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Rapid drug-screening of clandestine tablets by MALDI-TOF mass spectrometry

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Abstract

A novel method for the rapid screening of clandestine tablets for drugs by MALDI-TOF mass spectrometry is described. In this method, cetrimonium bromide (CTAB), a surfactant, is added to the conventional α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution used in preparing the MALDI samples. This procedure allows very clean mass spectra to be collected for amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), caffeine, ketamine and tramadol. The method was used successfully in the rapid drug-screening of some actual clandestine tablets, which had been seized from the illicit market, and can serve as a good complementary method to GC/MS for use in forensic analysis.
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Keywords: Rapid drug-screening; Clandestine tablet; MALDI-TOF mass spectrometry; CHCA; CTAB

1. Introduction

Because of the increasing use of illicit drugs, a rapid and accurate analytical technique for their detection is desirable. A number of analytical methods have been commercially developed for their identification, including a fluorescence polarization immunoassay [1], an immunochromatographic assay [2], and thin layer chromatographic analysis [3]. Each of the above methods has unique advantages and disadvantages with respect to sensitivity, precision and simplicity of use. More simple methods, such as the use of drug/narcotic detection kits, aerosol sprays/cans or collection paper dispensers are also commercially available. However, these tests provide only a quick cursory examination and are not legally acceptable as scientific proof. Thus far, GC/MS is the officially prescribed method and constitutes the most popular and powerful technique for the analysis of illicit drugs such as amphetamine and analogs thereof [4–15]. Because of this, GC/MS analysis has dominated of the field of "drug-screening" for many years and, as a result, a huge database is available in most commercial libraries. In fact, most clandestine tablets contain multi-components, including methamphetamine, MDMA, ketamine, as well as other so-called "designer drugs". For the analysis of such complicated mixtures, the method used should have high degree of accuracy. Although GC/MS analysis meets this need, in the actual experimental procedures, it is necessary to choose an optimal GC column and to determinate an appropriate temperature program for use in the separation. In addition, the major ionization source used in GC/MS is electron impact (EI). This is considered to be difficult for sample soft-ionization. Hence, it is necessary to derivatize the analytes prior to their injection into the GC system. All of these procedures are time consuming. Thousands of samples are frequently involved in routine testing and, as a result, a rapid and soft-ionization method which is also reliable and complementary to GC/MS for use in forensic analysis would be highly desirable.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) is a soft-ionization method, and has become a very popular and powerful tool in the analysis of biomolecules [16–24]. Recently, the analysis

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of low-mass ($m/z < 500$) molecules using this method has also been reported [25–32]. One of these methods is the use of a mixture of the surfactant of cetrimonium bromide (CTAB) and the conventional matrix of α-cyano-4-hydroxycinnamic acid (CHCA) to prepare the MALDI samples [25]. During the MALDI process, the presence of CTAB could suppress the matrix-related ion background. As a result, the low-mass range can be obtained. Otherwise, the use of porous silicon [33] or carbon nanotubes [34] can also provide desirable results in the low mass range. In this study, we now report, for the first time, on the rapid drug-screening of clandestine tablets based on MALDI-TOF mass spectrometry. Optimal conditions, such as concentrations of the surfactant of CTAB and CHCA for use, were investigated. Actual clandestine tablets seized from the illicit market were identified by MALDI-TOFMS. The results obtained by laser ablation-TOFMS and GC/EI/MS methods were also compared and these findings are reported herein.

2. Experimental

2.1. Reagents

Cetrimonium bromide (CTAB) and acetonitrile were obtained from Acros (New Jersey, USA). α-Cyano-4-hydroxycinnamic acid (CHCA) and caffeine were purchased from Aldrich (St. Louis, MO, USA) and Hayashi Pure Chemical Industries Ltd. (Osaka, Japan), respectively. Amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) standards were obtained from Radian International (Austin, TX, USA). The ketamine and tramadol (*cis*-2-dimethylaminomethyl-1-3-methoxyphenyl cyclohexanol) standards and all of the test samples of clandestine tablets were generously donated by Command of the Army Force of Military Police, Forensic Science Center, Taiwan.

