

SURFACE IONIZATION MASS SPECTROMETRY. ANALYSIS OF POST-MORTEMES FOR FATAL POISONING BY CHLORPROMAZINE

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ABSTRACT: In this paper the results of the investigation of post-mortems (stomach, intestine, kidney and urine) are presented for the chlorpromazine poisoning, using methods of surface ionization-mass spectrometry (SI/MS) as well as chemical, optical, TLC and GC/MS methods widely used in analytical toxicology. The SI mass spectra obtained show the high efficiency of the ionization of chlorpromazine and its metabolites: monodesmethyl-, sulphoxid-, and hydroxichlorpromazine. The high selectivity of SI to nitrogen bases and few lines of the SI mass spectra make it possible to identify, with a high accuracy, the samples without their chromatographic separation.

KEY WORDS: Surface ionization; Chlorpromazine; GC/MS.

Problems of Forensic Sciences, vol. XLIII, 2000, 231–236
Received 9 September 1999; accepted 16 May 2000

INTRODUCTION

At present, the derivatives of phenothiazine (strong neuroleptics), owing to their wide application in medicine and frequent misuse, were studied by different physical and chemical methods such as spectrophotometry, TLC, GC/MS, GC/MS/MS. The data base of GC and MS with electron ionization was published. The sensitive detection and identification of phenothiazine derivatives and their metabolites in biosolutions and tissues is the actual problem of analytical toxicology.

The SI/MS studies of some phenothiazine derivatives – chlorpromazine, tioproperazine and periciazine – show that owing to the high sensitivity and selectivity of the SI method, it is sufficient to have 1–5 ng of chlorpromazine for the reliable identification [1]. The high sensitivity and of the SI/MS method and its selectivity for strong neuroleptics, phenothiazine derivatives, make it possible to analyze them in mixtures including biosolutions and tissues without preliminary chromatographic separation.

The object of this work is to study SI/MS of post-mortems – stomach, intestine, kidney and urine – for the chlorpromazine poisoning, demonstrate the analytical possibilities of SI/MS for determination and identification of psychotropic preparations in post-mortems, demonstrate the high analytical possibilities of SI/MS, as compared to the results of the sample analysis by optical, TLC and GC/MS (Hewlett-Packard HP-6890) methods.

EXPERIMENTAL

The optical measurements are made with spectrophotometer CФК-2 (dish – 5.108, lightfilter – green, $\lambda = 490$ nm). The solution of chlorpromazine in the range of (10÷300) $\mu\text{g/ml}$ was used to build the calibration curve.

In SI/MS experiments a standard mass-spectrometer MI-1201B modernized for surface ionization (SI) studies was used. An oxidized tungsten band with the size of $1 \times 12 \times 0.02$ mm³ was used as a thermoemitter. The thermoemitter temperature was scanned in the range of 600÷1200 K. The samples were evaporated from the Knudsen quartz cell.

TLC and GC/MS studies were carried out using “Silufol” chromatographic plates and chromato-mass-spectrometer HP-6890.

Extraction procedure

100 g of grinding materials of a mortem human body were soured by the 10% HCl up to pH 2.0–3.0, then the mortem material was extracted by 100, 50 and 50 ml of acetonitrile for 30, 15 and 15 minutes while the mechanical shaking. Acetonitrile extracts were filtrated through a paper filter moistened by distilled water into a separating funnel (1000 ml) with 500 ml of the 2.5% water solution of sodium sulphate. The content in the separating funnel was shaken to obtain a homogeneous solution, soured by the 6 N HCl up to pH 2.0–3.0 (according to a universal indicator) and 3 times (for 10 minutes) extracted by ether (100 ml). The ether layer was separated. After the extraction by ether, the acid water acetonitrile solution was mixed with the 50% solution of sodium hydroxide up to pH = 13.0 (according to indicator “Phan”), 3 times (for 10 minutes) extracted by ether (100 ml). The ether extracts were put into a flask and evaporated under vacuum by a rotor evaporator at 40°C up to a volume of 35–40 ml. The obtained extract was filtrated into a 50 ml flask through a paper filter with the diameter of 5–6 cm containing 1.5–2.0% anhydrous sodium sulphate. The evaporating flask and the filter were washed with 15–10 ml of ether, which were added with the filtrate in the measuring flask (50 ml). The content of the measuring flask was again extracted from the ether phase by the 0.5 N HCl (10, 10, 10, 5 and 5 ml). The sulphate re-extract was put in a measuring flask with the volume 50 ml. The re-extract was mixed with the HCl solution up to 0.5 N. Aliquote of the 25 ml re-extract was put into a separating funnel,

mixed with the 50% solution of sodium hydroxide and 3 times extracted by ether. The extracts were mixed, filtrated through a paper filter containing 1 g of anhydrous sodium sulphate and dried in a small porcelain bowl. The residue was studied.

Up to 70% of chlorpromazine from urine can be extracted by this method.

