Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 1125–1133
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Application of surface ionization methods for highly sensitive and selective analysis of benzodiazepine derivatives

Usman Khasanov*, Utkur Rasulev, Dilshod Usmanov, Saida Iskhakova, Ulugbek Nabiev

Arifov Institute of Electronics, Akademgorodok, 700125 Tashkent, Uzbekistan

Received 3 May 2004; received in revised form 16 July 2004; accepted 24 July 2004

Abstract

In this work, the results of studying the possibilities of surface ionization mass spectrometry (SI/MS) and atmosphere pressure thermodesorption surface ionization (APTDSI) spectroscopy for high-sensitivity and selective detection and identification of psychotropic preparations of benzodiazepine derivatives – medazepam, diazepam and chlordiazepoxide – are presented. It has been established that the SI mass spectra of benzodiazepines have a small number of lines and are significantly different from those obtained by electron ionization (EI) and that the molecules of benzodiazepines can be ionized by surface ionization with high efficiency ($\geq$100 times and more than by EI) and the current density increases from diazepam to medazepam.

It has been found that the APTDSI spectra of benzodiazepine derivatives have two characteristic maxima connected with energy of sublimation and desorption.

The ionization efficiency is several C/g (Coulomb per gram), the linear range of the concentration dependence is 3–4 orders of a magnitude. The results obtained by the SI/MS and APTDSI methods have been compared with those obtained by the conventional TLC and GC/MS with electron ionization.

Keywords: Surface ionization mass spectrometry; Atmosphere pressure thermodesorption surface ionization spectroscopy; Benzodiazepine derivatives

1. Introduction

Widely used in medical practice, the psychotropic preparations are non-volatile and polar compounds, as well as thermally unstable ones owing to large molecular mass. Information obtained by studying such compounds is defined both by a way of ionization and by their chemical nature.

Now for analytical purposes, the diverse ionization methods of non-volatile thermally unstable polar compounds are used in gas phase: electron ionization, photon ionization, ionization under a beam of accelerated ions and atoms, chemical ionization and spray methods. In a combination with various methods of separating the mixtures and the methods of ion separation, they allow qualitative and quantitative analysis of different complex mixtures, including bio-solutions [1]. Nevertheless, development of selective and highly sensitive methods and devices of express detection and identification of the trace amounts of medicinal preparations, including psychotropic ones, widely used in medical practice in particular frequently abused preparations remains an important problem of pharmacology, toxicology, narcology and criminalistics [2].

Unique selectivity and high efficiency of the surface ionization (SI) of the nitrogen bases draw special attention to this way of ionization and to development on its basis of various selective and sensitive devices.

The phenomenon of the SI of organic compounds consists in formation of positive multiatomic ions in the process

* Corresponding author. Tel.: +998 71 162 54 74; fax: +998 71 162 87 67.
E-mail address: ariel@ariel.uzsci.net (U. Khasanov).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.09.026

of particle thermodesorption from the solid surface [3]. The degree of surface ionization with the formation of positive ions:

$$\alpha = \frac{\nu^+}{\nu^0} \quad (1)$$

where ν^+ is the flux of desorbed ions, ν^0 the flux of desorbed neutral particles of a specific kind, can be expressed by the well-known Saha–Lengmur formula

$$\alpha = A \exp\left[\frac{e(\varphi - V)}{kT}\right] \quad (2)$$

where A is the statistical weight ratio of the states of charged and neutral particles at temperature T of the solid, e the electron charge, φ the electron work function and V the ionization potential of the particles. As seen from Eq. (2), the surface ionization is selective with respect to V and φ. The selectivity of the surface ionization with respect to V is the underlying principle for selective detectors, having a high sensitivity to certain substances only.

The key rules of ion formation which allow predicting both a composition of the ions to be formed and efficiency of the SI ionization process in accordance with the molecule structure [4,5] were revealed. It was found that the molecules of organic compounds containing a nitrogen heteroatom (particularly amines, hydrozines and their derivatives) including a variety of physiologically active nitrogen bases are more effectively ionized with a value of the ionization coefficient close to unity (e.g., for tertiary alkylamines with $\beta = 0.2$–0.5 where β is the ionization coefficient). It is important that the molecules of organic solvents (ketones, alcohols, saturated hydrocarbons, ethers, etc.), as well as the molecules of simple gases containing air are not ionized practically. This fact has become a scientific ground for development of highly sensitive and selective SI devices operating both in vacuum and in air to detect and identify the trace amounts of physiologically active nitrogen bases in different mixtures, including bio-solutions [4–8].