2.2. Sample preparation

Tablets were ground into a fine powder and approximately 30 mg was dissolved in 3.0 mL 0.2N KOH solutions by shaking for 5 min. The solution was extracted with 3.0 mL of ethyl acetate (containing diphenylamine at 0.5 mg/mL as the internal standard) by shaking for 5 min. The mixture was centrifuged for 5 min and a 2.0 mL aliquot of the organic layer was transferred to an autosampler vial. The sample was analyzed (see GC/MS procedure above) on the day of extraction. For the MALDI-TOF experiments, the tablet power (1 mg) was dissolved with in methanol (1.0 mL). After 2 min of sonication and a 2 min centrifugation at 5000 rpm

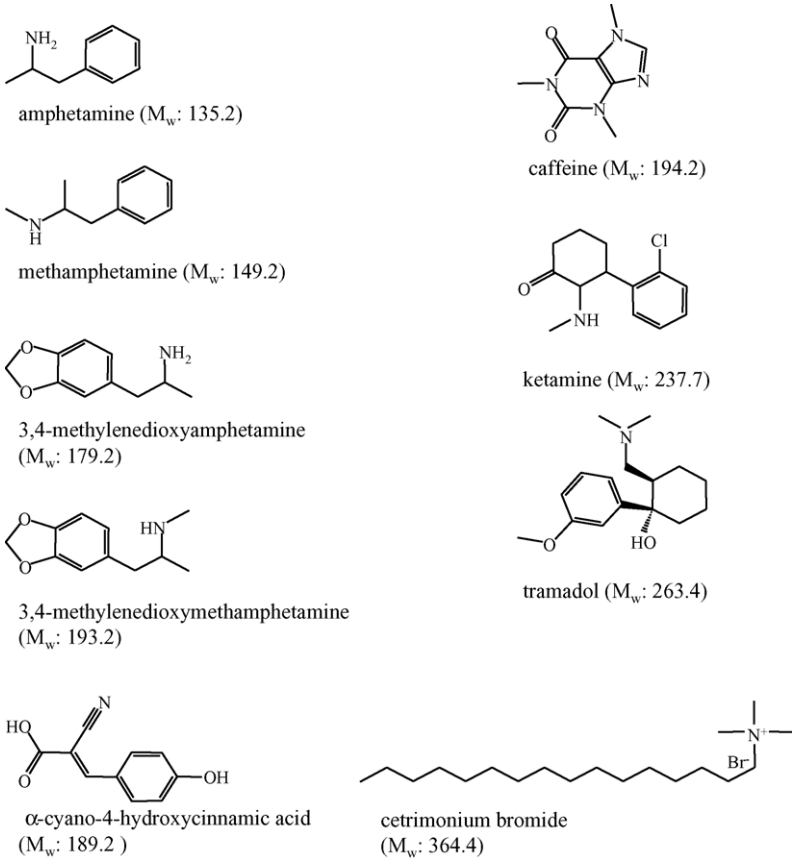

Fig. 1. Molecular structures of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), caffeine, ketamine, tramadol, α-cyano-4-hydroxycinnamic acid (CHCA) and cetrimonium bromide (CTAB).

at room temperature, the upper layer was collected and was then used directly in the analysis.

2.3. MALDI-TOFMS apparatus

The linear type of time-of-flight mass spectrometer (TOFMS) used in these experiments was a modified Wiley-McLaren design (R.M. Jordan Co., Grass Valley, CA); the flight distance was 1.1 m and the mass resolution was ~300. The instrument was equipped with a 4-in. turbo (flight tube) and a 6-in. diffusion (ionization region) pump system to maintain the vacuum below $\sim 5 \times 10^{-7}$ Torr during the experiments. All mass spectra were obtained using 355-nm radiation from a GCR-170 Nd:YAG laser system (Spectra-physics, Mountain View, CA). The accelerating voltage used was 10 kV. A stainless steel target was used as the MALDI substrate and the samples were deposited directly on it. The instrument was equipped with a video camera, displaying the image of the sample on a monitor, thus permitting the laser to be focused on a specific spot within the area of the target. The ions formed by MALDI were produced in a field-free region and then directly migrated toward the detector. A 25 mm triple microchannel plate (MCP) was used for ion detection. Data were recorded using a LeCroy 9350A digital oscilloscope (500 MHz) and processed by means of a personal computer. A time-of-flight range of 0–20 μs was generally used as the mass acquisition period, which corresponds to a *m*/*z* range of 0–627. All spectra were obtained as 200-shot averages. Mass calibration was conducted using fragments produced from a CHCA standard (*m*/*z*: 122.08, 146.04, 164.05, 172.04, 190.05, 294.07, 335.10 and 379.09; flight times: 8.828, 9.654, 10.227, 10.474, 11.008, 13.697, 14.616 and 15.547 μs), according to the following equation, $(m/z)^{1/2} = aT + b$, where T is the flight time of various ions.

