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Short communication

Analysis of omnoponum by surface-ionization mass spectrometry and liquid chromatography tandem mass spectrometry methods

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ABSTRACT

This paper provides the development of analytical capabilities of surface-ionization mass spectrometry (SI/MS) and high performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS) for narcotic analgesic omnoponum, which perfectly exemplifies a mixture of opium alkaloids. It has been revealed that the investigated opiates solution, omnoponum, is ionized by the surface ionization (SI) method with high sensitivity. In the SI mass spectrum, M^+, $(M-H)^+$, $(M-H-2nH)^+$, $(M-R)^+$ and $(M-R-2nH)^+$ ion lines, where M is a molecule, H is the hydrogen atom and R is a radical, were observed. These ion lines consist of combined omnoponum mixture SI mass spectra, i.e. morphine, codeine, thebaine, papaverine, and narcotine. Moreover, while the study of omnoponum by HPLC/MS/MS methods has attested that the mixture really consists of 5 components, it has been demonstrated that the SI/MS method can be utilized for the analysis of this mixture without the necessity of its chromatographic separation.

1. Introduction

Detection and identification of trace amounts of opium alkaloids, opiates and other illicit drugs in various samples including complex mixtures or matrices is a major analytical issue. Nowadays, various physical and chemical methods are employed in order to tackle this problem [1]. Recently Pothier and Galand for instance, have investigated the analysis of opium alkaloids still using thin layer chromatography (TLC) [2]. In addition, numerous more sophisticated and sensitive chromatographic methods and techniques have been developed lately. These include gas chromatography (GC) [3,4], HPLC [5] and capillary electrophoresis (CE) [6]. Most of these methods can be applied for the analysis of abused drugs in biological samples.

The most sensitive of the analytical approaches are those in which the detection is based on the compound ionization and mass selective analysis. Take chromatography–mass spectrometry in the case of GC–MS using electron impact ionization (EI) for example. GC–MS has a crucial advantage within the field of screening for unknown: EI-generated mass spectra are very reproducible and several spectral databases have been developed for identification purposes [7,8]. Alternatively, recent reports have described the analysis of opiates in biofluids using soft ionization techniques such as electrospray ionization by LC–ESI-QToF mass spectrometry [9].

The phenomenon of the SI of organic compounds consists in the formation of positive polyatomic ions in the process of particle thermodesorption from the solid surface and depends on the probability of isoenergetic exchange of electrons between the particle and the solid. More detailed description of the SI phenomenon is provided in the work [10]. It was revealed that the molecules of nitrogen-containing bases (particularly amines, hydrazines and their derivatives) including a variety of physiologically active ones are more effectively ionized with a value of the ionization coefficient close to unity. However, the molecules of organic solvents (ketones, alcohols, saturated hydrocarbons, ethers, etc.) as well as the molecules of simple gases which compose air are not virtually ionized. By now more than 500 organic and bioorganic compounds have been studied by SI/MS with the emitters from diverse materials, and the method has begun to be employed in forensic toxicology [10–12]. The SI mass spectra of these compounds have a small number of lines and consist of the ion lines M^+, $(M-H)^+$, $(M-H-2nH)^+$, $(M-R)^+$ and $(M-R-2nH)^+$, corresponding to the products of chemical reactions in the adlayer. Having said that, the SI method has been deployed for designing and development of novel highly-selective and highly-sensitive SI detectors [13]. In the work [14] authors were developing GC/SIOMS (Gas Chromatography/Surface

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Ionization Organic Mass Spectrometry) methods for determination of trace amounts of triethylamine in air, while the paper [15] presents results of SIOMS study of clinically important drugs.

Opium alkaloids are referred to N,N-heterocyclic compounds. In the study [16] the SI mass spectra of opium alkaloids, namely morphine, codeine, thebaine, papaverine, and narcotine, during their adsorption on the oxidized tungsten surface were investigated as well as their ionization mechanisms were determined.