By surface ionization mass spectrometry (SI/MS), over 500 organic and bioorganic compounds, including amines, their derivatives and different physiologically active substances, were studied with the emitters from diverse materials [4–8]. The SI mass spectra of these compounds have a small number of lines in comparison with EI/MS. In a majority of the cases, not molecules themselves but the products of their chemical transformation on the emitter surface are ionized. Therefore, the SI mass spectrum consists of lines of the ions $(M-H)_\beta^+$, $(M-R)_\beta^+$ (where M is the molecule, H the hydrogen atom, R the radical). They are formed from corresponding radicals on the emitter surface in amine adsorption with an establishment of coordination bonds with an adsorbent through a non-divided pair of the electrons of the nitrogen heteroatom, which results in weakening and subsequently breaking the β-bond relative to the nitrogen heteroatom. The dehydrogenization processes can precede their ionization on the emitter surface. In this case alongside with desorption of the ions $(M-H)_\beta^+$, $(M-R)_\beta^+$, the ions $(M-H-2nH)_\beta^+$ and $(M-R-2nH)_\beta^+$ can be desorbed. The intensity of lines of such ions, in the case of the SI of alkylamines, is always significantly lower than that of corresponding lines $(M-H)_\beta^+$, $(M-R)_\beta^+$. Exception is dehydrogenization resulting in ring aromatization. For the SI of the *N*,*N*-heterocyclic amines the intensity of lines of the ions $(M-H-2nH)_\beta^+$ and $(M-R-2nH)_\beta^+$ can be higher than that of $(M-H)_\beta^+$, $(M-R)_\beta^+$ [5]. The number of elimination hydrogen atoms is connected with the number of the saturated bonds in the heterocycles. For example, for the SI of allomatrine alkaloids [5], there is a theoretical possibility of ion formation with elimination of up to 13 hydrogen atoms, in fact in the SI mass spectrum all lines of the ions $(M-H-2nH)_\beta^+$, from $(M-H)^+$ to $(M-13H)^+$, corresponding to full aromatization of all cycles are observed. All these ions have the saturated bonds and non-shared electrons. They can be represented as ions with the positively charged quadrivalent nitrogen atom with the sp^2-hybrid orbitals.

The molecules of the nitrogen bases containing, except the nitrogen heteroatom, the π-electrons of conjugate bonds, the aromatic sextet, can be adsorbed on the surface by means of these adsorption centers. Accordingly, the heterogeneous reactions can go through other channels with formation of ionization particles produced not only by the decay on the β-bonds [9].

As stated above, the SI is one of the methods capable of forming the ions in air. The SI mass spectra of organic compounds obtained in vacuum and in air are practically identical and consist mainly of the quasi-molecular $(M+H)^+$, $(M-H)_\beta^+$ and $(M-R)_\beta^+$ ions. Developed and used in production, the SI sensors operating in air were intended to detect the trace amounts of volatile amines (from 10^{-14} g/s) for the problems of ecology, health care, and control and management of technological processes [5]. However, for registration of non-volatile physiologically active nitrogen bases having vapor pressure 10^{-10}–10^{-7} Torr at a room temperature these sensors are not good. For those purposes, an atmospheric pressure thermodesorption surface ionization (APTDSI) sensor was developed and created [6,10,11]. This sensor is based on temperature-programmed evaporation of the molecules of the sample under analysis from the evaporator to the emitter surface and allows not only registration of the trace amounts (from 10^{-12} g) of narcotics in the extracted bio-samples, but also identification in accordance with more probable temperature of substance evaporation taken from the database of the thermodesorption spectra and determination of the substance amount with high accuracy in accordance with the calibration curves.

This work is devoted to studying the possibilities of the SI/MS and APTDSI methods to analyze the psychotropic preparations – derivatives of benzodiazepine – in comparison with the conventional TLC and GC/MS with electron ionization.

2. Experimental

2.1. Instrument

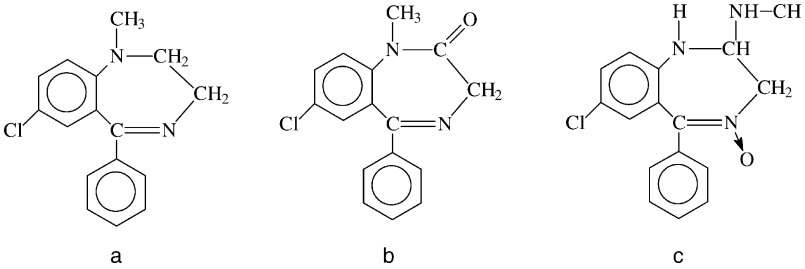
SI/MS experiments were carried out using a modified static magnetic mass spectrometer MI-1201B. An oxidized textured tungsten band (1 mm × 12 mm × 0.02 mm) was used as the thermo-emitter. Emitter temperature T_{em} was scanned within the range of 600–1200 K, evaporator one T_c = 80–140 °C. A flow of the substance under analysis to the emitter was formed by direct evaporation from a quartz Knudsen cell.

