Designer drug analysis by forensic MALDI techniques

The versatility of MRMS for forensic analysis.

Abstract

The phenethylamines derivatives, known as NBOMes, N-bomb or Smiles, are potent hallucinogens, which are often sold as blotter paper. Herein, matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) and MALDI mass spectrometry imaging (MALDI Imaging) were coupled to a Magnetic Resonance Mass Spectrometer (MRMS, classically known as FT-ICR MS), a high mass accuracy, high resolution mass spectrometer, and used to analyze seven blotter papers of NBOMes containing 25I-NBOH (m/z 414) and 25I-NBOMe (m/z 428).

Keywords:
NBOMes, MALDI Imaging, MRMS

Introduction

The term designer drug refers to a synthetic version of an illicit drug modified to potentialize or create new psychoactive effects. Some of the new psychoactive substances (NPS) are often sold impregnated on blotter paper, where each small square is sold as a dose. They have colorful images printed on its surface, as shown in Figure 1 and are administrated sublingually.

Mass spectrometry (MS) has been widely used in forensic investigations of NPS, being extremely versatile, due to the fact that it allows the use of different sources of ionization such as paper spray, direct sample analysis (DSA), electrospray ionization (ESI), desorption atmospheric

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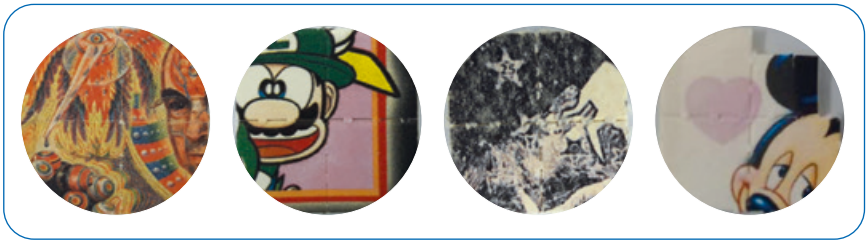

Figure 1: Blotter papers containing illicit substances.

pressure photoionization (DAPPI), easy ambient sonic-spray ionization (EASI) and matrix-assisted laser desorption/ionization (MALDI). Among the cited methods, MALDI MS can be considered a rapid analysis strategy for the identification of NPS as well as their metabolites. In the forensic context, mass spectrometry imaging has been used to investigate the chemical distribution on a surface of interest, mainly in the detection of drugs in hair, tissues, blood and fingerprints present on banknotes. The main challenge of the MALDI Imaging technique lies in the sample preparation, being the choice of the organic matrix, which is used in the desorption and ionization process of the analyte of interest, a crucial step in sample preparation that can have a significant effect on the outcome results of an imaging experiment.

In this study, analysis of blotter papers containing NBOMes was explored using the laser desorption ionization (LDI), MALDI and MALDI Imaging techniques combined with Magnetic Resonance Mass Spectrometry (MRMS), classically known as Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS).

Experimental

Seven blotter papers were supplied by the Civil Police of the State of Espírito Santo (PC-ES), Brazil. They were cut and fixed on a stainless steel plate with the aid of double-sided tape. Matrix application was evaluated, being performed in two ways: (i) small volumes (15 µL) of matrix was directly spotted onto the surface of the blotter paper using an automatic pipette (Transferpette®); and (ii) with a sprayer assisted by an ESI probe (Bruker Daltonics, Bremen, Germany). In both cases, the process was optimized using the CHCA matrix at a concentration of 25 mg/mL. After optimization of the matrix application method, the ionization efficiency was evaluated in function of the four matrices studied (DHB), CHCA, sinapinic acid (SA and (TCNQ)) and their respective concentrations (from 5 to 25 mg/mL).

