

Laser ablation and ionisation by laser plasma radiation in the atmospheric-pressure mass spectrometry of organic compounds

A.V. Pento, S.M. Nikiforov, Ya.O. Simanovsky, A.A. Grechnikov, S.S. Alimpiev

Abstract. A new method was developed for the mass spectrometric analysis of organic and bioorganic compounds, which involves laser ablation with the ionisation of its products by laser-plasma radiation and enables analysing gaseous, liquid, and solid substances at atmospheric pressure without sample preparation. The capabilities of this method were demonstrated by the examples of fast pharmaceutical composition screening, real-time atmosphere composition analysis, and construction of the mass spectrometric images of organic compound distributions in biological materials.

Keywords: mass-spectrometry of organic compounds, laser ablation, laser plasma radiation.

1. Introduction

At the present time, one of the most important lines of development of laser-assisted mass spectrometry consists in the development of techniques for the chemical analysis of substances and materials at atmospheric pressure without sample preparation. These techniques are extremely topical in biology and medicine in the analysis of the composition of biological tissues and liquids, in safety systems for the real-time detection of narcotics and explosives, in pharmacology for identifying biologically active medicine components, and for real-time performance-enhancing drug detection.

In the majority of known techniques, laser radiation is employed for the ablation of the sample under examination or for the desorption of compounds adsorbed on its surface. For the subsequent ionisation, use is usually made of traditional techniques, in particular, electrospray [1] and chemical [2] ionisation. Another method involves matrix-assisted laser desorption/ionisation (MALDI) at atmospheric pressure [3]. These ionisation techniques are profitably employed in the mass spectrometric detection of ionic, polar, and highly basic compounds, but they are inefficient in the ionisation of the chemical compounds of other classes.

A more universal method is the combination of laser ablation with photoionisation by hard UV radiation [4]. To effect such ionisation, use is usually made of krypton UV lamps, which emit photons with a maximum energy of 10.6 eV.

An alternative approach may consist in the use of a laser plasma produced at the surface of a laser-irradiated target. It is commonly known that the laser plasma is a high-power source of hard UV radiation [5]. The plasma emission spectrum may be shifted to the desired domain by selecting the target material and the parameters of laser radiation as is done, for instance, in the production of integrated circuits [6]. The laser-to-plasma radiation energy conversion coefficient may be as high as several tens of percent, making it possible to develop a high-power radiation source with the use of relatively compact lasers. Lastly, the plasma source can be produced directly inside the ionisation chamber and does not require additional windows, which limit the spectral radiation range.

Therefore, a laser plasma is expected to be a highly efficient instrument for producing the ions of organic compounds which are in the gaseous state. To identify volatile compounds of, for instance, different organic impurities in the air, the air sampling should be organised in such a way that the air flow passes near the target which the plasma is formed on. In the analysis of solid samples, the organic compounds should be transformed to the gas phase, for instance, by way of laser ablation. In this case, the use of sharply focused laser radiation for vaporising the sample permits obtaining the picture of spatial compound density distribution over the sample and designing a mass spectrometric microscope with a spatial resolution of several tens of micrometres.

Optically pumped solid-state lasers are the candidates of choice for the plasma production. They provide a high pulsed power for a pulse duration of less than 1 ns, a pulse repetition rate of up to several kilohertz, and extreme stability and reliability. In the harmonics generation or parametric frequency conversion the plasma production and sample vaporisation (ablation) may be effected in a broad spectral range with the use of one laser.

The present work is concerned with the investigation of ionisation of organic compounds by laser plasma radiation at atmospheric pressure, the sensitivity evaluation of the laser mass spectrometer, and the realisation of the mass spectrometer–microscope that relies on the laser ablation of a solid organic sample and the ionisation of vaporisation products by laser plasma radiation.

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2. Experiment

Our experimental setup is schematised in Fig. 1. In our experiment, use was made of a time-of-flight mass analyser (reflector) (1), which was developed at the Institute for Analytical Instrumentation of the Russian Academy of Sciences (St. Petersburg), with the introduction of ions through a gas-filled transport quadrupole from the atmospheric pressure domain. The gas-dynamic atmospheric input into the mass analyser provides an input gas flow rate of $\sim 2 \text{ cm}^3 \text{ s}^{-1}$ at atmospheric pressure. The mass analyser has a mass resolution $m/\Delta m \approx 5000$ for a mass evaluation uncertainty $\delta m = 2 \times 10^{-5} m$. For mass spectra processing, advantage was taken of the SaldixScope code [7], which provided real-time processing.

