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## Original Article

# Direct and rapid characterization of illicit drugs in adulterated samples using thermal desorption electrospray ionization mass spectrometry



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## ARTICLE INFO

## Article history:

Received 16 July 2018

Received in revised form

10 December 2018

Accepted 11 December 2018

Available online 8 January 2019

## Keywords:

Thermal desorption electrospray ionization mass spectrometry

Illicit drugs

Direct analysis

## ABSTRACT

Foods and drinks have been adulterated with illicit drugs to facilitate criminal activities. Unfortunately, conventional analytical methods are incapable of rapidly characterizing these drugs in samples, as serious interferences from sample matrices must be removed through tedious and time-consuming pretreatment. Ambient ionization mass spectrometry (AMS) generally does not require sample pretreatment and is thus a suitable tool for directly and rapidly detecting illicit drugs in samples in different physical states. In this study, thermal desorption electrospray ionization mass spectrometry (TD-ESI/MS), an AMS technique, was utilized to efficiently characterize illicit drugs spiked in samples including drinks, powders, and jelly candies. To perform sensitive analysis, the mass analyzer was operated in multiple reaction monitoring mode to monitor the molecular and fragment ions of the target analytes. The time required to complete a typical TD-ESI/MS analysis was less than 30 s. The limits of detection (LODs) for illicit drugs were found to be 100 ppb in drinks, 100–1000 ppb in instant powders, and 1.3–6.5 ng/mm<sup>2</sup> on stamp surfaces. FM2 and nitrazepam laced in the inner layer of a jelly candy were detected by TD-ESI/MS, showcasing the advantage of the technique for direct and rapid analysis as opposed to conventional methods.

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<https://doi.org/10.1016/j.jfda.2018.12.005>

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## 1. Introduction

Analyzing illicit drugs is an important aspect of criminal investigation. For instance, illegal drugs such as ketamine, 3,4-methylenedioxymethamphetamine (MDMA), mephedrone, FM2, and nitrazepam have been spiked in candies, drinks, foods, instant coffees, milk teas, herbal tea packs, and gummy bears, which have been previously found at crime scenes. These illegal drugs are commonly detected using chemical color tests [1], Fourier transform infrared spectroscopy [2,3], or portable Raman spectroscopy [3–5], as well as techniques like gas chromatography mass spectrometry (GC/MS) [6–8] and liquid chromatography mass spectrometry (LC/MS) [7–9]. These techniques have been indispensable in forensic laboratories for their chemical identification capabilities but unfortunately require laborious and time-consuming sample pretreatment to remove interfering matrices from complex samples before these drugs can be characterized. Additionally, the presence of proteins, peptides, lipids, sugars, dyes and fibers in the sample matrices can interfere with the results of conventional color tests and spectroscopic examinations.

Alternatively, ambient mass spectrometry (AMS) benefits from easy sample introduction, fast analyte desorption, and ionization and sensitive detection of chemical compounds [10–13]. The analytes on solids and in liquids can be rapidly characterized using AMS, which in contrast to conventional techniques requires only minimal or no sample preparation. For these reasons, several AMS techniques have been applied in forensic laboratories to conduct direct and sensitive analysis. For example, electrospray laser desorption ionization (ELDI), desorption electrospray ionization (DESI), and easy ambient sonic spray ionization (EASI) have been used to characterize trace ink molecules on questioned documents and distinguish authentic and counterfeit banknotes based on their surface chemical compositions [14–19]. Trace explosives such as 1,3,5-triazine, triperoxide, and trinitrotoluene on various surfaces have been detected by DESI, direct analysis in real time (DART), and flame-induced atmospheric pressure chemical ionization (FAPCI) [20–22]. ELDI, DESI, DART, and desorption FAPCI have been used to characterize the active ingredients in drug tablets, powders, and ointments without sample preparation [23–26]. Moreover, DART and DESI have been used to characterize drugs and metabolites in biofluids such as dried whole blood and serum [27–29]. Drinks laced with illegal drugs such as flunitrazepam, gamma-hydroxybutyric acid ( $\gamma$ -GHB), ketamine, and methylone are often encountered in real criminal cases; they can be rapidly characterized by using DART, DESI, and proton transfer reaction mass spectrometry (PTR-MS) that do not require tedious sample pretreatment [30–33].