The LDI, MALDI and MALDI Imaging analyses were performed on a 9.4 T Solarix mass spectrometer (Bruker Daltonics, Bremen, Germany), equipped with a smart beam-II™ laser (355 nm) and MALDI source. All analyses were performed in positive ionization mode in a spectral range between *m/z* 200 and 1500, and each analysis was the result of 100 laser shots per pixel, with laser focus setting small (~ 30 µm), laser frequency of 200 Hz and laser power of 33%. The LDI and MALDI images were acquired in a spatial resolution of 300 µm and processed with FlexImaging 3.0 software (Bruker Scientific LLC).

Results and Discussion

LDI-MS analysis

Initially, blotter papers, samples S1-S7, seized by PC-ES, were analyzed by LDI-MS, and their respective spectra are observed in Figures 2A-G. Blotter papers exhibited a similar spectral profile, with detection mainly of 25I-NBOMe (in samples S1-S6, Figures 2 A-F), that has *m/z* 428.07208 and 450.05406 (in protonated form, $[C_{18}H_{22}INO_3 + H]^+$, and as a sodium adduct $[C_{18}H_{22}INO_3 + Na]^+$, respectively) with mass errors of less than 2 ppm. The 25I-NBOH molecule, $[C_{17}H_{20}INO_3+H]^+$, $[C_{17}H_{20}INO_3+Na]^+$ and $[C_{17}H_{20}INO_3+K]^+$ ions, of *m/z* 414.05731, 436.03944 and 452.01346, and error = 3.02, 3.28 and 3.36 ppm, respectively, on the other hand, was detected only on the blotter paper S7, Figure 2G.

MALDI MS and MALDI Imaging analyses

A crucial step in the sample preparation for a MALDI Imaging experiment is the application of the matrix, which must be deposited as a homogeneous layer on the surface of the sample, maintaining the natural arrangement of the analytes in the sample.

In the images generated by MALDI Imaging (Figure 3 A-C), the application of the matrix with sprayer assisted by an ESI probe proved to be more efficient in the ionization of ion of *m/z* 414 (Figure 3C), detecting a higher signal intensity of the compound of interest on the blotter paper surface when compared to the automatic pipette method (Figure 3B) as well as the LDI-MS technique, i.e., without the use of matrix (Figure 3A). Besides, a higher sensitivity, measured by the TIC values for the ion of *m/z* 414, was observed using the matrix sprayer in the MALDI mass spectrum (Figure 3F), which presented a higher value of TIC ($8.0x10^7$). This behavior indicates that the matrix sprayer had a higher ionization power when compared to the mass spectra obtained by the automatic pipette (TIC $5.7x10^7$, Figure 3E) and without the use of