In experiments on APTDSI spectroscopy of pharmaceutical preparations, the device "Iskovich-1" was used. In brief, the devices consist of an air-flowing surface ionization detector connected with a temperature-programmed evaporator of samples. The emitter is heated up to a necessary constant temperature of ionization by means of electric current. An evaporator is graphitized metallic cup heated by electric current according to a given mode of the temperature on time. The amounts 1–3 μl of a solvent under analysis are put on the cup. Evaporated from the band, the sample molecules move with airflow to the surface ionization detector where the molecules are ionized by SI. This device "Iskovich-1" is different from [6,10,11] by connecting with a PC and having a database of thermodesorption spectra of psychotropic preparations and calibration curves. In experiments, the emitter temperature was 700 K, air velocity 50 L/h, evaporator temperature scanning from a room temperature to 500 °C with a linear rate of 10 K/s.

GC/MS analysis was performed with a chromato-mass spectrometer HP-6890 with MCD. A capillary column with 5% phenylmethylsiloxan was used. Injector temperature was 280 °C, heater temperature was programmed within the range of 150–200 °C, helium gas-carrier velocity was 3 mL/min. For EI/MS, electron energy was 70 eV, emission current 0.8 mA. For TLC the chromatographic plates "Silufol" were used.

2.2. Samples

The commercial samples of tablets of medazepam (Rudotel) (a), diazepam (Sibazon) (b) and chlordiazepoxide (Chlozepide) (c) were used in experiments:



The tablets of medazepam, diazepam and chlordiazepoxide with an active component 10, 5 and 5 mg, respectively, were ground and solved with ethanol, then the solutions were filtered and tested chromatographically with GC/MS. From those solutions 5 mg/mL each, the solutions with the concentration 500 μg/mL, 100 μg/mL, 10 μg/mL, 500 ng/mL, 100 ng/mL, 10 ng/mL were prepared to measure the calibration curves and LOD. The solutions were put on the chromatographic plates, cup of the APTDSI spectroscope and injector of the GC/MS with "Agilent" syringes with 5 mL in volume. The LOD for GC/MS was supposed to be the substance concentration when the substance is not identified. High selectivity of the SI/MS method allowed use of the commercial samples with no preliminary separation.

3. Results and discussion

3.1. Surface ionization mass spectrometry

The SI mass spectra of medazepam, diazepam and chlordiazepoxide obtained with the emitter from oxidized tungsten are presented in Fig. 1. The chromatogram (a) and EI mass spectrum (b) of medazepam obtained with the chromato-mass spectrometer are given in Fig. 2. It is seen that the SI and EI mass spectra significantly differ from each other, except the ion lines $(M-H)^+$. The SI mass spectrum has a relatively small number of lines but sufficient information for diagnostic purposes. This is explained by high selectivity of the SI with respect to the ionization potential V of the desorbed particles. In the case of electron ionization, the molecule themselves are ionized. In the case of surface ionization, mainly the products of the heterogeneous reactions of adsorbed molecules occurring on the hot emitter surface are ionized. The chlorine atom in the molecules makes it easier to identify the ion lines in accordance with its characteristic isotopic relations.

It is seen that the ion composition in the SI mass spectra of benzodiazepine derivatives, in accordance with the SI regularities [4,5], contains the lines of quasi-molecular ions $(M-H)_\beta^+$ and $(M-H-2nH)_\beta^+$ with m/z = 271, 269, 267 for medazepam and m/z = 281, 283, 285 for diazepam.

The ion lines with m/z = 298 and 300 for chlordiazepoxide are observed in the mass spectra as traces. That can be con-