In this work analytical capabilities of both surface-ionization mass spectrometry and highly effective liquid chromatography–mass spectrometry with tandem mass spectrometry for analgesic omnoponum, which exemplifies a narcotic opiates solution and has important practical applications, has been developed for the first time.

2. Experimental

2.1. Chemicals

Methanol (HPLC grade) was provided by Biosolve B.V. (Valkenswaard, the Netherlands), while formic acid was supplied by Acros Organics (Acros Organics, New Jersey, USA). Ultra-pure water was obtained using a Synergy 185 system designed by Millipore (Bedford, MA, USA). A commercial sample of omnoponum (Russia) was provided by the Center of Forensic-Medicine Expertise of the Republic of Uzbekistan and dissolved in ethanol and methanol for the SI/MS and HPLC/MS/MS study respectively at a concentration of 1 mg/ml. The sample was dissolved in 200 μl (100 ng/ml) of the chromatographic solvent, consisting of 14.95% methanol, 84.95% ultra-pure water, and 0.1% acetic acid, 10 μl of which were inserted into the column. An artificial mixture of opiates was a commercial sample of narcotic analgesic omnoponum, consisting of hydrochlorides of 5 opium alkaloids with a ratio of morphine to other alkaloids being 48–50% to 32–35%. The investigated one-percent omnoponum solution consisted of 6.7 mg of morphine, 2.7 mg of narcotine hydrochloride, 0.36 mg of papaverine hydrochloride, 0.72 mg of codeine hydrochloride and 0.05 mg of thebaine hydrochloride [17]. For the SI/MS study of omnoponum a special quartz evaporator was filled with 8 μl of the substance at a concentration of 1 mg/ml.

2.2. Surface-ionization mass spectrometry instrumentation and conditions

The SI/MS experiments were conducted using a MX-1320 magnetic field mass spectrometer ("NauchPribor", Orel, Russia) upgraded for SI research [18]. The thermoemitter was an oxidized tungsten wire with 58 mm in length and 200 μm in diameter. Operating vacuum amounted to $1.0–3.0 \times 10^{-6}$ Pa. The molecular flows of the studied compounds were directed to the thermoemitter by means of a special quartz evaporator, introduced into the ionization chamber through a vacuum lock [18]. The evaporator temperature T_{evap} varied within the range of 315–415 K. The thermoemitter temperature was regulated within a range of T_{em} = 600–1250 K. For T_{em} > 800 K, the temperature was measured with a VIMP-015 M optical micropyrometer ("Kalibr", Moscow, Russia); for T_{em} < 800 K, measurements were executed using the extrapolation of the dependence of thermoemitter temperature on its ohmic resistance [19].

2.3. Reference compounds

The opium alkaloids morphine, codeine and thebaine were provided as a donation jointly by the United Nations Development Programme (UNDP) and United Nations Industrial Development Organization (UNIDO) for the Center of Forensic-Medicine Expertise of the Republic of Uzbekistan. Pure samples of papaverine and narcotine were purchased at Grace–Alltech Associates (Deerfield, IL, USA). All the samples of morphine, codeine, thebaine, papaverine, and narcotine were dissolved in methanol at a concentration of 1 mg/ml.

2.4. HPLC/MS/MS instrumentation and conditions

The HPLC/MS/MS apparatus which was used during the experiments consists of the Waters Alliance 2790 Separation Module (Milford, MA, USA) and a quadrupole time-of-flight (QTOF) mass spectrometer assembled by Micromass (Manchester, UK). The system is operated by Masslynx software by Micromass (Manchester, UK). The electrospray ionization source was a Z-spray® functioned in the positive ion mode. The cone voltage optimized values ranged from 28 to 40 V. Source block and desolvation temperatures constituted 120 and 350 °C respectively. The capillary voltage was set at 3200 V. The protonated molecules $[M+H]^+$ were selected in the first quadrupole and transported to the hexapole collision cell, which used argon as a collision gas. The scan ranged from m/z 100 to 420 per second. In MS/MS mode argon was also used as a collision gas with the collision energy ranged from 29 to 39 eV. The Hypersil BDS phenyl column 2.1 mm I.D., 100 mm in length and a particle size of 3 μm (Alltech, Lokeren, Belgium) at 30 °C was used. Both organic and aqueous eluents contained ammonium formate (10 mM, pH 5). A linear gradient was carried out starting from 5% to 15% methanol in water for 21 min and at a flow rate of 0.2 ml/min. The system then returned to its initial conditions in 0.5 min and equilibrated for 6.5 min, amounting to a total run time of 28 min.