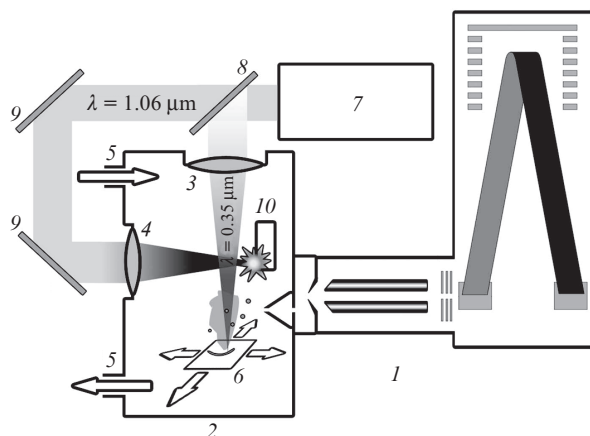


Figure 1. Schematic representation of the experimental facility intended for the mass-spectrometric analysis of solid organic samples without sample preparation, with the use of laser ablation and ionisation of ablation products by laser plasma radiation (see notations in the text).

An atmospheric-pressure hermetic chamber (2) containing a sample was placed in front of the intake of the mass analyser. The chamber was fitted with windows for the injection of laser radiation. The chamber was blown with air or purified gases (99.999-pure nitrogen and argon) through pipes (5). This made it possible to avoid in mass spectra the emergence of the ion peaks of trace organic compounds present in the atmospheric air. The sample under investigation was accommodated in the chamber on a Thorlabs MTS25-Z8 two-axis stage (6), which provided its displacement at 2- μm increments.

An RL-03/355 (ELS-94 Ltd.) diode-pumped solid-state Nd:YAG laser (7), equipped with a harmonic generator, with a pulse duration of 0.37 ns and a pulse repetition rate of 500 Hz was employed for sample vaporisation and plasma production. To vaporise the sample under investigation, use was made of the third harmonic of laser radiation with a wavelength of 0.35 μm and a pulse energy of up to 80 μJ . With the help of an interference mirror (8) and a lens (3) with a focal length of 40 mm this radiation was focused to a spot with a characteristic size of 30 μm onto the sample surface. The fundamental radiation with a wavelength of 1.06 μm , for which mirror (8) was transparent, was focused, using mirrors (9) and a lens (4), onto a metal target (10) to produce plasma on it. The target (10) was made in the form of a cylinder 10 mm in diameter and mounted on the shaft of a motor, which rotated it. This rotation practically eliminated the effect of erosion of the target material in the region of plasma production.

3. Results and their discussion

In the first series of experiments we determined the mechanism of ion formation with the use of the laser plasma as the ioniser. Laser plasma is a broadband UV radiation source with a spectrum extending to the soft X-ray domain, which enables ionising virtually all chemical compounds. Nevertheless, it turned out that the main channel of ionisation by the radiation of the laser plasma at atmospheric pressure was the attachment of protons H^+ by neutral molecules. It is believed that water molecules are the proton source under these conditions.

Figure 2a shows a typical air mass spectrum in the domain corresponding to water ions. One can see that it possesses two main peaks with mass-to-charge ratios $m/z = 18$ and 19, which may be interpreted as the lines of molecular H_2O^+ ions and protonated H_3O^+ molecules. Furthermore, the mass spectrum exhibits a peak with $m/z = 17$, which corresponds to OH^+ ions. Figure 2b depicts the same portion of the mass spectrum obtained on addition of heavy water vapour into the chamber. Observed in the spectrum are new peaks, which correspond to the ions D_3O^+ and D_2O^+ as well as to D_2HO^+ , DH_2O^+ , DHO^+ , and OD^+ . In this case, the peaks of organic compounds are recorded as the peaks of deuterated molecules. These data testify that the radiation of the laser plasma provides single-photon ionisation of water molecules, which have an ionisation potential of 12.6 eV [8]. It may be readily suggested that the resultant primary molecular ions enter into ion–molecule reactions with water molecules with the forma-