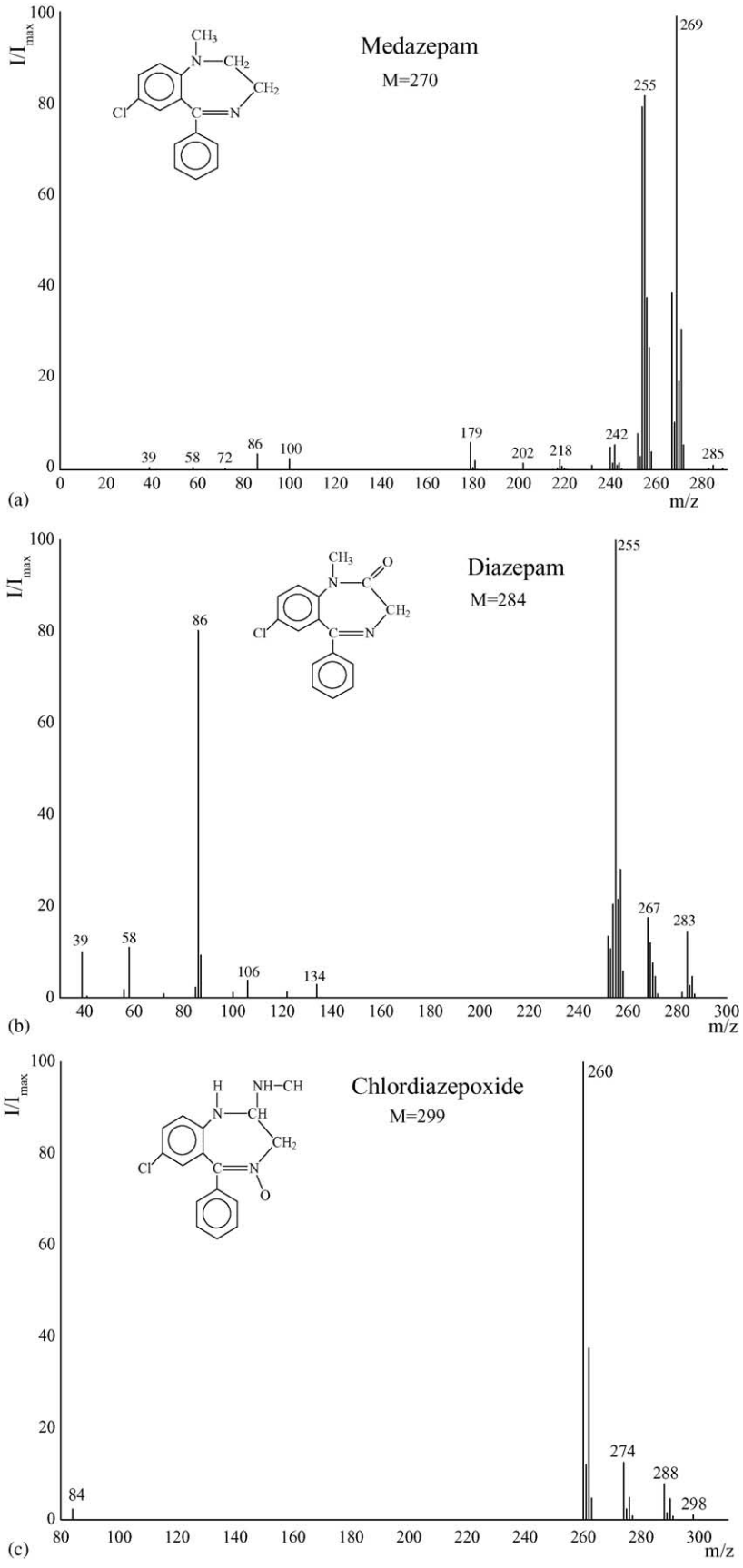


Fig. 1. The SI mass spectra of medazepam, diazepam and chlordiazepoxide.

nected with elimination of the oxygen atom having a coordination bond with the nitrogen atom. The base lines in the mass spectra of medazepam, diazepam and chlordiazepoxide are the ion lines with *m*/*z* 269, 255 and 260, respectively. As seen from the spectra, owing to the presence of two or more adsorption centers the molecules of benzodiazepine derivatives undergo different heterogeneous transformations. With a character of the adsorption center the products of the heterogeneous reaction yield have a diverse structure. For example, the ion $(M-H)^+$ for medazepam has three different structures (a, b, c) while the $(M-3H)^+$ ion has the only structure (d).