3. Results and discussion

3.1. Surface-ionization mass spectrometry

The SI mass spectrum of omnoponum on the oxidized tungsten thermoemitter is provided in Fig. 1, while the SI mass spectra of some reference compounds, namely morphine and codeine are supplied in Fig. 2. It can be seen from the SI mass spectrum of omnoponum that the spectrum is composed of ion lines which conform to the corresponding SI mass spectra of morphine, codeine, thebaine, papaverine, and narcotine molecules. The m/z 339 ion

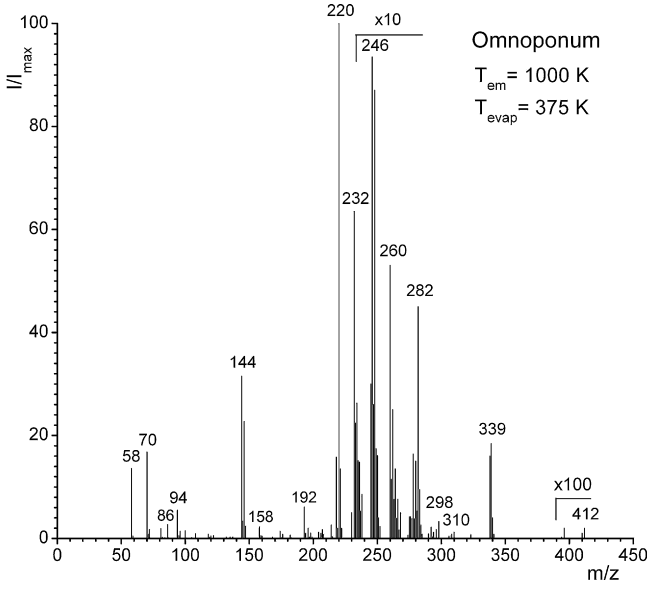

Fig. 1. The SI mass spectrum of omnoponum.

