Rapid screening of pharmaceutical drugs using thermal desorption – SALDI mass spectrometry

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Abstract. A novel approach to the rapid screening of pharmaceutical drugs by surface assisted laser desorption-ionization (SALDI) mass spectrometry with the rotating ball interface coupled with temperature programmed thermal desorption has been developed. Analytes were thermally desorbed and deposited onto the surface of amorphous silicon substrate attached to the rotating ball. The ball was rotated and the deposited analytes were analyzed using SALDI. The effectiveness of coupling SALDI mass spectrometry with thermal desorption was evaluated by the direct and rapid analysis of tablets containing lidocaine, diphenhydramine and propranolol without any sample pretreatment. The overall duration of the screening procedure was 30÷40 sec. Real urine samples were studied for drug analysis. It is shown that with simple preparation steps, urine samples can be quantitatively analyzed using the proposed technique with the detection limits in the range of 0.2÷0.5 ng/ml.

1. Introduction

The development of laser-assisted mass spectrometric techniques has resulted in new facilities, approaches and methods in the field of organic mass spectrometry. Surface-assisted laser desorption-ionization (SALDI) [1] is an excellent example, which holds great promise for analytical applications. In SALDI the gas-phase ions are formed from molecules deposited on a particular surface substrate that is irradiated with a pulsed laser. That process does not require the entrainment action of an added matrix compound for desorption [2]. Wide variety of solid state materials, such as graphite [1], activated carbon [3], titanium oxide (4) and zinc oxide [5], have been evaluated as a possible platform for SALDI with different degrees of success. However silicon materials, in particular, porous, nanocrystalline and amorphous silicon (a-Si) are so far the most commonly used substrates for SALDI.

The traditional approach to the analysis of organic and bioorganic samples by SALDI includes deposition of liquid solution of analytes as a droplet on the SALDI-active substrate. The droplet is dried and the sample support inserted into the mass spectrometer for analysis. The main problems with this approach are very poor repeatability from substrate to substrate, inhomogeneous distribution of analyte on the substrate surface and strong influence of matrix effects.
The implementation of gas-phase deposition SALDI as a gas chromatography/mass spectrometry (GC/MS) instrument was recently demonstrated [6]. In this method, individual, GC-separated compounds are adsorbed on the SALDI substrate and subsequently ionized and desorbed by the laser pulse. Gas-phase introduction of analytes was shown to be associated with high ionization efficiency, up to 1%. However, applying GC/SALDI MS for screening of pharmaceutical drugs is difficult because the analysis is time-consuming.

This work presents a novel approach for reproducible and quantitative analysis of solid and liquid organic and bioorganic samples by SALDI using gas-phase analyte introduction. Such approach is based on the combination of SALDI mass spectrometer with rotating ball interface [7] and thermal desorption chamber. Legal pharmaceutical products containing lidocaine, diphenhydramine and propranolol were used to demonstrate the effectiveness of proposed technique for the rapid screening of pharmaceutical drugs without a sample pretreatment or chromatographic separation step. Also, real urine samples were studied for drug analysis.

2. Experimental
The experimental setup is schematically shown in figure 1.

The mass spectrometer was a lab-built, time of flight reflectron (0.9 m) with the orthogonal ion injection via gas-filled radiofrequency quadrupole (3 MHz, 1 kV). The ions were detected by a secondary electron multiplier ETP 14882 (ETP Pty Ltd). A 40-mm-diameter, rotating stainless-steel ball forms the interface between the sample deposition chamber, where analytes were deposited on the SALDI active substrate, and the ion source, where the deposited analytes were ionized and desorbed by laser irradiation for the subsequent mass spectrometric analysis. Rotation of the ball is actuated by a computer-controlled stepping motor.

A diode-pumped Nd:YAG laser with near-diffraction limited beam quality, 0.5 ns pulse duration, was operated at 300 Hz. A frequency-tripling crystal yielded a 355 nm laser pulse of about 100 μJ. The laser pulse energy was varied in the range of 10-30 μJ using an attenuator. In order to more efficiently utilize the analyte, the laser focus was scanned over a 1×2 mm² sample deposition area using a computer-controlled, dual-mirror scanner. The laser focus moved approximately 0.08 mm between successive laser shots, and about 300 individual mass spectra were obtained from different points on the probed area of the SALDI active substrate.
Amorphous Si (α-Si) substrates were deposited on monocrystalline silicon by standard RF sputtering of Si in a low pressure (10³ Torr) Ar atmosphere, using a turbo-molecular pumped, lab-built apparatus [2]. The thickness of the deposited films measured using quartz crystal microbalances was approximately 0.3 µm. Two α-Si substrates were installed on opposite sides of the rotating ball. Analytes were deposited using thermal desorption on the substrate, the ball was rotated 180°, and the analytes were ionized and desorbed by laser irradiation for the subsequent mass spectrometric analysis. For temperature programmed thermal desorption, a resistively heated nickel-chromium wire wrapped around the stainless steel vaporizer tube was used to slowly warm the vaporizer. The temperature was monitored using a chromel-alumel type K thermocouple placed in contact with the vaporizer. Vaporizer was positioned at 2 mm distance from the surface of α-Si substrate.

Pharmaceutical tablets containing lidocaine, propranolol and diphenhydramine were obtained commercially. Small amount (less than 1 µg) of a tablet, removed using a scalpel, was used for the rapid screening of pharmaceutical drugs. To obtain liquid solution of analyte a tablet was firstly grinded to a powder. After dissolution in high-purity water with added sodium hydroxide the drug was extracted with ethyl acetate. Then the extract was filtered and dried, and analyte was purified by crystallization and dissolved in water methanol (1:1) mixture.