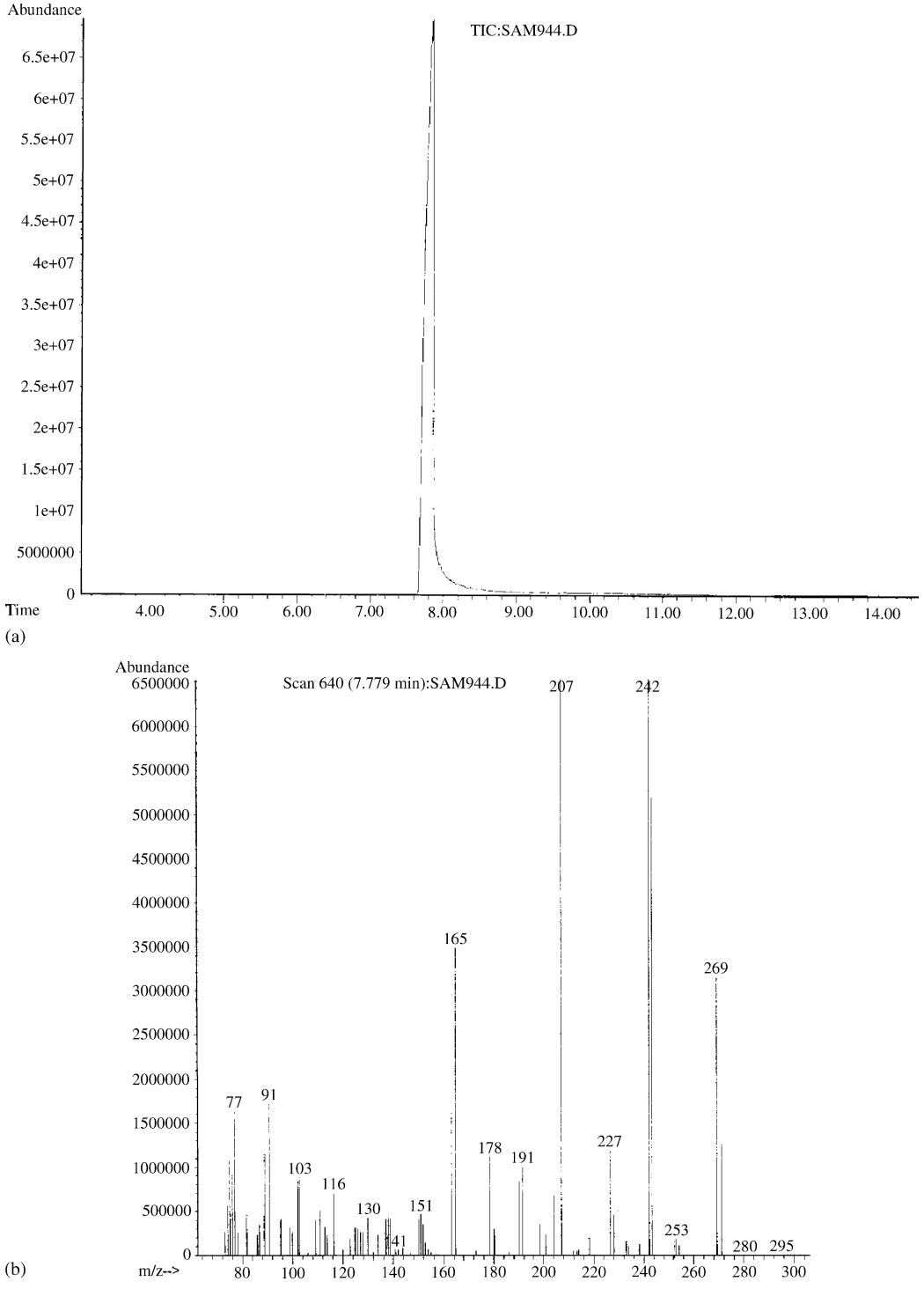
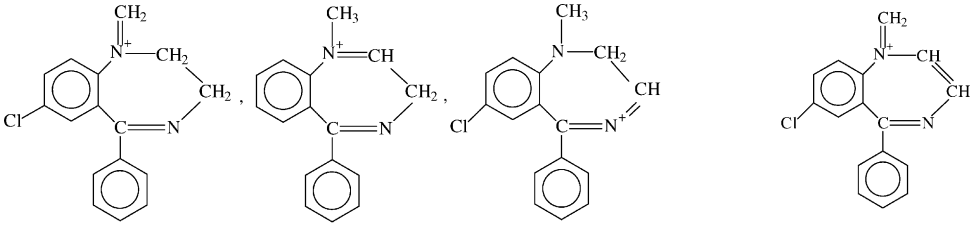


Fig. 2. The chromato-mass spectra of medazepam: chromatogram (a) and EI mass spectrum (b).



The $(M-3H)^+$ ion is formed by adsorption of the medazepam molecule only with the nitrogen atom being in a tertiary position with the methyl radical.

In the SI mass spectra of the benzodiazepine derivatives, there are ion lines with odd mass alongside with the ion lines with even mass. According to the nitrogen rule, they correspond to the structures of the ions having the even and odd numbers of nitrogen atoms, respectively, and that is one of the most important moments for determining the ion structure. For example, the presence of the ion lines in the SI mass spectrum with *m*/*z* 252, 254, 255, 256, 257 corresponds a superposition of the ion lines having different structures with one nitrogen heteroatom for even lines and two nitrogen heteroatoms for odd ones with the Cl atom for both cases, respectively. Additional evidence, i.e. the presence, in the SI mass spectrum, of the "metastable" ions correspondent to the mono-molecular decays of the vibrationally excited initial ions on their way in the mass spectrometer from the emitter to the collector [5]. The ion decays $m^+ \rightarrow m_1^+ + m_2$ taking place in the acceleration zone of the ion source give, in the mass spectrum, a line of the fragmentary ion m_1^+ widening in the direction of small masses or small energy. The same decays taking place in the no-field place of the mass spectrometer, between the accelerating electrode of the source and zone of the magnetic field, result in the diffusive ion lines with apparent mass $m^* \approx m_1^2/m$. According to this pair of lines it is possible to easily and unambiguously register the decays. For example, for medazepam in the mass spectra the following decays are observed:

