon). Identification of these metabolites in the urine may be an indicator of exposure to organophosphorus compounds and can be performed through a GC/MS analytical method with an ion trap and electronic ionization.

The dimethoate is rapidly metabolized, mainly through the initial splitting of the C-N bond to obtain dimethoate carboxylic acid and, eventually, a number of tiophosphate and phosphate esters. The minor quantitative elimination way involves the oxidative metabolism to produce the oxygen analogue of dimethoate, omethoate. The parent compounds represents 1-2% of the dose excreted in the urine.[5]

MATERIALS AND METHOD

The research was performed on a GC-MS Saturn 2000 Varian system composed of gas chromatograph model Varian CP – 3800 and mass spectrometer Varian Saturn – 2000.

Establishing optimal working conditions and functional parameters for the development of a GC/MS method are important steps in the development of a GC/MS method for the separation and spectral identification of dimethoate in urine.

ANALYTICAL CONDITIONS

GC – an instrument equipped with a ion trap detector; GC – gas-cromatograph has the role of taking in the sample and separate the mixture of substances composing it.

GC/MS parameters
Injector temperature: 300° C
Carrier gas: He • Column flow: 1.2 ml/min
Separation time: 50 min
Ionization mode: electron impact (70eV)
Ionization current: 20 µA
Ionization temperature: 170° C
Detection mode: full scan
Manifold temperature: 80° C
Ion trap temperature: 170° C
Interface temperature GC-MS: 260° C

The separation of the compounds was realized by the active layer of the DB-5MS capillary column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm). The optimal conditions for chromatographic separation and detection were established following the study on the compound's retention time's dependency on the structures of the said substances.

The temperature program of the gas-chromatograph's column's furnace is presented in Table 1.

Table 1: The column gas furnace temperature program.

Temperature (°C)	Rate (°C/min)	Hold (min)
140	0.00	1
290	5.00	19.00

MS – mass spectrometer – has the role of analysing molecules that come out of the gas-chromatograph through their unique mass spectre. Thus, the molecules that come out of the GC can be identified by the user. Mass spectrometry established the relative abundance of ions resulted from the ionization process of an organic molecule. The method is used in chemical analyses, in the analysis of some quantum processes or in the separation of certain chemical elements. The determined mass represents the m/z ratio (mass to charge) of the atom or group of atoms from which the ions resulted.

Mass spectrometer parameters
Manifold temperature = 800° C
Ion trap temperature = 1700° C
Ionization current = 20 µA
Acceleration tension = 70 eV
Working times
0 - 5 min – closed filament
5 - 37 min acquisition in mass domain 50 - 450 amu.
Acquisition domain 50 – 400 amu
Segment setpoint
Scan time = 1 sec/scan
Multiplier offset = 0 V
Emission current in FS 10 µA
Ion threshold 1 count

Scanning parameters for ions formed in the trap are presented in Table 2.

Table 2. Scanning parameters for ions formed in the trap.

Low Mass (m/z)	High Mass (m/z)	Ionization Storage Level (m/z)	Ionization Time Factor (%)
10	99	48.0	100
100	249	48.0	100
250	399	48.0	100
400	650	48.0	100

Normally, the mass spectrometer operates in the domain in which the analyzed substances will be found. When coupling it with a gas-chromatograph, substances no greater than 450 amu will be sent to the mass spectrometer, as those with greater molecular weight cannot be vaporized in the chromatographic column.

Thus, the maximum acquisition domain will be 50 – 450 amu, as below 50 amu an acquisition is not typical, since the atmosphere in the apparatus has a rich spectre up to 44 amu.

The use of the full scan technique (FS) is very good, since following the obtaining of the mass spectre, it can be compared with mass spectres already existant in dedicated spectral libraries. In this case though, the duration of a ion's analysis is of 4ms, in case it is used as an acquisition time for a scan of 1 s.

Confirming the identity of the compounds relies on comparing the mass specter and ratio of reference

ions' abundance for each analyzed substance identified in the sample with those of standards using the mass specter library. To identify the obtained specters, the following were used: Pfleger – Maurer – Weber specter library (PMW), specialized for compounds of interest in toxicology as well as specter libraries NIST2000 and Wiley6. If a mass spectre obtained from the sample cannot be compared with mass spectres in specialize spectral libraries, the identification of these compounds in biological matrices would not be possible to perform.