RESULTS AND DISCUSSION

Qualitative studies with solutions of BiJ and KJ, phosphorous-molybdenum acid and concentrated H_2SO_4 and TLC studies (benzene-dioxan-ammonia – 50:50:1, $R_f=0.63$) show that the accident took place owing to chlorpromazine poisoning. The quantitative measurement of chlorpromazine by optical methods show: stomach – 0.14 mg/g; intestine – 0.16 mg/g; kidney – 7.6 μ g/g, and urine – 0.01 mg/ml (HCl souring with the following ether extraction). In Figure 1 the SI mass spectra of a commercial chlorpromazine sample (Hungary) is presented, and in Figure 2 the intestine extract under identical temperature conditions for the emitter, which allows the observation of all basic ion lines of compounds under study in the whole mass spectra. For low temperatures of the emitter, the emission of quasimolecular ions $(M-H)^+$ and $(M-3H)^+$ (where M is the molecule, H is the hydrogen atom) is more intensive. For the comparison, in Figure 3 the chromatogram and in Figure 4 the EI mass spectrum of the intestine extract are presented.

It is seen from the SI mass spectra (Figure 1 and Figure 2) that chlorpromazine and its metabolites – monodesmethyl-, sulphoxid- and hydroxichlorpromazine – are ionized by the SI mechanism with the high efficiency. The small number in and the special character of the SI mass spectra allow the reliable identification in the extract of

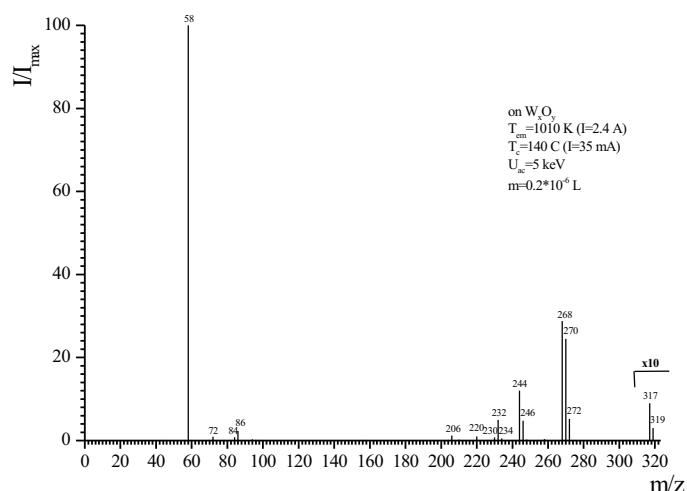


Fig. 1. SI mass-spectra of chlorpromazine.

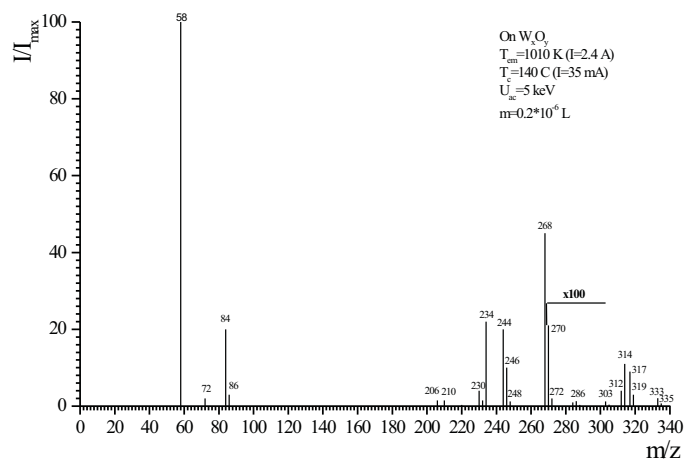


Fig. 2. SI mass-spectra of intestine extract.

post-mortem materials. The comparison of the SI mass spectra of chlorpromazine and intestine extract show that in the mass spectra, together with the lines of quasimolecular ions $(M-H)^+$, $(M-3H)^+$ chlorpromazine with $m/z = 317$; 319 a.u.m. and its metabolites: sulphoxichlorpromazine and hydroxichlorpromazine with $m/z = 333$; 335 a.u.m. desmethylchlorpromazine with $m/z = 303$; 305 a.u.m., there are lines of ions $(M-R_i)^+$, where R_i is the radical. The detachment of the alkylamine radical R_i from phenothiazine nucleus for chlorpromazine is kept for its metabolites. A series of lines an ion with $m/z = 312$; 314 a.u.m. is not identified the mass number of which corresponds to the odd number of nitrogen heteroatoms. The ion lines corresponding to didesmethylchlorpromazine are observed as noise owing to the great value of the molecule ionization potential, which can be explained by the alkylamine transformation from tertiary to initial. The GC/MS study of intestine extracts show this method, in our case, allows the detection of chlorpromazine only (the line with $R_f = 12.790$ min.). There is no identification with metabolites because other lines in the chromatogram correspond to hydrocarbons and acids. In the range of great mass the SI and EI mass spectra of chlorpromazine with the basic line of ammonium ions with $m/z = 58$ a.u.m. coincide, in the range of small mass there are few lines owing to selectivity in the SI mass spectra as compared to the EI mass spectra.

CONCLUSION

The estimations show that the sensitivity of the SI/MS method is more two orders higher than that for the traditional GC/MS method with electron ionization. The high sensitivity and selectivity of the SI/MS method allow the detection of the ultra-trace amounts of

chlorpromazine and its metabolites in extracts without their preliminary chromatographic separation.

Fig. 3. The chromatography and EI mass-spectra of chlorpromazine.

Reference:

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Fig. 4. The chromatography and EI mass-spectra of intestine extract.