Atmospheric pressure imaging mass spectrometry of drugs with various ablating lasers

K A Moshkunov[1], S S Alimpiev[1], A A Grechnikov[2], S M Nikifirov[3], A V Pento[1] and Ya O Simanovsky[3]

[1] General Physics Institute of RAS, Vavilov str., 38, 119991 Moscow, Russia
[2] Vernadsky Institute of Geochemistry and Analytical Chemistry of RAS, Kosygin str., 19, 119991 Moscow, Russia
[3] Advanced Energy Technologies, Novaya str., 100, 143025 Skolkovo, Russia

E-mail: moshkunov@gmail.com

Abstract. The atmospheric pressure mass spectrometric detection efficiency of organic species (tofisopam and verapamil) was measured by means of the laser ablation of dried solution drops containing known amount of the analyte. Ablated molecules were ionized by an atmospheric pressure laser plasma cell and then introduced in the TOF mass-spectrometer. The spot was formed by dripping 2 μl of solution on the stainless steel substrate and consequent drying. Then it was scanned by an intense ablating beam of various lasers (CO_2, Nd:YAG and femtosecond fiber laser) until the spot was completely eroded during the non-stop MS-analysis of ablated material. The sensitivity was defined as the ratio of the total ion current integral of the relevant mass peaks to the amount of molecules in the spot. All the tested lasers are suitable for the ablation and subsequent MS-detection of organic species in dried solution spots given enough power deposition is provided. The measured sensitivity values reach 0.1 ions/fg of tested analytes.

1. Introduction

The rapid expansion of mass spectrometry methods in the field of biology and medicine was driven by the discovery of soft non-destructive ionization methods [1] and the elaboration of high-resolution mass spectrometers [2]. Further development in the field led to the appearance of techniques that could operate with biological tissues with no preliminary procedure of preparation of probes, which cannot be studied by vacuum methods, giving rise to atmospheric pressure mass spectrometry (AP-MS).

The commonly employed atmospheric pressure method is matrix assisted laser desorption ionization MALDI [3,4]. It utilizes the ionization process by the evaporation of organic-containing matrix, which ionizes a wide variety of organic molecules. However, this requires the preparation of the sample and in the case when a study of a solid object is to be performed it can only analyse the very top layer (few microns). Surface assisted laser desorption ionization SALDI [5] analyses species in vacuum. The most basic method of ionization – electro spray [6] works at atmospheric pressure but requires the dissolution of an object in study. The ability to perform measurements in atmosphere and without sample preparation is crucial for many studies of organic tissues when depth profiling is required. Direct analysis in real time DART [7] technique allows atmospheric pressure mass analysis but is still limited to the surface layer of a sample.

The most efficient way to circumvent most of these issues is to separate the ionization from the ablation stage so that the measurement does not depend on a setup geometry, type of material and its laser absorption properties or matrix effects. Several modern techniques satisfy the need: laser assisted electro spray ionization LAESI [8], laser ablation atmospheric pressure photo ionization LAAPPI [9] for example. It utilizes laser ablation to form the flux of neutral particles, while the ionization is performed by a reaction chain driven by UV radiation or corona discharge.

The method developed in our laboratory relies on ionization by UV radiation produced by a laser plasma [10], which works with an air flow that contains molecules to be analyzed and does not depend on the way this flow was produced – either by various types of ablation, evaporation of the sample, or initially gaseous probes. The advantage of the technique is that the sensitivity of the detection does not rely on the probe type and can be calibrated for a wide range of applications. This allows the calibration of the sensitivity towards exact compounds by introducing known amount of molecules in the device. A spot produced by a drying of a drop contains well known amount of analyte. Laser ablation of it provides the required calibration stream.

However, the influence of the ablating laser parameters on the sensitivity of the method is still unclear. Wavelength, laser pulse energy and duration can affect the way the neutral flow is formed [11]. Ablation efficiency, analysed molecules fragmentation or formation of clusters affect the final amount of molecules available for ionization and subsequent mass analysis. We studied the ablation of organic-containing spots thin enough to ensure the evaporation to be governed by heating of underlying steel substrate which does not ablate itself. Three laser types were tested – surgical CO_2, Nd:YAG, and femtosecond fiber laser Fianium so that a wide range of wavelengths (355 nm – 10.6 μm) and fluences (0.065 – 1 J/cm^2) is covered.

2. Experimental

The setup consists of a time-of-flight mass spectrometer (Q-TOF reflectron MX 5311 developed by the Institute for Analytical Instrumentation of the Russian Academy of Sciences (St. Petersburg)) with attached modular atmospheric ionization device, target holder and an ablating lasers. The scheme of the atmospheric interface is presented in figure 1.