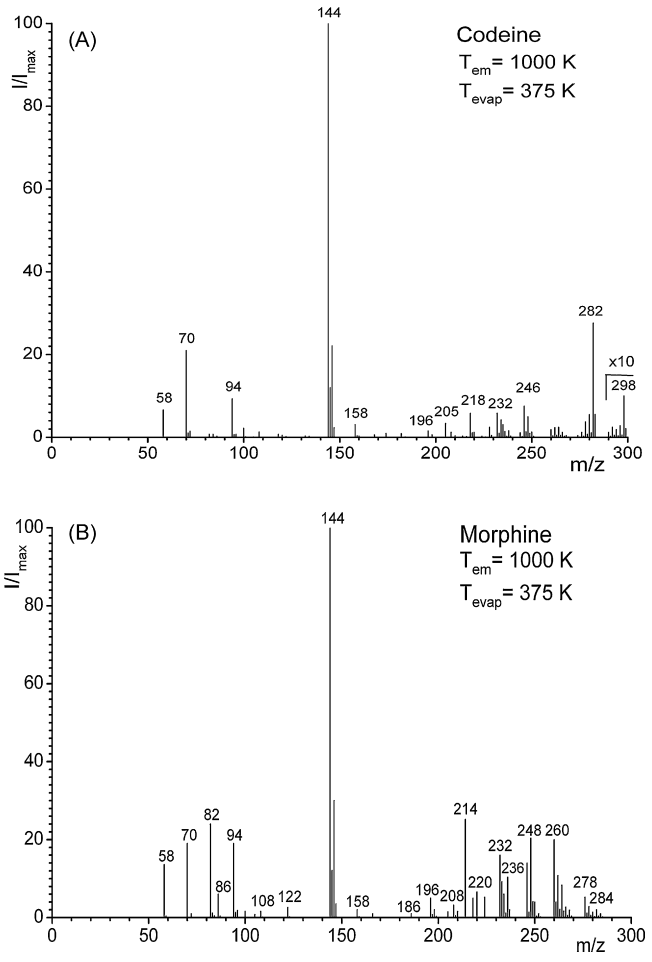

Fig. 2. The SI mass-spectra of reference compounds: (A) morphine, (B) codeine.

line correspond to the papaverine M^+ molecular ion, whereas ion lines at m/z 284, 298, 310, 338, 412 match morphine, codeine, thebaine, papaverine, and narcotine quasimolecular $(M-H)^+$ ions respectively. In the omnoponum mass spectrum, like in the separate adsorption mass spectra of opiate molecules, a series of $(M-H-2nH)^+$ ion lines, which were created by the elimination of up to 9 hydrogen atoms for both morphine (at m/z 282, 280, 278, 276) and codeine (at m/z 296, 294, 292, 290), 7 for thebaine (at m/z 308, 306, 304), 3 for narcotine (at m/z 410), was witnessed [16]. The series of morphine, codeine, and thebaine $(M-H-R-2nH)^+$ ion lines overlap with each other and represents the sum of their ion currents. For example, within the ion series at m/z 284–276, the m/z 284 line is the $(M-H)^+$ ion line of morphine, while the rest of morphine lines from the $(M-H-2nH)^+$ series overlap with codeine $(M-H-2nH-17)^+$ at m/z 282–276 and thebaine $(M-H-2nH-34)^+$ at m/z 282–276 ion lines.

In the omnoponum mass spectrum under thermoemitter temperatures $T > 900$ K the main ion line was one of hydrocotarnine at m/z 220, which was produced as a result of the product fragmentation of narcotine molecules on the thermoemitter surface. Although the density of the line made up 1.5×10^{-9} A/cm^2 when the cell temperature was $T_{evap} = 375$ K, and temperature of thermoemitter amounted to $T_{em} = 1000$ K, under $T_{em} < 900$ K the m/z 144 ion line with N-phenylmethylpyridinium structure, which is typical exclusively for SI morphine-like molecules, was main [16]. Under $T_{evap} = 405$ K and $T_{em} = 750$ K its density accounted for $\sim 1 \times 10^{-8}$ A/cm^2. All of the formed ions have an even number of electrons and can be presented as ions with quadrivalent positively charged nitrogen atoms.

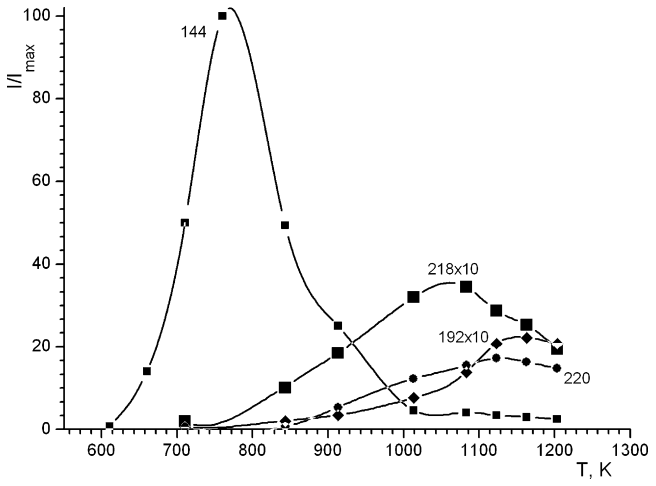

Fig. 3. The dependencies of some ion currents of omnoponum components upon emitter temperatures. The ion currents at m/z 218 and 192 are magnified tenfold.