$$269^+ \rightarrow 233^+ + 36, \quad m^* = 202.5$$

$$269^+ \rightarrow 240^+ + 29, \quad m^* = 216.5$$

$$269^+ \rightarrow 252^+ + 17, \quad m^* = 205.5$$

The line intensity m^* for emitter temperature $T = 1120$ K is 1.5, 0.1 and 1%, respectively. Nevertheless, despite the great number of decays in different channels, the initial ion $(M-H)^+$ remains a base line in the SI mass spectrum even for high temperature. Found in the SI mass spectrum, the ion lines with *m*/*z* 285 and 287 correspond to a hydro-oxidized molecule of medazepam and is an impurity of the active component in the tablets "Rudotel".

The temperature dependencies of currents for a number of medazepam ions are presented in Fig. 3. They demonstrate that the dependencies of ion currents on *T* is due to the dependence of the SI coefficient on *T*, as well due to the temperature dependence of the yield of chemical reactions in the adlayer defining the concentration of the ionized particles on the surface.

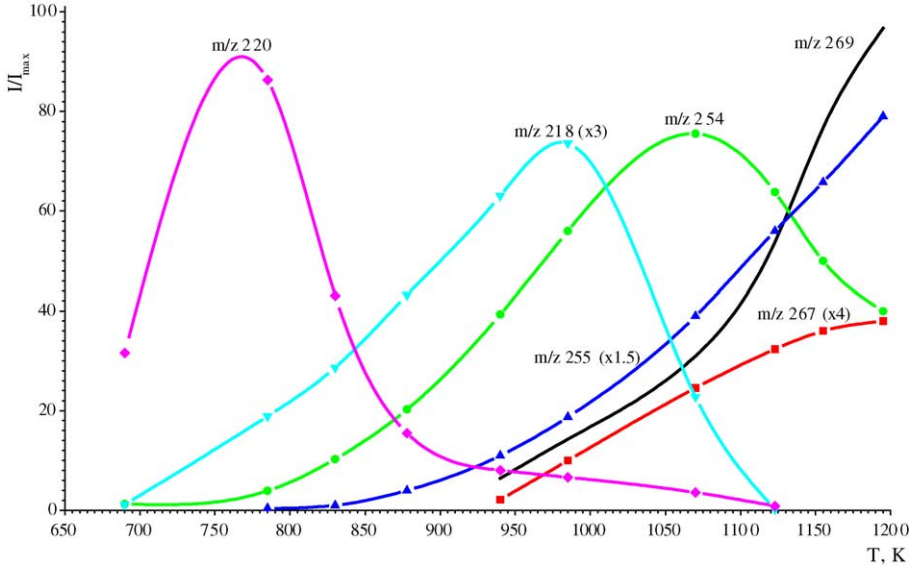


Fig. 3. The temperature dependencies of ion currents for medazepam.

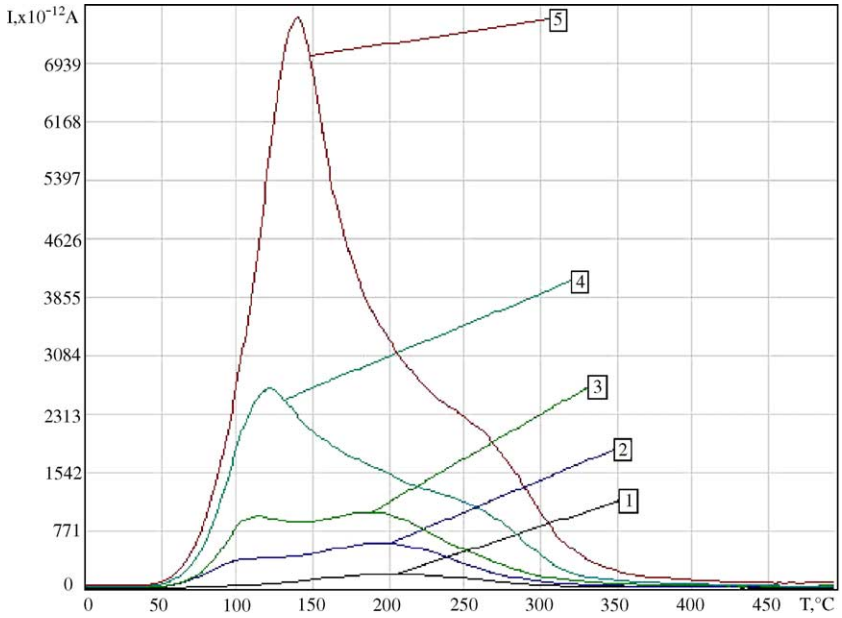


Fig. 4. The APTDSI spectra for different amounts of medazepam: 1 – 9 ng, 2 – 30 ng, 3 – 60 ng, 4 – 120 ng, 5 – 250 ng.