Although the molecular ion signals for illicit drugs in samples can be detected using AMS techniques, high-throughput analysis is generally impractical. Samples are placed near the ionization source for AMS analysis, restricting the size, shape, and portability of the sample — analysis of samples that are oversized, irregularly shaped, or immovable is thus non-ideal. To overcome this problem, it is necessary to use an AMS technique that combines easy sampling and high-throughput analysis to detect trace illicit

drugs in samples that have different physical states and properties.

Thermal desorption electrospray ionization mass spectrometry (TD-ESI/MS) is an AMS technique previously developed for high throughput analysis [34]. Trace chemical compounds on solid or in liquid samples are conveniently collected on a sampling probe that is then inserted into a heated oven to thermally desorb analytes on the probe. The analytes are then delivered by a nitrogen flow into an ESI plume for ionization. Since a sampling probe can conveniently collect trace amounts of analytes regardless of sample's state, TD-ESI/MS has been applied to characterize explosives, residual pesticides, and plasticizers on various sample surfaces and in different solutions [34–38]. The relative standard deviations (RSDs) were between 7.5 and 15.6% for phthalate standard solutions and between 2.77 and 6.81% for pesticides in oral fluid. The probe material can be tailored to the physical and chemical properties of the analyte to improve the efficiency of analyte sampling and thermal desorption; for example, materials such as solid phase microextraction (SPME) fibers have been combined with TD-ESI/MS to sample and quantify trace drugs in plasma for pharmacokinetic studies [39].

In this study, to implement a high-throughput and convenient AMS technique for forensic assays, probe sampling combined with TD-ESI/MS was used to expedite the analysis of illicit drugs regardless of sample shape, size, and physical state. Since sample matrices were either nonvolatile or thermally unstable compounds, they decomposed or were not desorbed in the TD-ESI source; therefore, interferences from these compounds during TD-ESI/MS analysis was minimized, making it possible to directly detect volatile drugs without sample pretreatment. We characterized illicit drugs laced in drinks, coffee powders, jelly candies, and on stamps, facilitating easy and fast sample collection using modified probes to collect analytes from solid surfaces, inside soft solid, or in solutions.

## 2. Materials and methods

### 2.1. Chemicals and solvents

Chloroform ( $\text{CHCl}_3$ ) and ethyl acetate (EA) were purchased from Macron Fine Chemicals (Center Valley, PA, U.S.A.). Formic acid and methanol were obtained from Sigma–Aldrich (St. Louis, MO, U.S.A.) and Merck (Darmstadt, Germany), respectively. Ethanol and acetic acid were purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.). Distilled deionized water was produced by a PURELAB purifier (ELGA, Marlow, U.K.). Standards for illegal drugs including 5-MeO-AMT, amphetamine (AM), methamphetamine (MA), mephedrone, para-methoxymethamphetamine (PMMA), 3,4-methylenedioxymethamphetamine (MDMA), methylone, 2-(4-chloro-2,5-dimethoxyphenyl)ethan-1-amine (2C-C), ketamine, nitrazepam, flunitrazepam (FM2), and lysergic acid diethylamide (LSD) were purchased from Cerilliant Corporation (Round Rock, TX, U.S.A.). Stock solutions of illicit drugs were prepared in MeOH. Drinks and powders for whole fat milk (Uni-President Enterprises Corporation), grape juice (Uni-President Enterprises Corporation), fruit and vegetable juice (Chou-Chin Industrial Co.), sugar-free green tea bags (Vitalon Foods

Company), 3-in-1 instant coffee packs (Nestlé Company), matcha tea packs (Starbucks), and gelatin powder (Tehmag Foods Corporation) were obtained from local supermarkets. Postage stamps were purchased from Chunghwa Post Co., Taiwan.