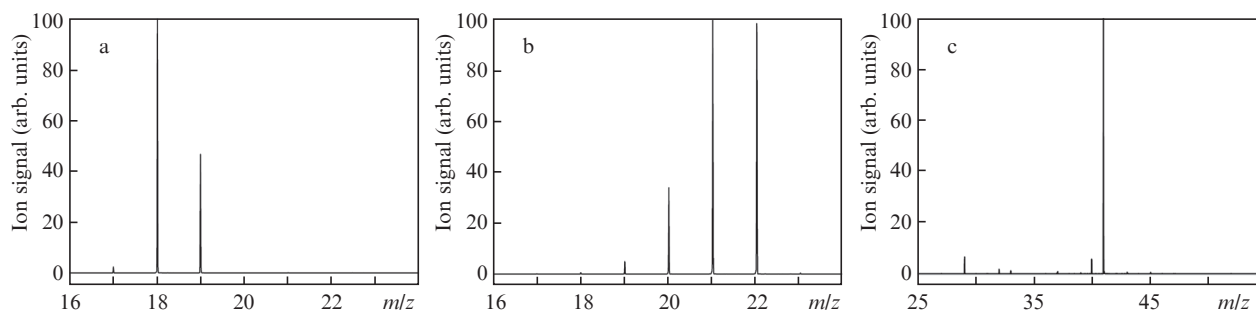


Figure 2. Mass spectra of the air containing water vapour (a) and of the air on addition of heavy water vapour (b), as well as the mass spectrum obtained on argon puffing into the sample chamber (c).

tion of hydroxonium ions H_3O^+ (the proton affinity energy is equal to 691 kJ mol^{-1} [9]), which in turn are efficient proton donors and effect the 'soft' ionisation of organic and bioorganic molecules. Also possible is the direct proton transfer from the molecular water ion H_2O^+ , which is a higher-efficiency proton donor than H_3O^+ owing to its lower proton affinity energy (593 kJ mol^{-1}).

This ionisation channel is not the only possible one as evidenced by the mass spectrum of Fig. 2c, which was obtained on puffing argon into the sample chamber. Apart from the peaks of Ar^+ and O_2^+ ions resulting from direct photoionisation (ionisation potentials of 15.75 and 14.01 eV), observed in this mass spectrum is the high-intensity peak of protonated argon ArH^+ with $m/z = 41$ as well as the peaks of protonated molecules of nitrogen N_2H^+ with $m/z = 29$ and of oxygen O_2H^+ with $m/z = 33$. The respective proton affinity energies of argon, oxygen and nitrogen are equal to 369, 421 and 494 kJ mol^{-1} [9]. This is indication that the second protonation channel may consist in the attachment of free protons produced under the action of laser plasma radiation on the water vapour present in the sample chamber. It is noteworthy that the introduction of heavy water vapour into the sample chamber gives rise to deuterated peaks of argon, oxygen, and nitrogen. The possibility of ionisation by protonation of molecules and atoms with so low a proton affinity energy is an advantage of the technique under development over other 'soft' ionisation techniques. For instance, the method of surface-assisted laser desorption/ionisation from silicon surfaces (SALDI) is applicable only for the ionisation of compounds with a proton affinity energy greater than 850 kJ mol^{-1} [10]. Chemical ionisation usually enables ionising compounds with a proton affinity energy greater than $750\text{--}800 \text{ kJ mol}^{-1}$ [11]. The MALDI technique [12] is characterised by about the same threshold values of proton affinity energy of the compounds under analysis.

In the second series of experiments we estimated the sensitivity of the technique under development in the detection of organic molecules. For this purpose, we employed a piezoresonance vapour microflow generator, which allowed us to obtain a controllable constant ultralow vapour flux of a broad class of chemical compounds [13]. Use was made of a mass-sensitive piezoelectric resonator for bulk acoustic thickness shear waves (AT-cut quartz) with an operating frequency of 18 MHz. The mass responsivity of the resonator was equal

to $5930 \text{ Hz } \mu\text{g}^{-1}$. The resonator electrode was coated with a thin film of a substance, whose evaporation (sublimation) resulted in an increase in the resonance frequency of the mass-sensitive resonator. Frequency measurement permits determining the flow rate of the molecules under analysis admitted to the chamber for sample injection. For such molecules we selected caffeine, whose room-temperature vapour pressure is equal to $3 \times 10^{-7} \text{ Torr}$ [14]. Its low evaporation rate provides a substance flow rate, which is stable for a sufficiently long period of time, for a low density of the molecules under analysis and thereby models the measurement conditions in the laser vapourisation of a sample with a low content of the target substance.