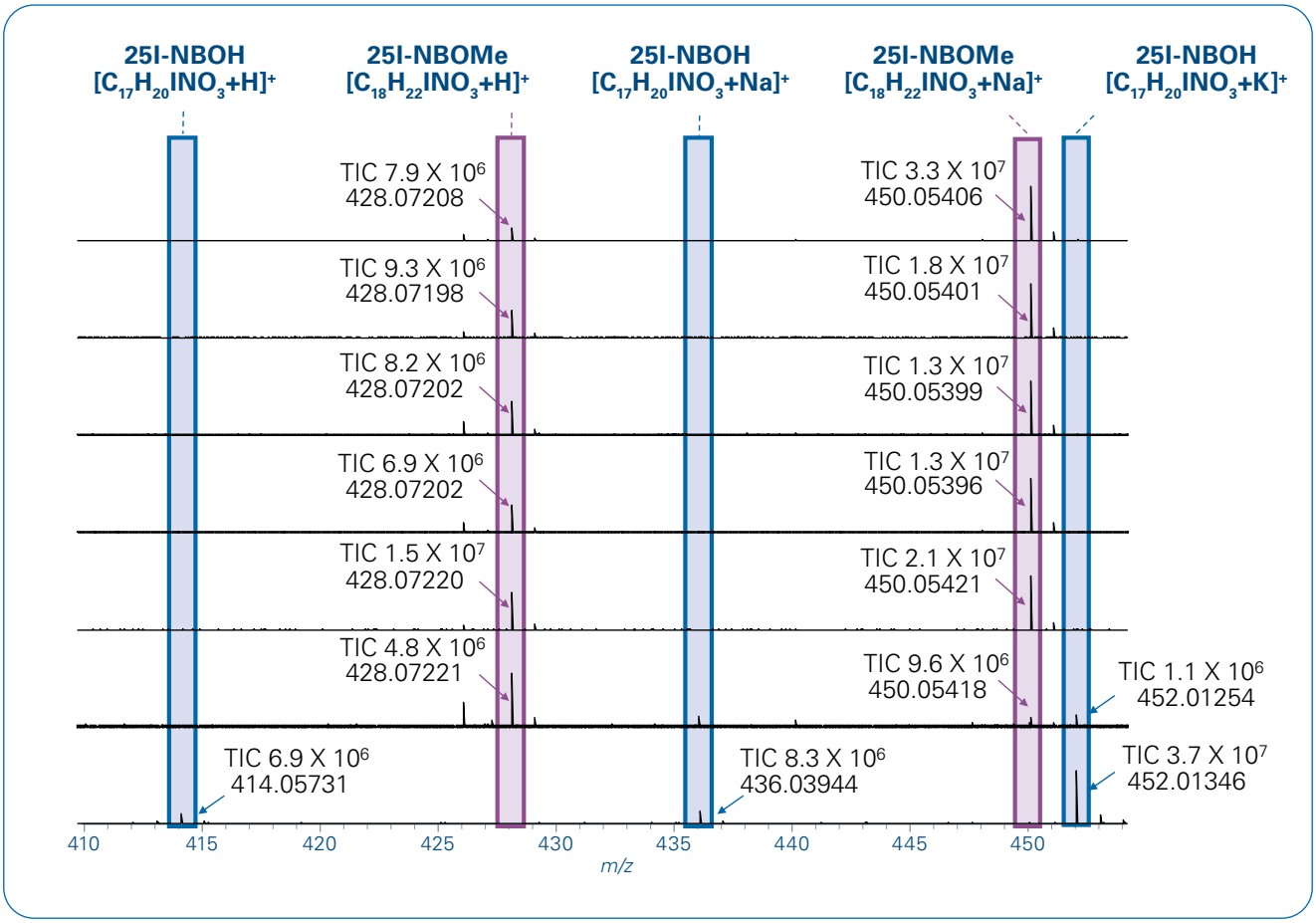

Figure 2: LDI mass spectra of seven blotter papers showing the presence of designer drugs: (A)-(F) 25I-NBOMe detected as $[C_{18}H_{22}INO_3 + H]^+$ and $[C_{18}H_{22}INO_3 + Na]^+$ ions at m/z 428 and 450, respectively, and (G) 25I-NBOH, detected as $[C_{17}H_{20}INO_3+H]^+$, $[C_{17}H_{20}INO_3+Na]^+$ and $[C_{17}H_{20}INO_3+K]^+$ ions at m/z 414, 436 and 452, respectively.

the matrix, which use is necessary to facilitate the ionization of the ion of interest (*m/z* 414) and decrease the suppression caused by the paper signal, *m/z* 575.07841 (TIC $3.0x10^7$, Figure 3D). One of the reasons for this efficiency may be related to the small droplets uniformly formed by the matrix on the surface of the sample during the process of deposition by the matrix sprayer.

To evaluate the best matrix and their concentration in the distribution study of 25I-NBOMe hallucinogen, *m/z* 428, the spatial distribution of this ion was measured on the surface of the blotter paper by MALDI Imaging from the individual deposition of four matrices: DHB, CHCA, SA and TCNQ, in the concentrations of 5, 10, 15, 20 and 25 mg/mL in small pieces (0.2 x 0.5 cm) of sample S5, Figure 4 A-D. The matrices were applied with the matrix sprayer.