## 2.2. Preparation of illicit drug-laced samples

Individual standard solution for each illicit drug was prepared at a concentration of 1000 ppm. To simulate drinks laced with illicit drugs, drug standards for MA, mephedrone, MDMA, methylene, 2C-C, nitrazepam, and FM2 were spiked into whole fat milk, fruit and vegetable juice, and sugar-free green tea, respectively. The drug-laced solutions were further diluted to produce sample solutions at different concentrations ranging from 1 ppb to 100 ppm. An aliquot of the solution containing amphetamine, PMMA, ketamine, and LSD was spiked in both the 3-in-1 instant coffee and matcha powders so that the concentration of each drug standard in the powder was between 100 ppb and 100 ppm. The solid powders were then ground and stirred to well mix the drugs in the powders. Standard solutions of 5-MeO-AMT, ketamine, MDMA, and LSD at concentrations from 25 ppb to 100 ppm (40  $\mu\text{L}$ ) were individually spiked onto the back of the stamp (32 mm  $\times$  24 mm, W  $\times$  L) so that the concentration of each illicit drug on the stamp surface was between 1.3  $\text{pg}/\text{mm}^2$  and 5.2  $\text{ng}/\text{mm}^2$ .

The drug-laced gummy bear was produced as follows: 8 g gelatin powder and 40 g grape juice were mixed in a glass cup and heated at 80  $^{\circ}\text{C}$  for 15 min to dissolve the gelatin. The temperature of the gelatin solution was then decreased to 35  $^{\circ}\text{C}$  in a water bath. The gelatin solution (1.5 mL) was mixed with a 100  $\mu\text{L}$  drug standard solution (100 ppm of FM2 and nitrazepam); the mixture was then poured in a bear-shaped silicone mold and placed in a refrigerator at 4  $^{\circ}\text{C}$  for 30 min. For the outer drug-free gelatin layer, 8 g gelatin powder and 40 g tea solution were mixed and heated at 80  $^{\circ}\text{C}$  for 15 min, after which the temperature of gelatin solution was cooled to 35  $^{\circ}\text{C}$  in a water bath; the solution was then deposited on a star-shaped silicone mold and the drug-laced gummy bear was put inside the mold and stored in a refrigerator at 4  $^{\circ}\text{C}$  for 30 min.

## 2.3. Solvent extraction of drug-laced samples

To extract illicit drugs in the liquid samples, an aliquot of the sample solution (200  $\mu\text{L}$ ) was mixed in 100  $\mu\text{L}$  chloroform: ethyl acetate: ethanol (3:1:1, v/v/v) solvent containing 1% NaOH (aq) and then vortexed for 15 s, after which the suspension was collected for TD-ESI/MS analysis. The illicit drugs mixed with the 3-in-1 instant coffee or matcha powder (10 mg) were extracted in 200  $\mu\text{L}$  chloroform: ethyl acetate: ethanol (3:1:1, v/v/v) solvent; after vortexing, the suspension was collected for TD-ESI/MS analysis. As for the extraction of illicit drugs from solid samples, the stamp was cut into quarters, where one of which was suspended in 200  $\mu\text{L}$  chloroform: ethyl acetate: ethanol (3:1:1, v/v) solvent containing 1% NaOH (aq). Fast solvent extraction was completed within 30 s. The extracts were analyzed as liquid samples, where 2  $\mu\text{L}$  of each extract was sampled onto the inoculating loop for TD-ESI/MS analysis.