To improve the precision of determining the flow rate of caffeine molecules, the measurement time was made equal to 10 h. The resonator frequency variation during this period was equal to 250 Hz, which corresponds to a resonator mass lowering of about 40 ng due to caffeine sublimation. Accordingly, the rate of this flow averaged over the experiment period was equal to 1.2 pg s^{-1} , or $3.7 \times 10^9 \text{ molecules s}^{-1}$. Figure 3 shows the mass spectra recorded prior to and after the microflow generator installation in the sample chamber. The data presented in the drawing suggest that the generation of the flow of caffeine molecules gives rise to the peak with $m/z = 195$, which corresponds to protonated caffeine molecules. The intensity of this peak is at least ten times greater than the background signal. Hence we can draw the conclusion that the detection threshold for caffeine vapour in the air is approximately equal to several tenths of a picogram. Although the resultant threshold is considerably inferior to the threshold reached earlier in the detection of the flow of caffeine molecules by the SALDI technique in a vacuum [15], the proposed technique of ionisation by laser plasma radiation enables detecting the trace amounts of organic compounds directly in the atmospheric air. In particular, considering that the injection device of the mass analyser provides an atmospheric air intake of $2 \text{ cm}^3 \text{ s}^{-1}$, for a standard mass-spectrum acquisition interval of 1 s the instrument is capable of detecting caffeine vapour in the air for a relative density of $\sim 3 \times 10^{-11}$, or $\sim 30 \text{ ppt}$. This conclusion is also borne out by the fact that a number of peaks of protonated organic molecules contained in trace amounts in the laboratory air were observed in the mass spectra recorded when the atmospheric air was blown through the sample chamber.

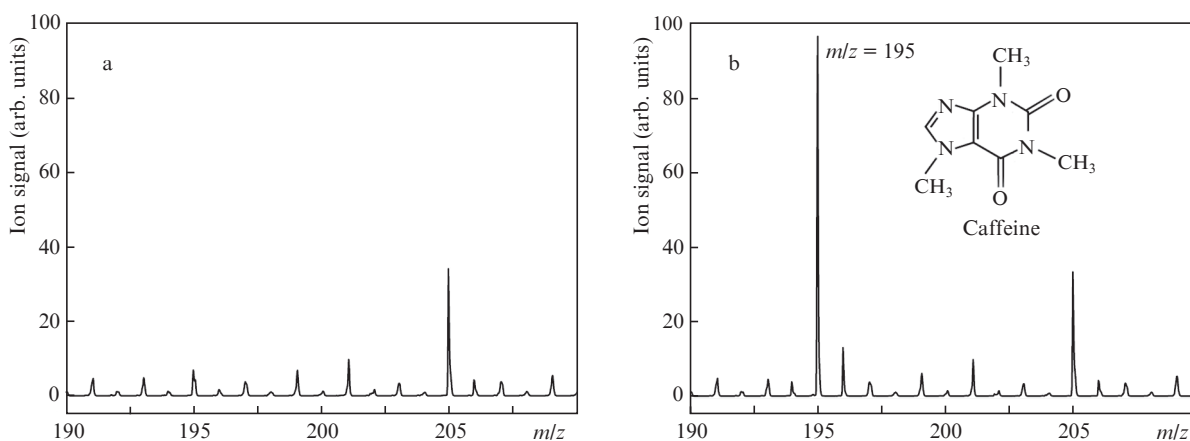


Figure 3. Mass spectra obtained prior to (a) and after (b) installing into the sample chamber the microflow generator with a deposited caffeine film.

In the third series of experiments we evaluated the capabilities of our technique for the analysis, without sample preparation, of solid samples containing different organic compounds. For the objects of investigation we selected medicine tablets with known reactant densities. In the laser vaporisation of these tablets the absorption of near-UV radiation is primarily due to pharmaceutical excipients, which are always present in the tablets. One of such substances is, for instance, talcum (minnesotaite), while the organic compounds themselves are transparent in this spectral region. The presence of absorbing excipients is responsible for the ablative vaporisation mode when medicine tablets are exposed to 355-nm laser radiation. Figure 4 shows the mass spectra recorded in the laser ablation of sulfadimethoxine and tofizopam tablets and the ionisation of vaporisation products by laser plasma radiation. One can see that the mass spectra exhibit the peaks of active pharmaceutical substances in the form of the peaks of protonated molecules.