3.2. Atmosphere–pressure thermodesorption surface ionization spectroscopy

The thermodesorption surface ionization spectra of medazepam, diazepam and chlordiazepoxide are given in Figs. 4–6. Each benzodiazepine showed characteristic T_{max}. The highest temperature was observed for chlordiazepoxide (215 °C), whereas medazepam and diazepam were ∼140 and ∼180 °C, respectively. It is seen from the spectra that the shapes depend on the amount of substances under analysis deposited on the evaporator (Figs. 4–6). For the great (≥0.1 μg) and small (<10 ng) amount of the substance deposited on the evaporator surface, one peak with different maximums is observed. For the intermediate concentration in the thermodesorption spectra, there are both peaks. Such a character of the dependence can be explained by a difference in the values of sublimation and desorption heat of the molecules from the evaporator surface [10,11]. For the great amount of the substance, molecule evaporation is defined by sublimation heat; for the small amount of the substance, all the molecule are adsorbed on the evaporator surface, and to be evaporated they have to overcome energy of desorption activation. For the intermediate concentration of pharmaceutical preparations when the number of sublimating molecules

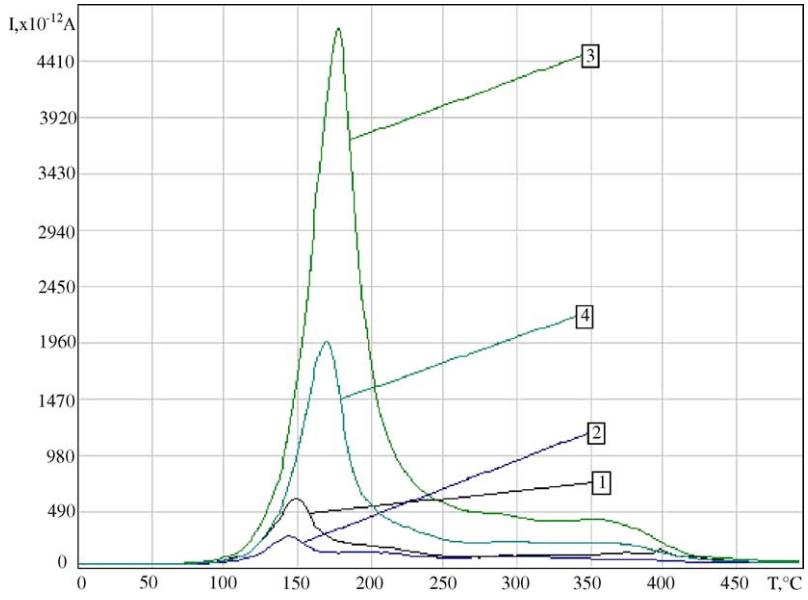


Fig. 5. The APTDSI spectra for different amounts of diazepam: 1 – 50 ng, 2 – 20 ng, 3 – 200 ng, 4 – 400 ng.

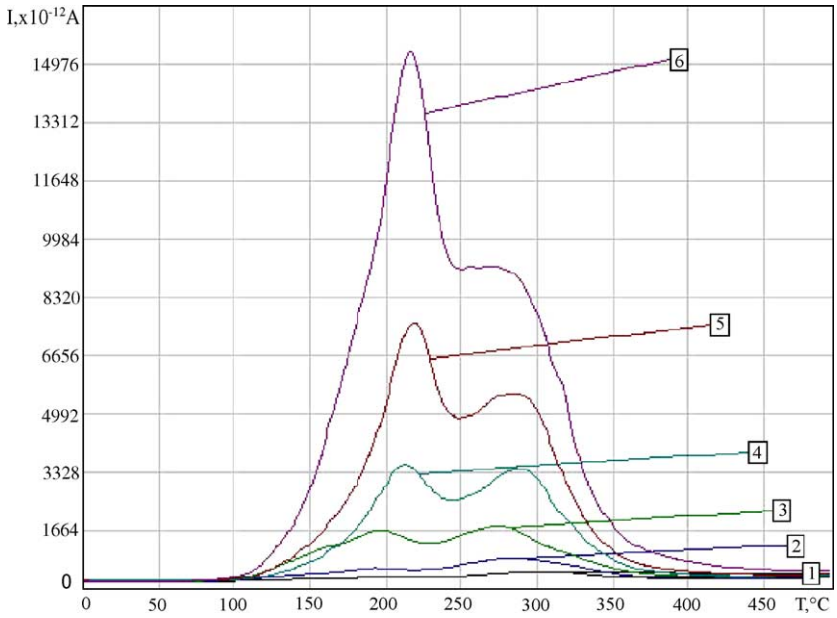


Fig. 6. The APTDSI spectra for different amounts of chlordiazepoxide: 1 – 30 ng, 2 – 50 ng, 3 – 100 ng, 4 – 200 ng, 5 – 300 ng, 6 – 500 ng.