## 2.4. Thermal desorption electrospray ionization mass spectrometry (TD-ESI/MS)

The samples laced with illicit drugs were (i) directly characterized by TD-ESI/MS or (ii) extracted in a solvent before TD-ESI/MS analysis. The TD-ESI/MS setup, which has been described in previous publications [34–36], consisted of a metal probe, heated oven, and ESI source. To directly collect illicit drugs on solid sample surfaces for analysis, a stainless steel inoculating loop was swept across the stamp surface (200  $\text{mm}^2$ ). Conversely, we used an acupuncture needle (0.27 mm in diameter, Ching Ming, Taiwan) to collect small amounts of liquid, gelatin, and powder samples. To collect analytes from liquid samples, the needle was simply dipped into each sample to a depth of 2 mm and then removed. For the double-layered jelly candy, the acupuncture needle was inserted into the sample to a depth of 5 mm and 12 mm to sample analytes from the outer and inner layers, respectively. Since it was difficult to collect the fine particles in powder samples with an acupuncture needle, the needle was first dipped into water and then inserted into the powder sample; wetting the surface of the needle enabled the adsorption of a small amount of the sample powder. In addition to direct sampling, analytes were collected from each sample via solvent extraction; an aliquot of the extract (2  $\mu\text{L}$ ) was deposited on the stainless steel inoculating loop with a pipette prior to TD-ESI/MS analysis. The metal probe was then inserted into the heated oven (300  $^{\circ}\text{C}$ ) to desorb and vaporize analytes. A stream of preheated nitrogen gas (2 L/min) was used to carry gaseous analytes into the ESI plume for post-ionization. The flow rate of the ESI solution (40% MeOH with 1% formic acid) was set at 160  $\mu\text{L}/\text{h}$  and the pressure of the nebulizing nitrogen gas was set at 4 psi. The TD-ESI unit was coupled to an Ultivo triple quadrupole mass spectrometer (Agilent, Santa Clara, CA, U.S.A.); the voltage of the MS capillary was set at +4.5 kV, the drying gas temperature was 280  $^{\circ}\text{C}$ , and the scan rate of the triple quadrupole mass analyzer was 500 ms/scan. The mass analyzer was operated in multiple reaction monitoring mode (MRM) to study the limits of detection (LODs, S/N  $\geq$  3) of the illicit drugs in the samples. Table 1 shows the fragmentor and CE voltages of the parent/fragment ion pairs used in this study.

## 3. Results and discussion

TD-ESI/MS combined with probe sampling was utilized to directly characterize illicit drugs in samples with different physical states. A stainless steel inoculating loop and acupuncture needle were utilized to collect samples.

### 3.1. Analysis of illicit drugs in drinks

In many cases of drug-facilitated sexual assault, illicit drugs such as FM2 and nitrazepam — also known as date rape drugs — are adulterated into the drinks to be administered to victims. Since the matrix compounds in drinks are usually complicated, tedious sample preparation is necessary to remove the matrix interference before traditional GC/MS and LC/MS analysis. However, most of the matrix compounds are

nonvolatile or thermally unstable, they are either undesorbed or decomposed during thermal desorption. This minimizes the interferences from the drink matrices during TD-ESI/MS analysis and direct characterization of volatile illegal drugs in drinks without sample pretreatment become possible. The TD-ESI mass spectra for whole fat milk, fruit and vegetable juice, and sugar-free green tea were first recorded. Even though the chemical composition of the fruit and vegetable juice was complicated, its TD-ESI mass spectrum (Fig. 1a) was dominated by ions derived from the decomposition products of sugars; such ions included dehydrated disaccharides ( $m/z$  289 and 325) and dehydrated monosaccharides ( $m/z$  127, 145, and 163). For whole fat milk, its TD-ESI mass spectrum was dominated by ions derived from creatinine ( $m/z$  114), dehydrated monosaccharides, dehydrated cholesterol ( $m/z$  369), and diglycerides ( $m/z$  355, 383, 411, and 439) (Fig. 1b). Protein molecules could not be desorbed or totally decomposed by thermally heating, their molecular ion signals were not detected on the TD-ESI mass spectrum. For sugar-free green tea, its TD-ESI mass spectrum was dominated by the caffeine ion ( $m/z$  195) (Fig. 1c).

Fig. 1d–f shows the TD-ESI mass spectra for the drug-laced drinks, wherein the concentration of each illicit drug was 100 ppm. Ion signals for the protonated illicit drugs  $[M+H]^+$  including those for MA ( $m/z$  150), mephedrone ( $m/z$  178), MDMA ( $m/z$  194), methylone ( $m/z$  208), 2C-C ( $m/z$  216), nitrazepam ( $m/z$  282), and FM2 ( $m/z$  314) were all detected in the three drinks without sample pretreatment. In addition, the fragment ions of MDMA ( $m/z$  163) and mephedrone or methylone ( $m/z$  160) were also detected on the mass spectra. The illicit drug ions were further characterized by MS/MS analysis for identification. The mass analyzer was operated in MRM mode to (i) monitor the parent/fragment ion pairs listed in Table 1, and (ii) to study the LODs of illicit drugs in the drinks. The S/N ratio of each analyte was determined based on the peak of first MRM transition shown in Table 1. The S/N ratios of some illicit drugs were higher than 10 due to their second MRM transitions were interfered by sample matrix (Table 2). The LODs of the illicit drugs were determined to be 100 ppb. Since much higher concentrations of these laced drugs are found in samples from real criminal cases, the LODs of using TD-ESI/MS to detect these illicit drugs are adequately low to enable rapid