These experiments also permit estimating the sensitivity of the proposed analysis technique. To do this, we measured the crater produced by the laser radiation on the tablet surface during one minute of irradiation. The crater diameter was equal to 55 μm for a depth of $\sim 40 \mu\text{m}$, which corresponds to a crater volume of 10^{-7}cm^3 . In this case, the flow rate of sulfadimethoxine molecules vaporised by the laser radiation was equal to $1.5 \times 10^{-9} \text{g s}^{-1}$. Since the signal-to-noise ratio in the recorded spectra exceeds 10^3 (Fig. 4), it is safe to assume that the experimentally recorded flux is 300 times higher than the detection threshold, which is equal to 5pg s^{-1} . This value of the threshold flux of the substance under detection is comparable to the value of the flux of caffeine molecules obtained earlier using a microflow generator. However, it is pertinent to note that this estimate ranks below the sensitivity estimate made using the microflow generator because of uncertainties in the evaluation of ejected substance flux from the crater dimensions. It is well known that large clusters and nanoparticles, which cannot be detected by a mass spectrometer, may be formed in this flow. This leads to an underestimation of the real sensitivity of the instrument and to an uncertainty in sensitivity estimates in the laser ablation. We note that the detection threshold estimates made in our work are only slightly inferior to the record detection thresholds of mass spectrometric complexes elaborated on the basis of sample ionisation

by electrospray and MALDI techniques, which lie in the $0.2\text{--}0.5 \text{pg s}^{-1}$ range, according to Refs [16, 17]. At the same time, it is common knowledge that these techniques necessitate a very long and tedious sample preparation.

In the fourth series of experiments we analysed solid organic samples with spatial resolution and constructed mass spectrometric images of the object being analysed. To investigate the feasibility of obtaining the mass spectrometric image of the sample under analysis and determine the spatial resolution in the mode of sample scanning, a human hair was selected as the model object. This choice is due to the following fact: in coloured hair tissue the absorption of the 355-nm wavelength radiation in use amounts to 10^3cm^{-1} and turns out to be sufficiently high for efficient ablation. Furthermore, a hair is a stable object, which retains its shape for a long time, and its diameter is easily determined using conventional measuring means.

Hairs are comprised of 3% water and of 97% keratin – a proteic substance. Keratin is a biopolymer, the product of polymerisation of cysteine, a sulfur amino acid. In the case of laser ablation of a hair, due to heating there occurs disintegration of the biopolymer molecules with the production of several fragments. In the ionisation of vaporisation products by laser plasma radiation we observed a characteristic mass spectrum containing more than 50 peaks with $m/z = 40\text{--}250$. For image construction we chose the peak with $m/z = 70$, which had the highest amplitude and supposedly corresponded to the main structural biopolymer fragment C_3NOH_4 .

Figure 5a shows the dependence of the amplitude of the ion peak with $m/z = 70$ on the stage displacement transversely to the hair axis at 2- μm scanning increments. The circle at the centre corresponds to the optical image of the hair 60 μm in diameter, which was obtained by conventional techniques. One can see that the mass spectrometric image has a spatial resolution of $\sim 30 \mu\text{m}$, which correlates well with the characteristic dimension of the spot of laser radiation which vaporises the sample. Figure 5b shows the mass spectrometric hair image recorded by way of sequential two-coordinate stage scanning at 2- μm increments.

We emphasise that the experiments outlined above are model-like and demonstration in nature. Their sole objective is to demonstrate the applicability of the proposed technique for the solution of the currently burning problem of con-