2.4. GC/MS apparatus

A gas chromatograph (Hewlett-Packard 6890 GC: Palo Alto, CA) equipped with a mass spectrometer (Hewlett-Packard 5973 mass selective detector) and an auto-injector (Model 7683) was used. A capillary column (30 m × 0.32 μm I.D.) with an HP-5 MS (5% diphenyl and 95% dimethylpolysiloxane) bonded stationary phase film 0.25 μm in thickness (Agilent Technologies, USA) was used. The temperatures of the inlet, quadrupole, injector and interface were maintained at 230, 150, 250 and 280 °C, respectively. The temperature program for the column oven was as follows: 70 °C for 1 min, a linear ramp to 200 °C at 15 °C/min and a 2 min hold. Finally, the temperature was ramped linearly to 260 °C at 20 °C/min with a 12.3 min hold. The total analysis time was 27 min. Helium carrier gas was used at a constant flow-rate of 1.0 mL/min (at splitless mode). Data were collected using the Hewlett-Packard Chem-Station software. The mass conditions were as follows: ionization energy, 70 eV; ion source temperature, 230 °C; full-scan, 40–450 amu at 1.84 scans per second.

3. Results and discussion

Fig. 1 shows the molecular structures of the major chemicals used in this study. Amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) are strong central nervous system stimulants, and are generally considered to be illicit drugs. Caffeine is not an illicit drug but it is also frequently found in clandestine tablets because it acts as a stimulant, increasing the heart rate and blood pressure, and also permits the use of a "low-cost". Ketamine is a non-barbiturate, rapid-acting disassociate anesthetic that is used on both animals and humans; it is being abused by an increasing number of young people as a "club drug," and is often distributed at parties. Tramadol is a centrally acting analgesic. We selected these chemicals, all of which are commonly found in clandestine tablets, as model compounds to evaluate the MALDI-TOFMS drug-screening method developed here. α-Cyano-4-hydroxycinnamic acid (CHCA) is a common matrix that is frequently used in MALDI-TOFMS. Cetrimonium bromide (CTAB), a surfactant, is used as a suppressant to reduce the CHCA fragments during the laser desorption ionization process and was also used in this study.

Fig. 2 shows mass spectra obtained by the MALDI-TOFMS method from the matrix CHCA (mass spectrum a)