identification. The repeatability of using TD-ESI/MS to analyze illicit drugs in drinks was studied by detecting drug-laced fruit and vegetable juice (1 ppm, each). The relative standard deviation (RSD,  $n = 5$ ) was calculated to be 19.5% for MA, 16.7% for mephedrone, 16.4% for MDMA, 16.5% for methylone, 22.8% for 2C-C, 23.1% for nitrazepam, and 12.1% for FM2.

### 3.2. Analysis of illicit drugs in instant powders

In addition to liquid samples, TD-ESI/MS was also utilized to analyze powder samples. An acupuncture needle was used to collect small amount of the particles from powder samples. The needle surface was wetted by dipped it into water first, it was then inserted into the powders for sampling. Fig. 2 shows the TD-ESI mass spectra for the 3-in-1 instant coffee and matcha tea powders laced with AM, PMMA, ketamine, and LSD, where the concentration of each drug was 100 ppm. The 3-in-1 coffee and matcha tea powders contained abundant caffeine, so that the ion signal of protonated caffeine ( $m/z$  195) was the dominant peak on their mass spectra. The 3-in-1 instant coffee powder also contained creamer powder, we therefore detected several ion peaks related to diglycerides ( $m/z$  355, 383, 411, and 439). In addition, protonated AM ( $m/z$  136), PMMA ( $m/z$  180), ketamine ( $m/z$  238), and LSD ( $m/z$  324) ions were detected in both powder samples. The LODs for AM, PMMA, ketamine, and LSD were between 500 and 1000 ppb in the 3-in-1 instant coffee powder and 100–500 ppb in the instant matcha powder (Table 3). The LODs for these illicit drugs were higher in the instant powders than those in its respective drinks indicating more serious matrix effects from the powder samples. Even so, TD-ESI/MS is still suitable for real sample analysis because previous forensic cases have found that the contents of illicit drugs laced in instant powders usually range from 1 to 6% ( $w/w$ ), which is a high enough analyte concentration for TD-ESI/MS. The RSDs ( $n = 3$ ) for the detection of drugs (1 ppm, each) in the matcha powder were between 15.1 and 38.3% (25.1% for ketamine, 15.1% for AM, 18.7% for PMMA, and 38.3% for LSD).