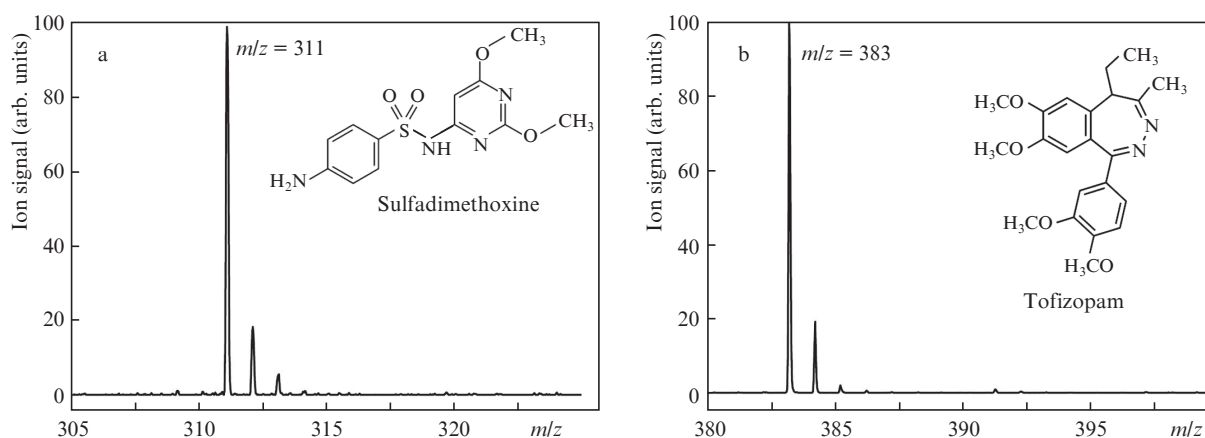


Figure 4. Mass spectra of sulfadimethoxine (a) and tofizopam (b) recorded by laser vaporisation and the subsequent ionisation of evaporation products with laser plasma radiation.

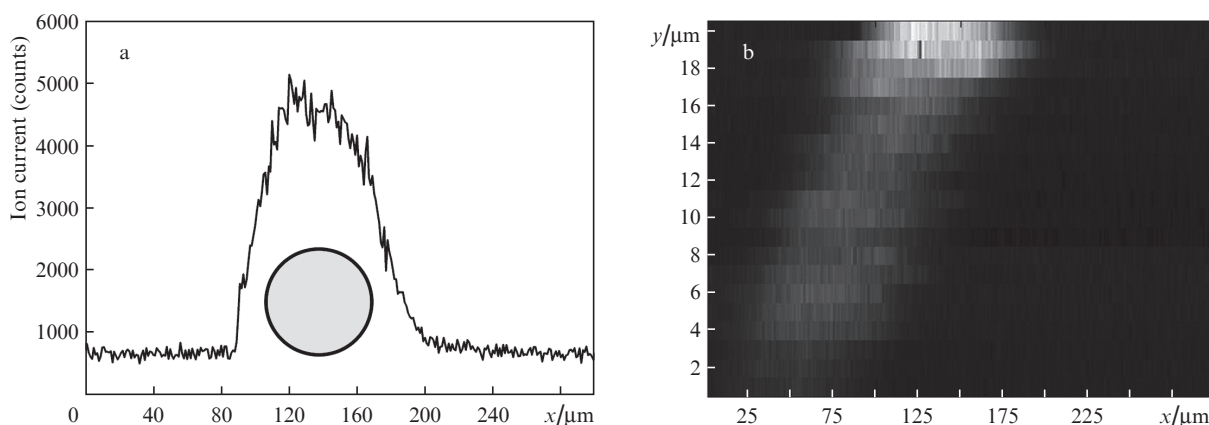


Figure 5. Dependence of the amplitude of the peak with $m/z = 70$ on the stage displacement x transversely to the hair axis (a) and mass spectrometric hair image obtained by two-coordinate stage scanning (b).

structuring mass spectrometric images of the composition of complex bioorganic objects. We also note that the spatial resolution achieved in this work is far from being the limiting one and may be substantially improved with the use of presently well-elaborated techniques of material laser ablation.

4. Conclusions

A new technique was developed for the mass spectrometric analysis of organic and bioorganic samples, which combines laser ablation with the ionisation of its products by laser plasma radiation. This technique permits analysing gaseous, liquid, and solid samples at atmospheric pressure without sample preparation. The compounds under examination are detected in the form protonated molecules. We demonstrated the possibility of ionisation by the protonation of molecules and atoms with low, less than 500 kJ mol^{-1} , proton affinity energies. The detection thresholds of our technique were evaluated in the analysis of gaseous and solid samples.

The capabilities of our technique were demonstrated by the examples of fast screening of pharmaceutical compositions, analysis of real-time atmospheric composition, and construction of mass spectrometric images of organic compound density distribution in biological materials.

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