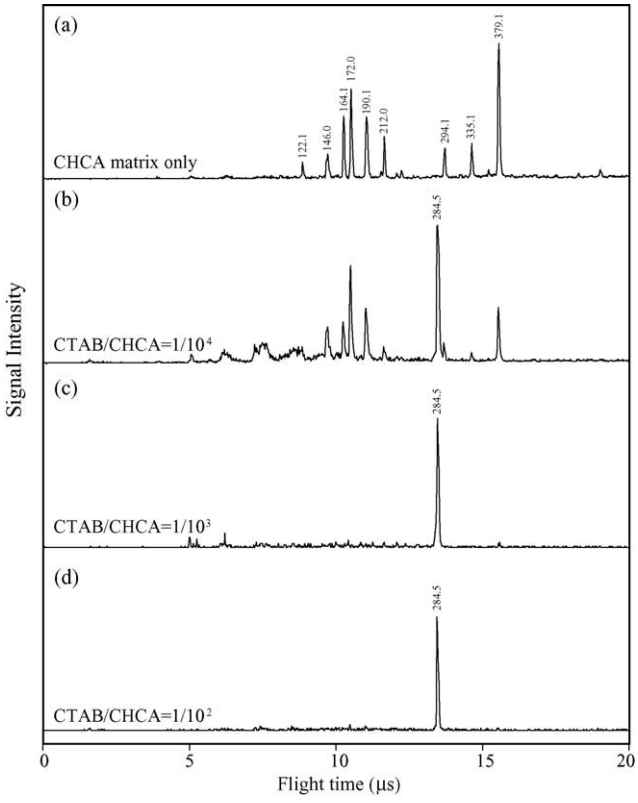

Fig. 2. MALDI-TOF mass spectra obtained from the matrix CHCA (mass spectrum a) and a mixture of CHCA and CTAB at different concentration ratios (mass spectra b–d; CTAB/CHCA = $1/10^4$, $1/10^3$ and $1/10^2$, respectively). The concentration of CHCA was 10 mg/mL in a mixed solution (water/acetonitrile = 50/50, v/v).

and a mixture of CHCA and CTAB for different concentration ratios (mass spectra b–d; CTAB/CHCA = $1/10^4$, $1/10^3$ and $1/10^2$, respectively). Herein, the concentration of CHCA was 10 mg/mL in a mixed solution (water/acetonitrile = 50/50, v/v). As can be seen from spectrum a, when only the matrix CHCA was present, the spectrum contained numerous strong peaks corresponding to matrix-related ions. These observed ion peaks, their corresponding flight times and possible ion forms are listed in Table 1. These data are in general agreement with previous literature reports [25,26], where a nitrogen laser (λ = 337 nm) was used, and were used to construct the mass calibration equation, as described above. As shown in mass spectra b-d, the use of the CHCA matrix suppressed the peaks, when it was added to CTAB at ratios of 1:10,000, 1:1000 and 1:100, respectively, by maintain the CHCA concentration at 10 mg/mL. Furthermore, only one major peak is observed in spectra c and d, corresponding to the $[CTAB\text{-}Br]^+$ ion (*m*/*z*, 284.53). It appears that, at an appropriate ratio of CHCA to CTAB, the addition of CTAB to CHCA is effective in suppressing CHCA-related matrix ion signals.

Table 1
Ions observed in the LDI mass spectrum of matrix CHCA using a Nd:YAG laser (λ = 355 nm) at different flight times (μs)

m/*z*	Flight times (μs)	Ion form
122.08	8.828	$[M + H\text{-}C_3H_2NO]^+$
146.04	9.654	$[M + H\text{-}CN\text{-}H_2O]^+$
164.05	10.227	$[M + H\text{-}CN]^+$
172.04	10.474	$[M + H\text{-}H_2O]^+$
190.05	11.008	$[M + H]^+$
294.07	13.697	$[2M + H\text{-}CO_2\text{-}C_2H_3N]^+$
335.10	14.616	$[2M + H\text{-}CO_2]^+$
379.09	15.547	$[2M + H]^+$

Fig. 3A shows the results for the suppressive effect of CTAB (1/1000 to CHCA) in the presence of amphetamine and related compounds (mass spectra a–g: amphetamine, methamphetamine, MDA, MDMA, caffeine, ketamine and