In the omnoponum mass spectrum, molecular decay lines of oscillating-excited $(M-H)^+$ papaverine and narcotine $(M-H)^+$, $(M-3H)^+$ ions at m/z 338 and 412, 410 respectively, which are accompanied by the detachment of methyl radicals and methoxy-groups, can be observed [16].

Temperature dependences of ion current (Fig. 3) are typical for the omnoponum components and correspond to the dependences obtained by separate adsorption of alkaloid molecules of the omnoponum components. The temperature dependences of the currents of a series of ions for omnoponum characterize heterogeneous reaction rates on the thermoemitter surface. As can be seen from the graph, under the thermoemitter temperature $T_{em} > 900$ K, the main peak was at m/z 220, which was created from the product of narcotine molecules fragmentation in the emitter surface, while at $T_{em} < 900$ K the main peak was at m/z 144, which is characteristic solely for SI of morphine-like molecules.

Ion lines, which conform to the corresponding products of heterogeneous association reactions on the thermoemitter surface between molecules of mixture components, has not been found in the omnoponum SI mass spectrum. This fact attests that molecules of mixture components do not interact with each other during their joint adsorption on the thermoemitter surface.

In order to investigate the additivity of omnoponum SI mass spectrum in identical experimental conditions, SI mass spectra of its components under separate adsorption as well as the cumulative ion currents for the each of the components were obtained. As a result, there were no new ion lines in the SI mass spectrum under joint adsorption (Fig. 1) which were not present in the mass spectra under separate adsorption (Fig. 2) and relating to products of associative reactions between the mixture components on the thermoemitter surface.

The total ion current of morphine (7.0×10^{-11} A), codeine (1.2×10^{-10} A), thebaine (2.5×10^{-10} A), papaverine (1.5×10^{-10} A), and narcotine (1.1×10^{-10} A) approximately equals (7.0×10^{-10} A) to the total ion current of omnoponum (6.8×10^{-10} A) within permissible experimental errors. Referring to the experimental results, it can be suggested that omnoponum SI mass spectrum, which consists of mixture of 5 opium components, corresponds to the sum of its components mass spectra, i.e. it is additive.

3.2. HPLC/MS/MS

The ESI Q-TOF CID mass spectra of the reference compounds, i.e. morphine, codeine, thebaine, papaverine, and narcotine are

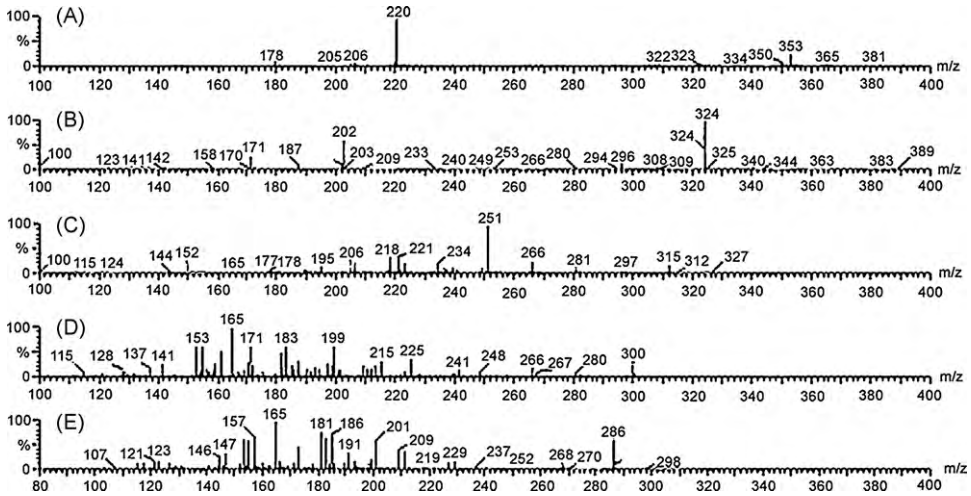

Fig. 4. The ESI Q-TOF CID mass spectra of reference compounds: (A) narcotine, (B) papaverine, (C) thebaine, (D) codeine, (E) morphine.