Table 1
The limits of detection and the efficiencies of ionization

Substances	LOD (pmol)	Ionization efficiency (C/mol)	Linearity
Medazepam	∼0.05	810	∼4.0 in order
Chlordiazepoxide	∼0.1	∼250	∼3.5 in order
Diazepam	∼1.4	∼56	∼3.0 in order

is comparable with the number of molecules desorbed from the evaporator surface, both peaks are observed in the curves of the thermodesorption spectra.

In Table 1, the limits of detection (LOD) and the ionization efficiency of molecules for the studied compounds, defined in accordance with the area of the dependence $I(t)$ in Coulomb, from 56 to 810 C/mol are given. They have a linear dependence on the substance amount and the range is defined by the limit of detection of pharmaceutical preparation molecules and emitter "poisoning" for the great amounts of the substance ≥ 1 μg [5]. The linear range of calibration curves is 3.0–4 in order. APTDSI detection is an express one and time for one analysis is not more than 3 min.

Thus, the high SI efficiency of medazepam, chlordiazepoxide and diazepam, special character of the mass- and thermodesorption spectra allow, using SI/MS and APTDSI spectroscopy, reliable identification of the trace amounts of these preparations with the limit of detection (from 10^{-12} g) significantly exceeded those for conventional TLC and GS/MS with electron ionization.

4. Conclusion

For the first time using SI/MS and APTDSI spectroscopy, the surface ionization of the molecules of psychotropic preparation – tranquillizers of medazepam, diazepam and chlordiazepoxide has been studied. They are ionized with the high efficiency by the SI. APTDSI detection allows not only detection of tranquillizers – benzodiazepine derivatives with the high efficiency (from ∼0.05 pmol) – but also determination of their amounts in accordance with the calibration curves having a wide linear range (3–4 in orders).

The high analytical possibilities of the SI/MS and APTDSI methods in combination with relative simplicity, non-expensiveness and expressiveness allow their use in narcological, toxicologic and criminalistic laboratories. The expressiveness of the APTDSI method for detection of the substance amount in the solution under analysis is more perspective direction of its analytical application to control a content of active components in the technological processes.

Acknowledgements

The work was performed with financial support of the Center for Science and Technology of the Republic of Uzbekistan under Grants 2.1.7 and II.13.29. The authors are very grateful to T.Kh. Islamov for the samples and to V. Pikhut for assistance in preparing this paper.

References

[1] W.G. Ewing, in: W.G. Ewing (Ed.), Analytical Instrumentation Handbook, 2nd ed., Marcel Dekker, New York, 1997, pp. 915–945.
[2] O.H. Drummer, Proceedings of the TIAFT 2001, Prague, 2001, pp. 1–7.
[3] E.Ya. Zandberg, U.Kh. Rasulev, B.N. Shustrov, Dokl. Akad. Nauk SSSR 172 (1967) 885–889.

[4] E.Ya. Zandberg, U.Kh. Rasulev, Russ. Chem. Rev. 51 (1982) 819–841.
[5] U.Kh. Rasulev, E.Ya. Zandberg, Prog. Surf. Sci. 28 (1988) 181–412.
[6] U.Kh. Rasulev, U. Khasanov, V.V. Palitsin, J. Chromatogr. A 896 (2000) 3–18.
[7] A. Ishii, K. Watanabe-Suzuki, H. Seno, Y. Katsumata, O. Suzuki, J. Ghromatogr. B 758 (2002) 117–128.
[8] T. Fujii, Eur. Mass Spectrom. 2 (1996) 91–114.
[9] U.Kh. Rasulev, U. Khasanov, U.O. Nabiev, T.Kh. Islamov, Uzbek J. Phys. 2 (2000) 63–70.
[10] U.Kh. Rasulev, U. Khasanov, S.S. Iskhakova, A.V. Mikhaylin, Int. J. Ion Mobility Spectrom. 4 (2001) 121–124.
[11] S.S. Iskhakova, A.V. Mikhaylin, U.Kh. Rasulev, Ya.R. Sagatov, U. Khasanov, Zhurnal Analiticheskoy Khimii (Russian) 59 (2004) 58–63.