RESULTS AND DISCUSSIONS

Dimethoate has moderate acute toxicity for mammals (for example, DL50 in mice and rats is 150 and 400 mg/ kg of bodyweight) (IPCS, 1989). Omethoate is approximately 10 times more toxic and a stronger cholinesterase inhibitor than dimethoate. Dimethoate is well distributed in the body's tissue and metabolized in the liver to omethoate (most probably through the enzyme system of P450 cytochrome) which is then quickly transformed into multiple dialkyl methyl phosphate metabolites, which are eliminated in urine within 1-2 days. Dimethoate is considered mutagenic, but it is not teratogenic. [1,10].

In order to verify the developed methods, they were applied on biological samples obtained from patients in the ATI II Clinical Toxicology Section, patients suspected of acute organophophorus compounds intoxication. The urine (aprox. 25 ml) undergoes liquid-liquid extraction procedures in order for the sample to be analyzed through the GC/MS system. For example, we present the medical applicability of this method in the case of an acute dimethoate intoxication.

The separation and identification of dimethoate or omethoate in urine corroborate with the enzymatic activity determinations for serum pseudo-cholinesterase and lead to the establishment of an analytical diagnosis in case of an acute dimethoate intoxication and the initiation of specific therapies for this type of intoxication (such as antidotes). Identifications are performed through the comparison of the mass specter obtained through the analysis of urinary extract samples with mass specters already existent in the database. This specter is obtained based on the molecular mass of the compound of interest which, following fragmentation, gives birth to specific spectral lines. These are shown in tables 3 and 4.

CASE REPORT

C.P., male, aged 19, no occupation

Admission reasons
- Coma
- Acute respiratory insufficiency
- Muscular fasciculation

APP – no significant case history

History – patient with no significant case history is found by his parents in a coma with respiratory dysfunctions and muscular fasciculation, symptoms that follow the voluntary ingestion of an organophosphorus pesticide. Near the young man, the parents found an unlabeled bottle containing a liquid with a smell particular to insecticides. They call the ambulance and the young man is transported to the Clinical Emergency Hospital.

In the Major Emergencies Department, the patient is in a coma with severe dyspnea and muscular fasciculation. The oropharyngeal secretions are vacuumed and orotracheal intubation and Ruben balloon ventilation are applied.

Table 3: Specific ionic fragments and spectral lines for dimethoate

Compound	Spectral line (m/z)	Chemical formula of ionic fragment
Dimethoate C5H12NO3PS2 (M=229)	157	– [M-72] - (CH3O)2PS.S+
	143	– [M-86] - (CH3O)(HO)PS.S+
	125	– [M-104]- (CH3O)2PS+
	93	– [M-136]- (CH3O)2P+

Figure 1: Mass spectre for dimethoate (Nist98 library)

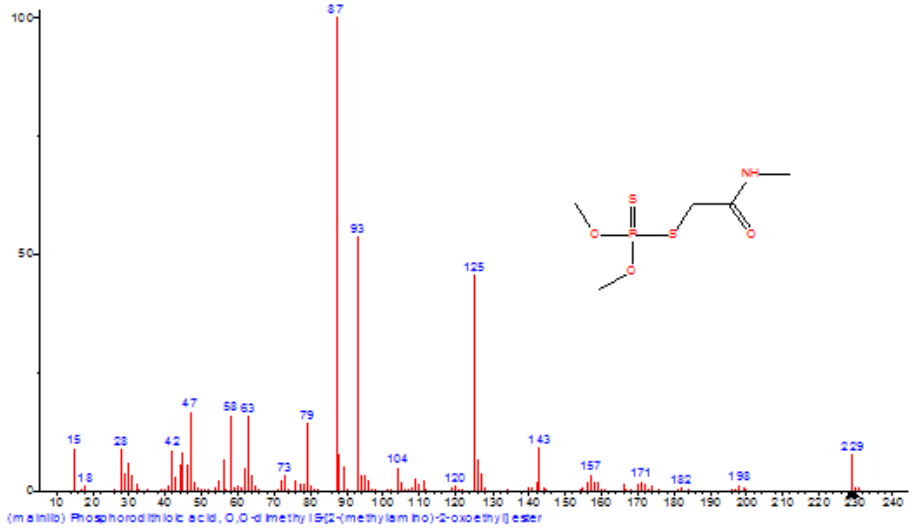


Figure 2: Mass spectre for omethoate (Nist98 library)