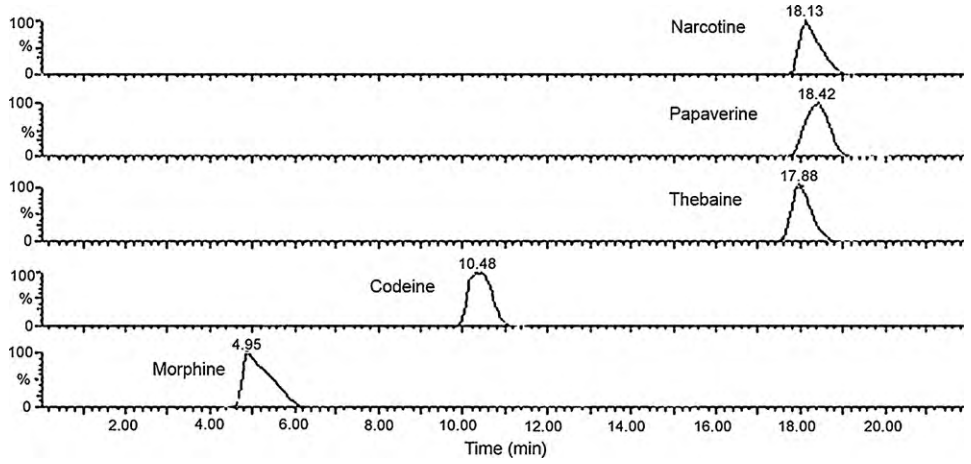

Fig. 5. The TIC chromatograms of omnoponum components.

provided in Fig. 4. The nitrogen heteroatom which is present in their structure makes them well suited for electrospray ionization in positive ion mode. The $[M+H]^+$ ion could be witnessed throughout the ESI mass spectra and detected with high sensitivity. All the compounds have been analyzed in MS and MS/MS (CID) mode. Morphine and codeine show a very similar fragmentation pattern as codeine contains only one additional methyl group. As is shown by the spectra, most fragments found in the morphine mass spectra were also detected in the case of codeine. In both mass spectra the fragmentary ion at m/z 165 is main, which is common for morphine-like structures. However, the ESI Qq-TOF CID mass spectrum for thebaine is by far different with the main peak at m/z 251. The presence of two double bonds in the second ring implies a completely different, but simpler fragmentation pattern compared to morphine and related analogues. In the mass spectra of morphine-like structures, weak intensity ions (fragments) formed by cleavage of the piperidine ring were witnessed. When it comes to the ESI Qq-TOF CID mass spectrum of papaverine, the main peak is at m/z 324, whereas for narcotine it is at m/z 220. The mass spectra of papaverine and narcotine comprise of relatively few ions. The chromatograms are shown for omnoponum components in Fig. 5. Chromatographic separation has revealed that omnoponum really consists of the above-listed substances, namely morphine, codeine, thebaine, papaverine and narcotine.

4. Conclusion

For the first time a mixture of opium alkaloids which consists of 5 components, namely narcotic analgesic omnoponum has been studied by SI/MS and HPLC/MS/MS methods. It has been revealed that under joint adsorption molecules of the components do not interact on the thermoemitter surface, i.e. ions caused by heterogeneous association reactions between the components on the emitter surface do not appear in the SI mass spectra of the mixtures. The mass spectrum consists of the sum of SI mass spectra of the each component and is additively added. The study of omnoponum by HPLC/MS/MS methods has attested that it really consists of 5 components. Thanks to high selectiveness of the SI/MS method, it is possible to analyze omnoponum, which consists of 5 components, without its preliminary chromatographic separation.

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References

[1] W.G. Ewing, in: W.G. Ewing (Ed.), Analytical Instrumentation Handbook, 2nd ed., Marcel Dekker, New York, 1997, pp. 915–945.