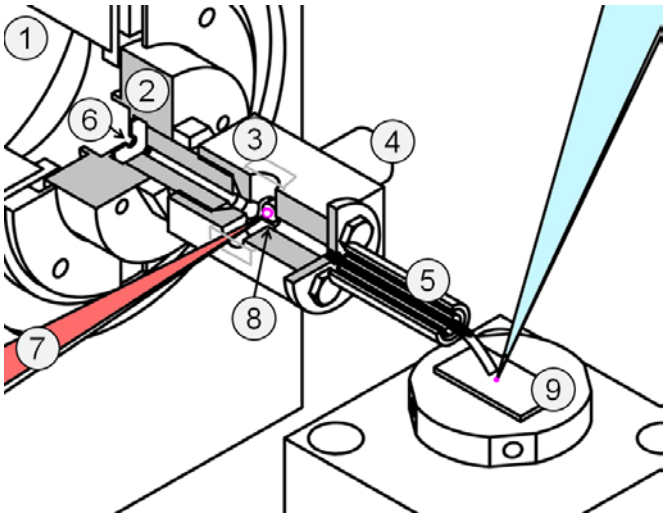

Figure 1. Atmospheric pressure interface. 1 – mass-spectrometer, 2 – diafragm holder and capillary, 3 – ionization cell, 4 – rotating metal target, 5 – heated capillary, 6 – diafragm, 7 – plasma igniting laser, 8 – plasma, 9 – sample, ablated by laser.

The sample is mounted on a two-axis scanning table with adjustable height so that samples with any height can be mounted with surface matching the firmly fixed ablating laser waist and the collection volume of the capillary. The ablated material is pumped by a capillary into an ionization

chamber. The heated surface of the capillary is kept at 200° C, so that clusters dissociate into separate molecules, which are ionized by a complex chain of processes caused by the radiation of the laser ignited by plasma on a metal (tungsten) rotating target [10].

The ion stream is then conducted through the 0.3 mm diafragm that separates atmosphere from vacuum.

Though we can perform measurements on any solid target, even organic tissues, the experiment was performed on a stainless steel plate. The water-alchogol solutions containing medical drugs: one – tofisopam (1 μg/ml) and other verapamil (1 μg/ml) were dripped in 2 μl drops on the substarte and dried. The resulting spot is 1-2 mm in diameter and the average thickness of few monolayers of dried analyte. The simple estimations show that laser absorption by thin layer of analyte is negligible. The laser is dominantly absorbed by the steel substarte, which heats up and desorbs the organic overlayer.

The dried drop spots can be ablated with excessive laser power ensuring that all the material is eroded and the known amount of analyzed molecules entered the atmospheric interface. Simultaneously mass spectra are aquired and the corresponding mass peaks are monitored and integrated. In our case both analyzed species are ionized by protonation and manifest as the $M+H^+$ masses along with dimers and trimers and isotopic variations. The dominant peaks are m/z 383 and 455 for tofisopam and verapamil correspondingly. The ratio of the integrated ion signal recorded during the laser scanning of the spot to the amount of deposited molecules gives the ionization efficiency and sensitivty of the device.

While the fundamental frequency of Nd:YAG was used for plasma ignition and ionization, the ablating lasers were varied. Three lasers were tested and their parameters are presented in Table 1.

Table 1. Ablating lasers parameters.

Laser type	**Fiber**	**CO_2**	**Nd:YAG (3^{rd} harmonic)**
Model	Fianium FP532-5	Lancet-1	RL-03/355
Wavelength	1064 nm	10.6 μm	355 nm
Average power	1.28 W	0.16 W	24 mW
Pulse duration	30 fs	0.01 s	300 ps
Pulse repetition rate	1 MHz	16 Hz	300 Hz
Pulse energy	1.28 μJ	10 mJ	80 μJ
Spot FWHM	50 μm	155 μm	100 μm
Energy density	0.065 J/cm^2	50 J/cm^2	1 J/cm^2

Mass spectra recorded during target ablation show a clear presence of tofisopam and verapamil – figure 2.

The results of dried spots scanning by three lasers and two analytes are presented in figure 3. It is a relief image of a spot containing 2 ng tofisopam or verapamil deposited on stainless steel plate. The intensity is obtained by mass spectrometric scanning of 4x4 mm frame. The images presented are the intensity of the net analyte signal plotted versus scanning coordinates (millimeters).

The mass spectra from the spots clearly indicate the presence of tofisopam and verapamil for respective scans. The noise for peaks related to tofisopam is very low and their background is negligible. A clear circle-like structure reproduces for every spot and is very characteristic. The results of the integration and sensitivity calculation are presented in table 2.