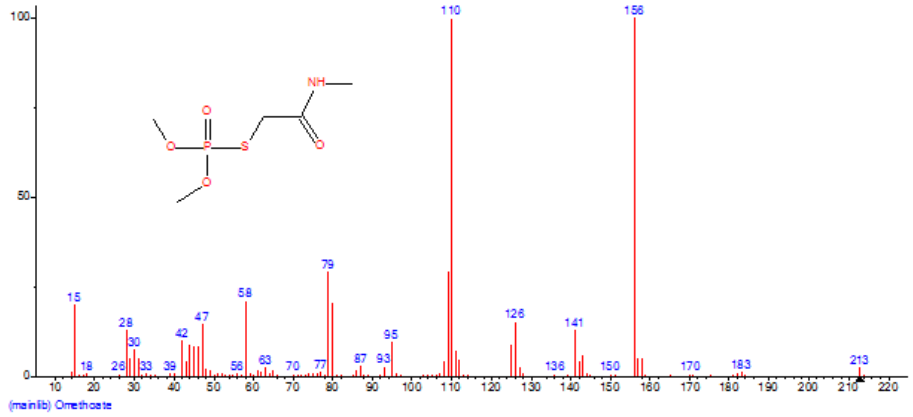


Table 4: Specific ionic fragments and spectral lines for omethoate

Compound	Spectral line (m/z)	Chemical formula of ionic fragment
Omethoate C5H12NO4PS (M=213)	156	– [M-57] - CH3NCO
	141	– [M-72] - (CH3O)2P=O.S+
	126	– [M-87] - (CH3O)2(HS)P+
	110	– [M-103] - (CH3O)2(HO)P+
	109	– [M-104] - (CH3O)2P=O+
	79	– [M-134] - (CH3O)(HO)P+

The determination of pseudocholinesterase activity detects a high degree of inhibition of 0.5 UI/ml. A urine sample is being taken for the toxicological examination and is sent to the Analytical Toxicology Laboratory. The patient is hospitalized in the ATI Toxicology Section.

Objective examination upon admission:

- Severe general condition;
- Reed IV coma; non-reactive;
- Pale, sweaty skin;
- Cyanotic extremities;
- Miotic, equal pupils, with slow photomotor pupil

reflex;
- Strong reflexes in all four members, with subintrant muscular fasciculation;
- At pulmonary level, bronchial rales bilaterally diffuse;
- Does not efficiently ventilate on IOT probe, so mechanical ventilation is applied;
- Diarrhea;
- Arterial Tension = 80/60 mm Hg;
- Subfebrile (t^o = 37.5).

ECG upon admission records sinus bradycardia (44/min) without conduction or repolarization.

The CT brain scan and lumbar puncture exclude a vascular etiology of the coma.

The analytical toxicological GC/MS examination of the urine:

Following the analysis of the urine, the total ion chromatogram that is shown in Figure 3 was obtained. In it, besides dimethoate, its metabolites can also be identified.

The substances identified through the above GC/MS method used for the urine analysis are shown in table 5.

The cardiopulmonary radiography detects a homogenous opacity situated in the inferior right pulmonary field.

Therapeutic measures

Stabilization
- Vacuuming tracheobronchial secretions
- Support ventilation
- Nasogastric intubation; gastric lavage;
- Central sub-clavicle catheter
- Parenteral fluids
    - glucose solution;
    - Ringer solution;
    - isotonic saline solution;
    - Gelofusine;

Support therapy
- Specific antidote – atropine under clinical surveillance
- Mucolytic – acetyl cysteine;
- Bronchodilators – miofilin;
- Antibiotherapy in combinations (penicilin G 1 milion UI/6 hours + gentamicin 80 mg/day + metronidazol 500 mg/day);
- Plasma – 4 units/day, for 3 days and 2 units/day for 2 days;
- Monitoring vital functions;
- Monitoring pseudocholinesterase activity.

The applied therapeutic measures, respiratory and general nursing are continued.

Clinical evolution

4 days from admission, the evolution is favorable; the patient is conscious; he is taken off the mechanical ventilation apparatus; he breathes spontaneously with the intubation probe and is extubated. His oral cavity is washed.

The cholinesterase activity is measured: 2.1 UI/ml.