The CHCA matrix showed to be more efficient in the ionization of 25I-NBOMe (Figure 4D), evidenced by the higher abundance and uniform distribution of the compound on the surface of the blotter paper, especially at concentrations higher than 10 mg/mL. This higher efficiency in the ionization of 25I-NBOMe, provided using the CHCA matrix, may be related to the interactions of the matrix with the analyte through hydrogen bonds. The CHCA matrix is generally applied in the ionization of compounds of lower *m/z* values, also commonly used in forensic analysis.

Ionization studies of psychoactive compounds intentionally added to absorption papers, which are illegally commercialized and seized by the local police, had their spatial distributions evaluated by MALDI Imaging methodology, that contemplated tests of matrix reagent application forms, varying their concentrations and chemical structures (four matrices were evaluated).

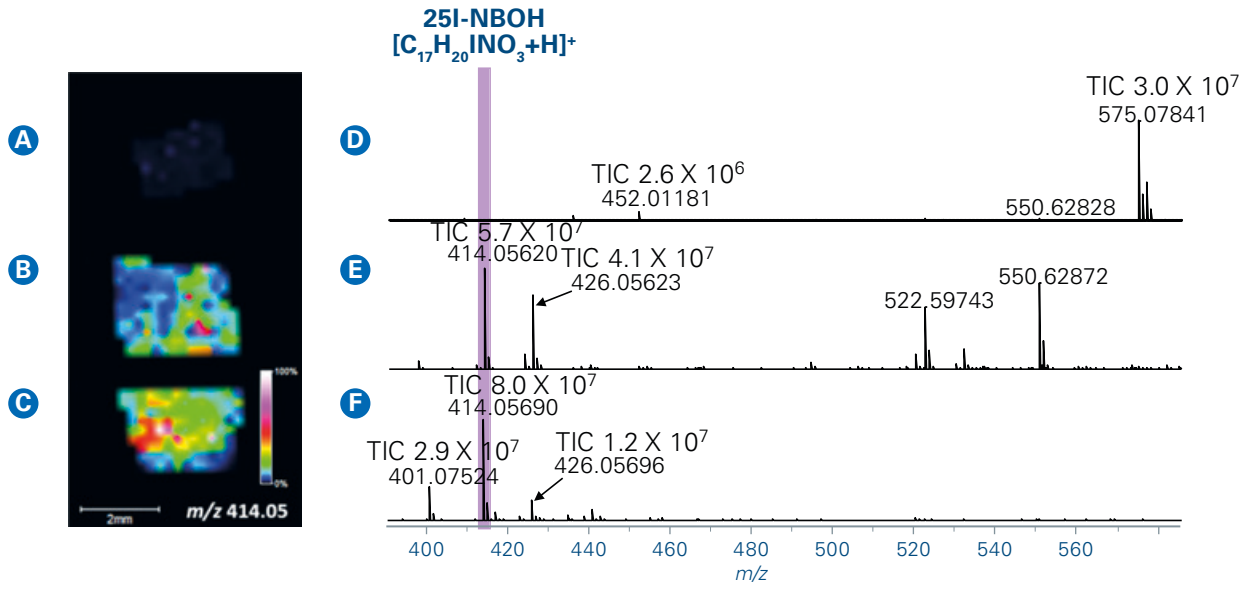

Figure 3: MS images showing the distribution of 25I-NBOH molecule at m/z *414 on the surface of sample S7: using* (A) *LDI, and* (B)-(C) *MALDI,* (B) *with CHCA matrix applied via automatic pipette and* (C) *with a sprayer assisted by an ESI probe. LDI* (D) *and MALDI* (E)-(F) *mass spectra of their respective MS images, Figure* (A) *and* (B)-(C)*, respectively.*