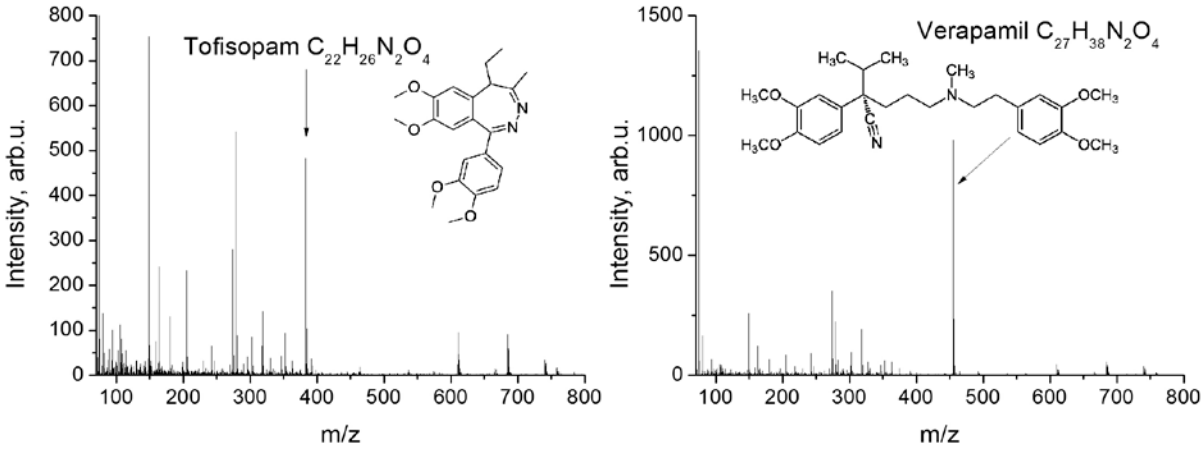

Figure 2. Characteristic mass spectra containing tofisopam and verapamil in dried spots.

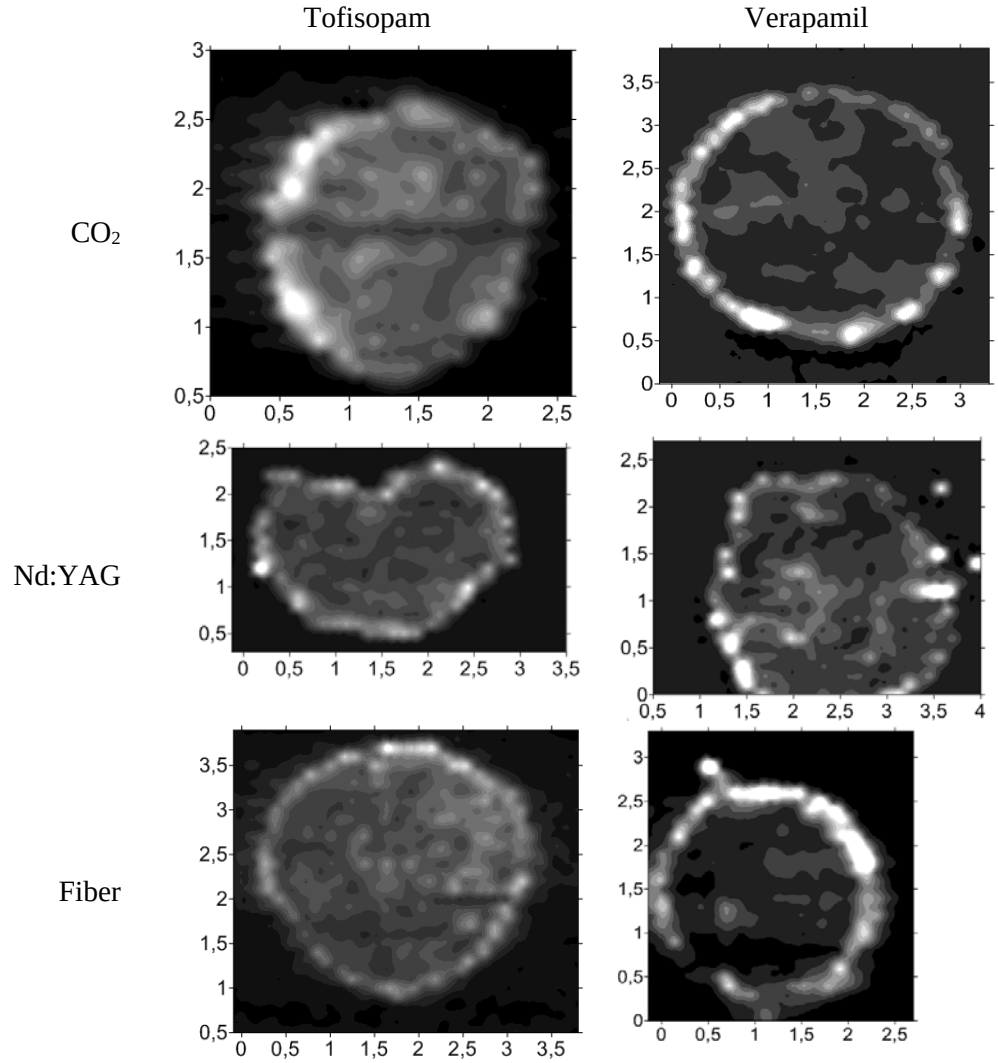

Figure 3. Mass-image (concentration distribution) of tofisopam and verapamil-containing dried spots by various ablating lasers. Image dimensions are millimetres.