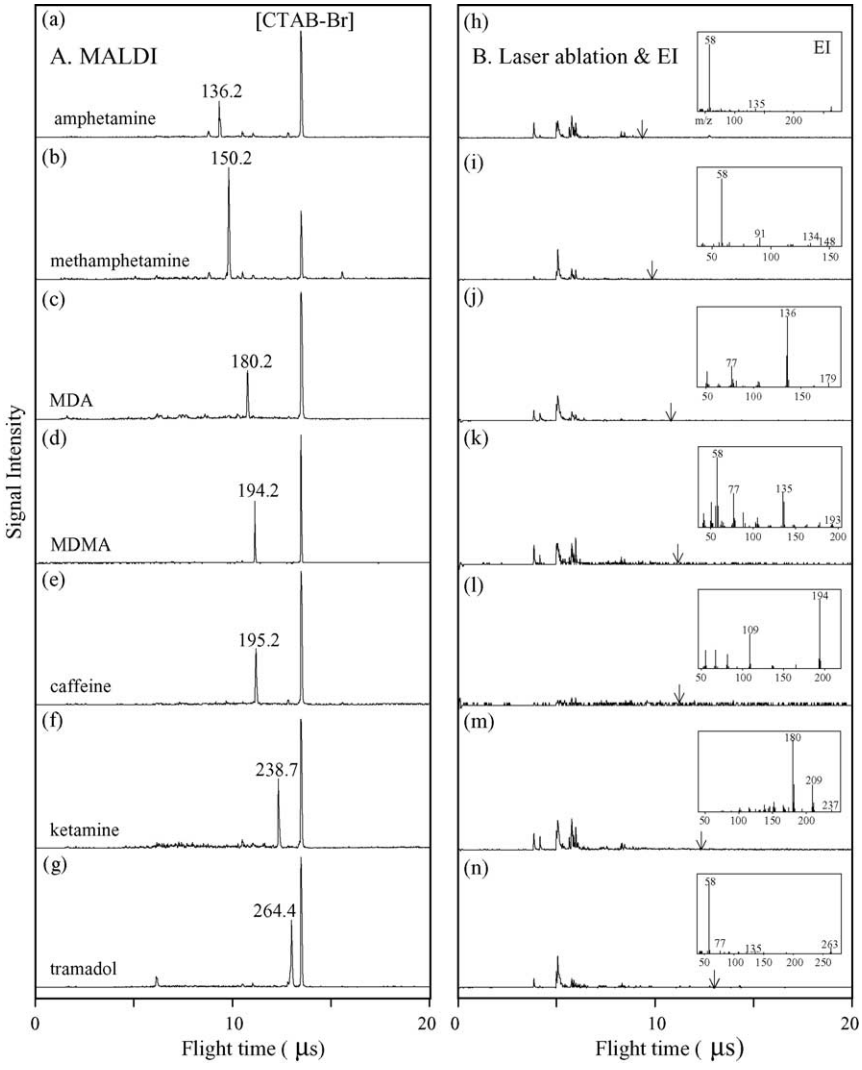

Fig. 3. MALDI-TOFMS (frame A), the suppression effect of CTAB (1/1000 to CHCA) in the presence of amphetamine and related compounds (mass spectra a–g: amphetamine, methamphetamine, MDA, MDMA, caffeine, ketamine and tramadol, respectively). Analyte concentrations were 20 ppm each. Comparison with results obtained by the laser ablation (frame B) and GC/EI/MS (the insets in frame B) methods, respectively, using the same analytes (mass spectra h–n).

Table 2
Comparison of the limit of detection (LOD, at S/N = 3) for MALDI-TOFMS (in this study) and GC/MS (reported data) for clandestine drugs

Compound	MALDI-TOFMS	GC/MS	Reference
Amphetamine	10 ng/mL	2.8 ng/mL	[36]
Methamphetamine	2 ng/mL	2.5 ng/mL	[36]
3,4-MDMA	2 ng/mL	1.2 ng/mg (hair)	[37]
3,4-MDA	10 ng/mL	3.1 ng/mg (hair)	[37]
Ketamine	5 ng/mL	4 ng/mL	[38]
Tramadol	5 ng/mL	8 ng/mL	[39]

tramadol, respectively). Herein, the concentrations of analytes were 20 ppm each. Clearly, except for the CTAB peak, only one major peak was detected for each analyte, which is corresponds to the parent ion peaks $[M + H]^+$. In fact, it is quite difficult to determined analytes quantitatively using MALDI without internal standard compounds. Efforts have been made to improve this, including the use of ionic liquid matrixes [35], in which the R.S.D. value is improved. For convenience, the results obtained in this study at a signal to noise ratio of 3 (S/N = 3) are shown in the Table 2. In the case of GC/MS, additional sample handling, such as extraction and derivatization procedures are necessary to achieve similar results [36–39]. For comparison, Fig. 3B shows the results obtained by laser ablation and GC/EI/MS methods, respectively. As shown in spectra h–n, the arrow-marks show the position of the flight times for each analyte that should have appeared; none of the characterized peaks were detected by the laser ablation technique.