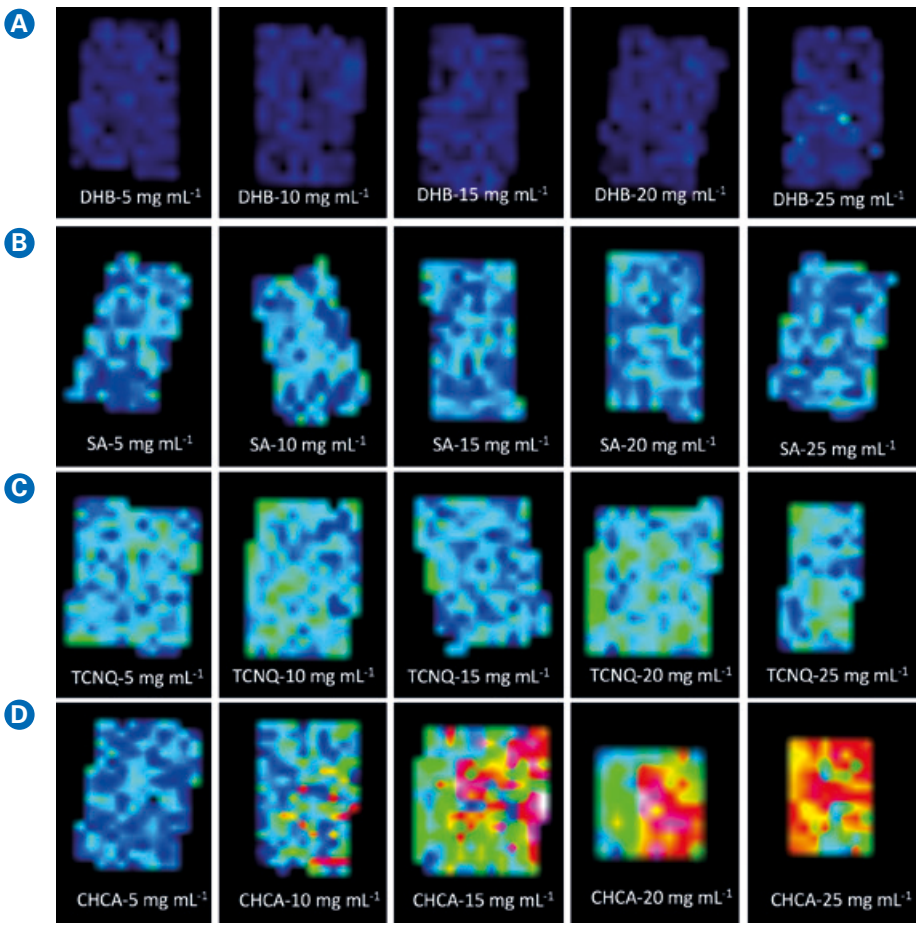

Figure 4: Positive MALDI MS images showing the distribution of 25I-NBOMe at m/z *428 on the surface of sample S5 coated with four different matrices:* (A) *DHB,* (B) *SA,* (C) *TCNQ and* (D) *CHCA in different concentrations (from 5 to 25 mg/mL).*

Conclusions

- The initial characterization by the LDI-MS technique in the analysis of blotter papers seized by PC-ES provided the identification of the psychoactive compounds 25I-NBOH and 25I-NBOMe, having *m/z* values of 414 Da and 428 Da, respectively, and mass accuracy lower than 4 ppm. The experiments of MALDI MS and MALDI Imaging were optimized, where among the application forms studied, the matrix sprayer showed to be the best method, compared to the use of the automatic pipette, providing a greater surface homogeneity, resulting in the detection of the monitored ions with more excellent uniformity and intensity. In the evaluation of the best matrix and concentration, the CHCA, in concentrations higher than 10 mg/mL, were more efficient in the ionization of compound 25I-NBOMe in comparison to the other matrices evaluated, presenting a uniform distribution of the drug throughout the blotter paper surface. These results have demonstrated the higher analytical power provided by the analyses of the MRMS due to its ability of determination of the elemental composition ($C_cH_hN_nO_o$) of the drugs compounds with high accuracy and resolving power.

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This Application Note is a condensed and revised version of the Microchemical Journal article "Designer drugs analysis by LDI(+), MALDI(+) and MALDI(+) imaging coupled to FT-ICR MS".

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[2] See references within.

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