3. Results and discussion

3.1. Screening of pharmaceutical drugs

Lidocaine, propranolol and diphenhydramine were selected as test analytes because of their high proton affinities, differences in chemical structure, and their interest as widespread drugs, that can give a good indication of the utility of thermal desorption - surface assisted laser desorption ionization. In the rapid screening mode, the vaporizer with studied samples was rapidly heated to 170°C followed by the ball rotation and the SALDI/MS analysis of deposited probes. The preliminary study showed that 10 mJ/cm² of laser fluence gives the best performance in the rapid screening mode.

Propranolol was detected as the protonated molecule at m/z 260, with no fragment ions. Figure 2 shows typical SALDI mass spectrum of lidocaine. Mass spectrum contains the protonated molecule, MH⁺, at m/z 235, and major fragment ion at m/z 86.

![Figure 2. SALDI mass-spectrum of lidocaine.](image)

The fragmentation of protonated lidocaine molecule is formed by the C–C bond cleavage, accompanied by hydrogen transfer from the amino group to the carbonyl group, yields a protonated amine with m/z 86 as it is seen in the Scheme 1.

![Figure 3. SALDI mass-spectrum of diphenhydramine.](image)
Figure 3 demonstrates typical SALDI mass spectrum of diphenhydramine. It is seen that diphenhydramine is more label compound, as compared with propranolol and lidocaine and gives three main peaks due to the protonated molecule, $\text{MH}^+$, at $m/z$ 256, and two fragment ions. The proposed fragmentation pathways are illustrated in scheme 2 and scheme 3. Major fragment ion at $m/z$ 167 is formed by the cleavage of the bond between oxygen and the carbon of the amino group, while minor fragment ion at $m/z$ 72 is formed by the cleavage of the bond between oxygen and the carbon of the diphenylmethyl substituent.

The resulting SALDI mass spectra are seen to contain structural information about analyte molecules. The simultaneous monitoring of fragment ion peaks and the protonated molecule peaks increases the ability to discriminate and identify drugs when complex probes are analyzed.

The overall duration of the screening procedure using thermal desorption – SALDI analysis with the rotating ball interface is 30–40 sec in comparison with the 10–20 min GC/MS runs routinely used in forensic analysis.

### 3.2. Urine analysis

With simple preparation steps, urine samples can be directly analyzed using thermal desorption/ SALDI MS. Liquid solution of analyte was added to the urine. Then 5 mg of NaHCO$_3$ and 0.5 mL of chloroform isobutanol (6:1) mixture were added to 0.1 mL of urine. Samples were vortexed for 2 min and centrifuged at 10000 rpm for 3 min. The extraction efficiency was found to be 95–98%. Some µL of the bottom organic layer was transferred into the vaporizer. The temperature programmed thermal desorption mode was used for analyte sampling. In this mode the temperature of the vaporizer was programmed for rapid heating to 120°C, then to 200°C with a rate of 10°C/min. Laser fluence was 25 mJ/cm$^2$.

Figure 4 shows the ion mass thermogram obtained for propranolol protonated molecules. The mass thermogram was a record of ion current response for one mass unit (m/z 260) over the time of analysis. It is seen that propranolol thermally desorbs over a relatively narrow temperature range after about 3 min from starting programmed thermal desorption. This is reflected in the ion signal trace, which has well-resolved maximum. Figures 4b and 4c present mass spectra in the region of mass-spectral peak of protonated molecule before the beginning of propranolol desorption and at the maximum of ion current. These were obtained by integrating the mass spectra over the one cycle of analyte deposition and laser desorption-ionization.

Evidently, peak desorption temperatures can be used as a guide for identification of the drugs, along with mass spectral information. It is especially important when analyte ions have near equal m/z values, because a vapor pressure difference gives separation of analytes for accurate mass spectral identification.
Figure 4. Mass thermogram of propranolol (MH)$^+$ ion. The lower insets show mass spectra obtained a) after 150 sec, b) after 220 sec. Concentration of propranolol in urine was 2 µg/mL.

The linearity of response was studied by varying the concentration of drugs in urine samples. Figure 5 demonstrates the dependence of analytical signal on the amount of propranolol in extract solution placed in the vaporizer. It is seen that the linear dynamic range extends over at least 3 orders of magnitude. The correlation coefficient found to be above 0.995. The relative standard deviation for five replicate analyses was less than 10%. Very similar results were obtained with lidocaine and diphenhydramine. The detection limits for studied drugs was estimated to be 0.2±0.5 ng/ml.

The obtained data clearly indicates the capability of proposed technique for the quantitative determination of low molecular weight drugs in urine samples.
Conclusions
Temperature programmed thermal desorption is coupled to a SALDI mass spectrometer with rotating ball interface for the first time. The application of such approach for drug detection is demonstrated. Lidocaine, propranolol and diphenhydramine were studied as test analytes. It is shown that the presented technique provides a simple and rapid screening method for tablets and other pharmaceutical products with total analysis time as short as 40 sec or less. Tested analytes are detected as protonated molecules with no or very structure-specific fragmentation. The schemes of fragmentation are proposed.

It is also demonstrated that the presented technique is applicable to the quantitative analysis of liquid biological materials such as urine. The detection limits of studied drugs were estimated to be 0.2–0.5 ng/ml.

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