[2] J. Pothier, N. Galand, Automated multiple development thin-layer chromatography for separation of opiate alkaloids and derivatives, J. Chromatogr. A 1080 (2005) 186–191.
[3] C. Meadway, S. George, R. Braithwaite, A rapid GC–MS method for the determination of dihydrocodeine, codeine, norcodeine, morphine, normorphine and 6-MAM in urine, Forensic Sci. Int. 127 (2002) 136–141.
[4] M Chiarotti, N. Fucci, Comparative analysis of heroin and cocaine seizures, J. Chromatogr. B 733 (1999) 127–136.
[5] P. Fernandez, C. Vazquez, L. Morales, A.M. Bermejo, Analysis of opiates, cocaine and metabolites in urine by high-performance liquid chromatography with diode array detection (HPLC-DAD), J. Appl. Toxicol. 25 (2005) 200–204.
[6] R. Weinberger, I.S. Lurie, Micellar electrokinetic capillary chromatography of illicit drug substances, Anal. Chem. 63 (1991) 823–827.
[7] P.A. Pfleger, O.H. Maurer, S.T. Mills, Mass-spectral and CG Data of drugs, Poisons, Pesticides, Pollutants and their Metabolites, 2nd ed., Wiley–VCH, Berlin, 1992, pp. 243–292.
[8] A.C. Mofatt, in: A.C. Mofatt (Ed.), Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post-Mortem Materials, 2nd ed., The Pharmaceutical Press, London, 1985, pp. 358–401.
[9] K.A. Mortier, K.E. Maudens, W.E. Lambert, K.M. Clauwaert, J.F. Van Bocxlaer, D.L. Deforce, C.H. Van Peteghem, A.P. De Leenheer, Simultaneous, quantitative determination of opiates, amphetamines, cocaine and benzoylecgonine in oral fluid by liquid chromatography quadrupole-time-of-flight mass spectrometry, J. Chromatogr. B 779 (2002) 321–330.
[10] U.Kh. Rasulev, E.Ya. Zandberg, Surface ionization of organic compounds and its application, Prog. Surf. Sci. 28 (1988) 181–412.
[11] E.Ya. Zandberg, U.Kh. Rasulev, Surface ionization of organic compounds, Russ. Chem. Rev. 51 (1982) 819–841.
[12] T. Fujii, Surface-ionization organic mass spectrometry: mechanism, Eur. Mass Spectrom. 2 (1996) 91–114.
[13] T. Fujii, H. Arimoto, New sensitive and selective detector for gas chromatography: surface ionization detector with a hot platinum emitter, Anal. Chem. 57 (1985) 2625–2628.
[14] T. Fujii, T. Kitai, Determination of trace levels of trimethylamine in air by gas chromatography/surface ionization organic mass spectrometry, Anal. Chem. 59 (1987) 379–382.
[15] T. Fujii, H. Arimoto, Y. Mitsutsuka, Surface ionization organic mass spectrometry of imipramine, desipramine, clomipramine, and lidocaine, Anal. Chem. 66 (1994) 1884–1889.
[16] D.T. Usmanov, U. Khasanov, U.Kh. Rasulev, Surface-ionization mass spectrometry of opium alkaloids, Chem. Nat. Comp. 39 (2003) 489–494.
[17] M.D. Mashkovskiy, Medical Remedies, 1st ed., Medicine, Tashkent, 1987 (in Russian).
[18] U.Kh. Rasulev, E.G. Nazarov, K.S. Tursunov, T.V. Arguneeva, Surface ionization mass spectrometry of quarterly salts of ammonium, Zhurnal Analiticheskoi Khimii 46 (1991) 1802–1811 (in Russian).
[19] U.P. Hill, S.M.A. Leccini, B.A. Petica, Temperature calibration of tungsten wires and ribbons and their bearing on heats of chemisorption, Trans. Far. Soc. 62 (1966) 229–232.