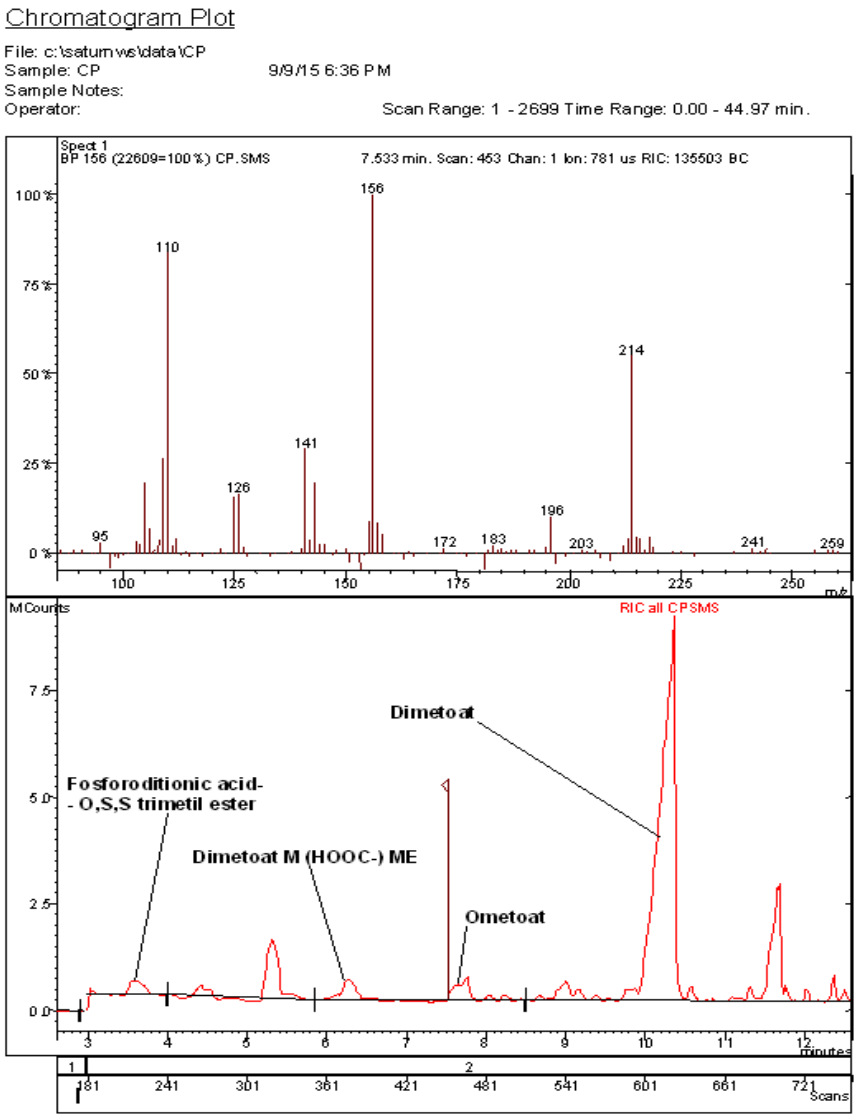
The antibiotherapy is continued 3 days from the extubation to solve his pulmonary affection.

The evolution is favorable. The patient is released 7 days from admission with no neurological damage.

Table 5: The substances identified in the urinary extract that was analyzed through the GC/MS method and their specific spectral lines.

Compound	MW	EI fragment ions (m/z)
Dimethoate	229	87, 125, 93, 79, 229
Omethoate	214	156, 126, 110
Dimethoate M (HOOC-) ME	230	93, 125, 198, 230
Phosphoditioic acid – O,S,S trimethyl ester	172	93, 109, 125

Figure 3: Dimethoate and its metabolites – omethoate, dimethoate M (HOOC-) ME and the phosphoditioic acid – O,S,S trimethyl ester. Omethoate mass specter.



CONCLUSIONS

The GC-MS method is the only method that can determine organophosphorus compounds in unknown matrices for the quality control of water, the environment and foods or to establish the analytical diagnosis in case of contamination/intoxication with organophosphorus compounds.

The mass spectrometer can acquire data through various methods. In “full scan” method (FS), the mass spectre obtained through electron impact ionization can be compared with mass spectres found in specialized libraries, making possible the identification of these substances in unknown matrices.

To reduce the number of false positive or false negative results, analytical procedures based on precision, accuracy, detection limits, error source identification are needed but also the expanding of existent spectre databases.

A quick and correct analytical diagnosis influences the quickness and correctness of specific therapy measures that can be applied in such a context.

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