Table 2. Sensitivity for tofisopam and verapamil for various ablation lasers.

Laser type	**Tofisopam, ion/fg**	**Verapamil, ion/fg**
Nd:YAG	0.110	0.004
Fiber	0.070	0.005
CO_2	0.033	0.008

4. Discussion

Recorded mass-spectra contain along with analyte-related peaks a lot of peaks that relate to molecules contained by ambient air. Nevertheless, the sensitivity of the method (table 2) is high and does not vary considerably with the laser type. The ionization occurs via a long chain of processes resulting in proton attachment to an analyte molecule. This process is often used in atmospheric ionization methods [12]. In our case, UV radiation generated by metallic laser plasma produces water ions [10] that start the reaction chain. The concentration of these proton donors is orders of magnitude higher than the concentration of analyte ions.

The mass-image of a dried spot in figure 3 exhibits a circular structure meaning that drying is accompanied with the concentration of solution additives at the periphery (liquid-solid-air border). The dried spots were barely visible before the experiment and disappeared completely after. Consecutive scans reveal very small amount of the analyte (tofisopam and verapamil) left after the first scan. Visual inspection of the stainless steel substrate reveals no damage to it and no metal ions were detected.

Several conclusions can be drawn on the basis of data in table 2. Firstly, neither average power nor laser fluence affect sensitivity considerably or there is any clear correlation. This is in accordance with the conception that the laser pulse removes all the material in the spot in few pulses. Excessive power is no more absorbed neither by the material deposited nor the temperature developed in steel substrate is enough to cause significant evaporation. Moreover, in the case of a dilute solution the evaporation process is believed to be caused not by laser absorption by the very material but rather by substrate that heats to the temperature that is enough to evaporate the organic-containing layer.

While there is no clear dependence on power parameters the sensitivity changes smoothly with wavelength. Tofisopam detection sensitivity is highest at lower wavelength and reduces with its increase and the verapamil sensitivity has a contrary dependence. The absolute values of sensitivity factor in the unknown ionization cross-section and no conclusion can be drawn from tofisopam to verapamil ratios.

5. Conclusions

Series of experiments on sensitivity calibration of atmospheric pressure mass spectrometer towards medical drugs (verapamil and tofisopam) were carried out. Known amount of molecules of the analyte was removed from the stainless steel surface by laser ablation of dried solution drops and consequently ionized by laser plasma. The resulting sensitivity reaches values 0.1 ion/fg. Investigation of the sensitivity dependence on ablating laser parameters revealed that the beam power characteristics are of low importance given the laser power is enough to ablate the thin layer of analyte from the steel substrate.

Acknowledgements

This work was supported by the Ministry of Education and Science of the Russian Federation (Contract No. 14.579.21.0020).

References

[1] Monge M E, Harris G A, Dwivedi P and. Fernández F M 2013 *Chemical reviews* **113** 2269
[2] Scigelova M and Makarov A 2006 *Proteomics* **6** 16
[3] Karas M and Hillenkamp F 1988 *Analytical chemistry* **60** 2299
[4] Laiko V V, Moyer S C and Cotter R J 2000 *Analytical Chemistry* **72** 5239
[5] Alimpiev S S, Grechnikov A A, Sunner J, Karavanskii V A, Zhabin S N, Simanovsky Ya O and Nikiforov S M 2008 *J.Chem.Phys*. **128** 014711
[6] Fenn J B, Mann M, Meng C K, Wong S F and Whitehouse C M 1989 *Science* **246** 64
[7] Cody R B, Laramée J A and Durst D H 2005 *Analytical Chemistry* **77** 2297
[8] Rezenom Y H, Dong J and Murray K K 2008 *Analyst* **133** 226
[9] Vaikkinen A, Shrestha B, Kauppila T J, Vertes A and Kostiainen R 2012 *Analytical chemistry* **84** 1630
[10] Pento A V, Nikiforov S M, Simanovsky Ya O, Grechnikov A A and Alimpiev S S 2013 *Quantum Electronics* **43** 55
[11] Vogel A and Venugopalan V2003 *Chemical reviews* **103** 577
[12] Venter A R, Douglass K A, Shelley J T, Hasman G Jr. and Honarvar E 2013 *Analytical chemistry* **86** 233