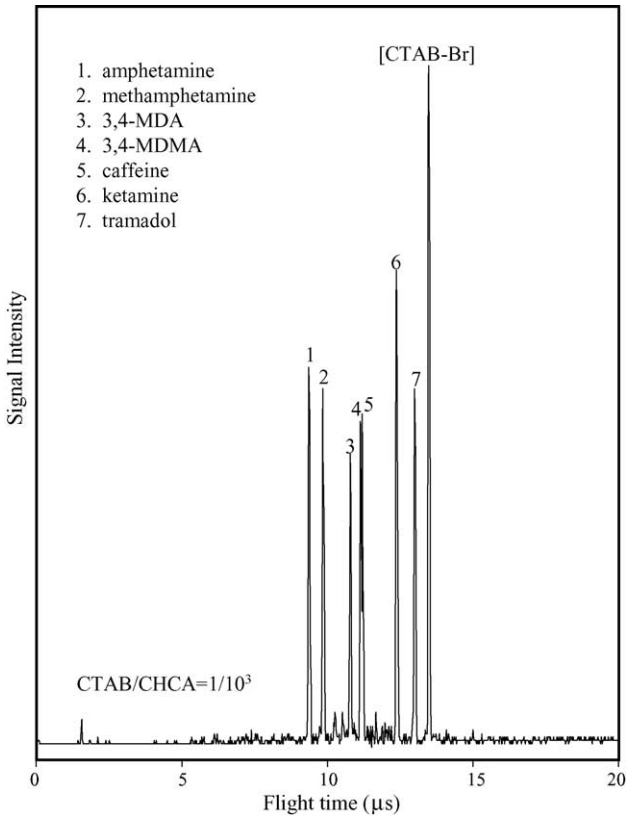

Fig. 4. The mass spectrum of a mixture of seven analytes obtained by MALDI-TOF mass spectrometry (CTAB/CHCA, 1/1000).

Table 3
Distribution of methamphetamine and related compounds by GC/MS, in 535 tablets seized from the illicit market during 2001

A	MA	MDA	MDMA	Caffeine	Ketamine	Tramadol	Numbers
√	√		√	√	√		2
√	√		√		√		7
√		√	√	√			8
√		√	√				9
√		√		√			1
	√			√	√		21
	√		√	√	√		19
	√				√		5
	√	√					3
	√		√		√		13
	√		√				3
	√		√	√			3
	√						1
		√	√	√			13
		√		√			1
		√	√				1
		√					22
		√	√	√	√		71
		√	√	√			236
			√		√		3
			√				52
				√	√		3
				√			23
					√		13
						√	2
Total							535

√: Detected; A: amphetamine; MA: methamphetamine; MDA: 3,4-methylenedioxyamphetamine; MDMA: 3,4-methylenedioxymethamphetamine.

The insets in Fig. 3B show mass spectra of the analytes obtained by GC/MS under the electron impact mode. All of the analytes were used directly without any derivative procedures. As a result, although some fragments were detected, they are difficult to distinguish from each other. It should also be noted that MDMA provided an imine fragment ($m/z = 58$), a major peak in the electron impact mass spectrum of amphetamine and analogs thereof. This is the reason for why it is necessary to derivatize the analytes when conventional GC/MS methods are used. Needless to say, each compound has unique properties and conditions for the subsequent individual derivatization are also different. All of these procedures make the analysis work complicated and difficult because the most recently obtained clandestine tablets were multi-component.

Fig. 4 shows a mass spectrum of the mixture of analytes obtained by MALDI-TOF mass spectrometry (CTAB/CHCA, 1/1000). Seven major peaks (peaks, 1–7) can be observed which correspond to each individual analyte. As a result, problems associated with the laser ablation and GC/MS methods can be resolved. Table 3 shows the results of the GC/MS analysis of 535 clandestine tablets, which were seized from the illicit market during 2001. The experimental conditions and methods are described above. Most of the clandestine tablets contained multi-components; some contained other compounds which are often referred to as “designer drugs” (data not shown). Such analysis work would take a huge amount of time to finish. Herein, we selected three clandestine tablets as examples and examined them by MALDI-TOF mass spectrometry. Fig. 5, frames A and B show the results obtained by the MALDI-TOFMS and GC/MS-TIC methods, respectively. In the mass spectra a–c, the peaks having flight times of 9.81, 11.13, 11.17 and 12.33 μs were assigned as methamphetamine, MDMA, caffeine and ketamine, respectively. In contrast to this, in the ion chromatograms d-f, recorded under the total ion current (TIC) mode, peaks having migration times of 6.18, 9.39, 12.57 and 12.83 min were assigned as methamphetamine,

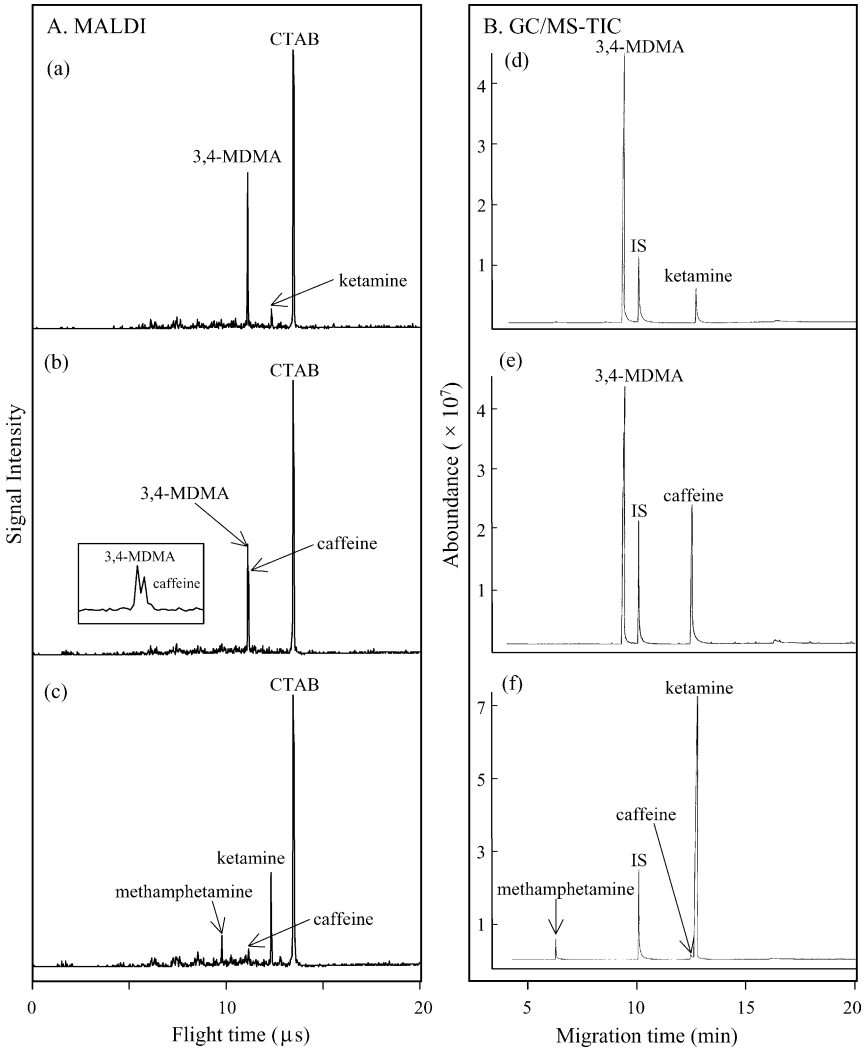

Fig. 5. Frames A and B show the results obtained by MALDI-TOFMS (mass spectra a–c) and GC/MS-TIC (mass spectra d–f) methods, respectively, for three randomly selected clandestine tablets.

MDMA, caffeine and ketamine, respectively, based on their mass spectra (data not shown). Thus, we conclude that MALDI-TOF mass spectrometry, which is clearly accurate, sensitive and rapid, can be considered for use in rapid drug-screening and is sufficiently reliable to be a complementary method to GC/MS for use in forensic analysis.

4. Conclusions

A CTAB/CHCA modified MALDI-TOFMS method can be successfully used for the rapid drug-screening of amphetamine and related compounds in clandestine tablets. The optimum MALDI conditions for the analysis of these analytes was achieved using a mixed matrix of CTAB/CHCA (1/100–1/1000) in a water-acetonitrile solution (50/50, v/v). The method was successfully applied to the analysis of clandestine tablets, and the distribution of these illicit drugs was determined. Moreover, the method proposed here provides results in less than a few seconds without any complicated pre-treatment for amphetamine related compounds; whereas GC/MS requires a derivatization and additional sample handling to achieve similar results.

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