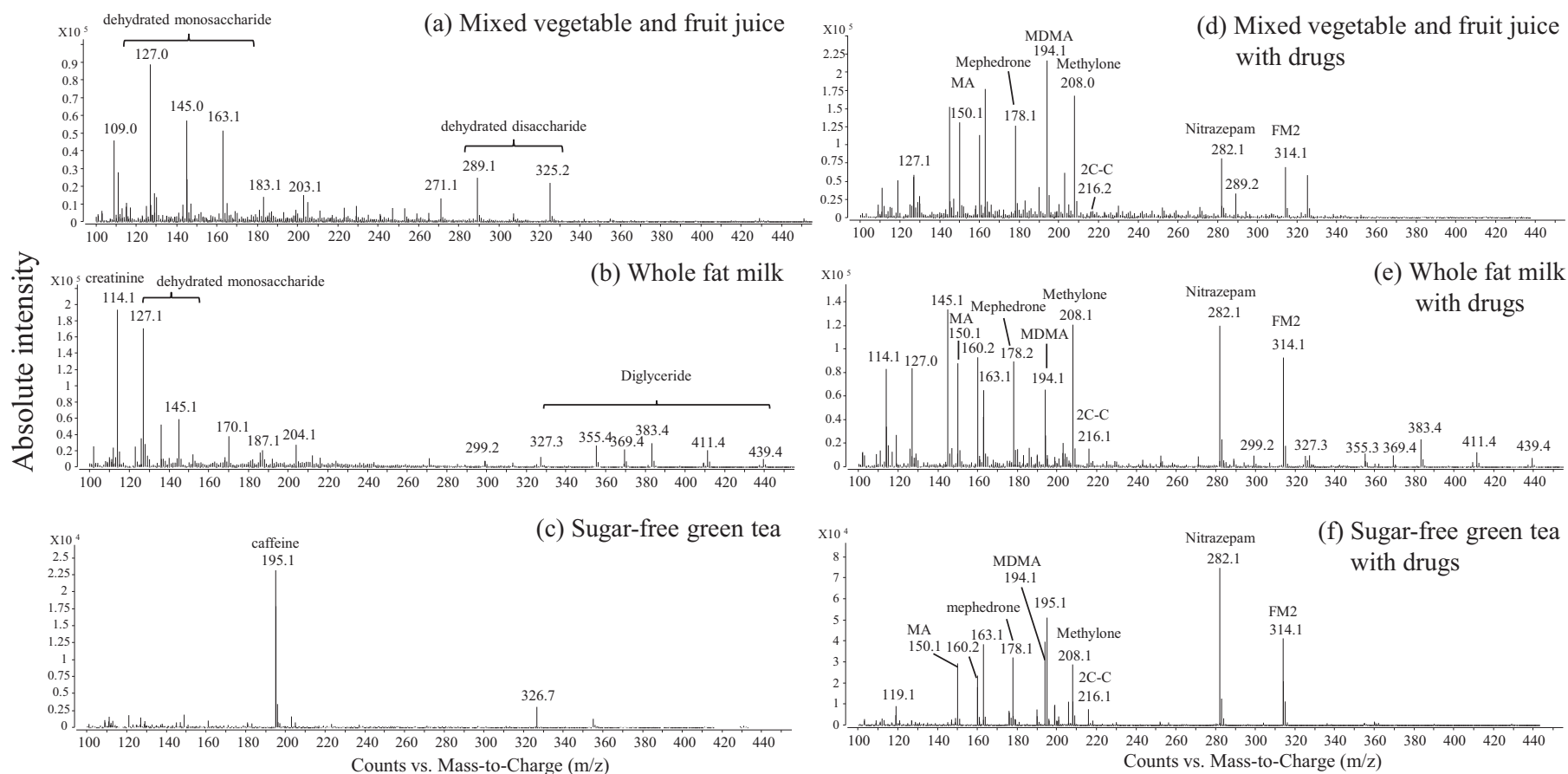
### 3.3. Analysis of illicit drugs on a stamp

Analyzing paper soaked in drug solutions is an important task in forensic science because illicit drugs such as LSD have been

**Table 1 – The fragmentor and CE voltages of parent/fragment ion pairs for multiple reaction monitoring (MRM) mode analysis.**

Compound	Precursor ion ( $m/z$ )	Product ion ( $m/z$ )	Fragmentor (V)	CE (V)
5-MeO-AMT	205.1	188.1 <sup>a</sup> , 147.0	96	2 <sup>a</sup> , 14
Amphetamine	136.1	91.0 <sup>a</sup> , 119.0	80	10 <sup>a</sup> , 2
Methamphetamine	150.1	91.0 <sup>a</sup> , 119.1	96	14 <sup>a</sup> , 2
Mephedrone	178.1	160.2 <sup>a</sup> , 145.1	96	2 <sup>a</sup> , 14
PMMA	180.1	149.1 <sup>a</sup> , 121.1	96	2 <sup>a</sup> , 14
MDMA	194.1	163.1 <sup>a</sup> , 105.0	96	2 <sup>a</sup> , 18
Methylone	208.1	160.1 <sup>a</sup> , 190.1	96	10 <sup>a</sup> , 2
2C-C	216.1	199.1 <sup>a</sup> , 184	96	2 <sup>a</sup> , 14
Ketamine	238.1	125.0 <sup>a</sup> , 207.1	112	22 <sup>a</sup> , 6
Nitrazepam	282.1	236.1 <sup>a</sup> , 180.1	144	18 <sup>a</sup> , 38
FM2	314.1	268.2 <sup>a</sup> , 239.1	160	22 <sup>a</sup> , 30
LSD	324.2	223.1 <sup>a</sup> , 208.1	144	18 <sup>a</sup> , 26

<sup>a</sup> The product ion fragmented at its respective collision energy (CE).

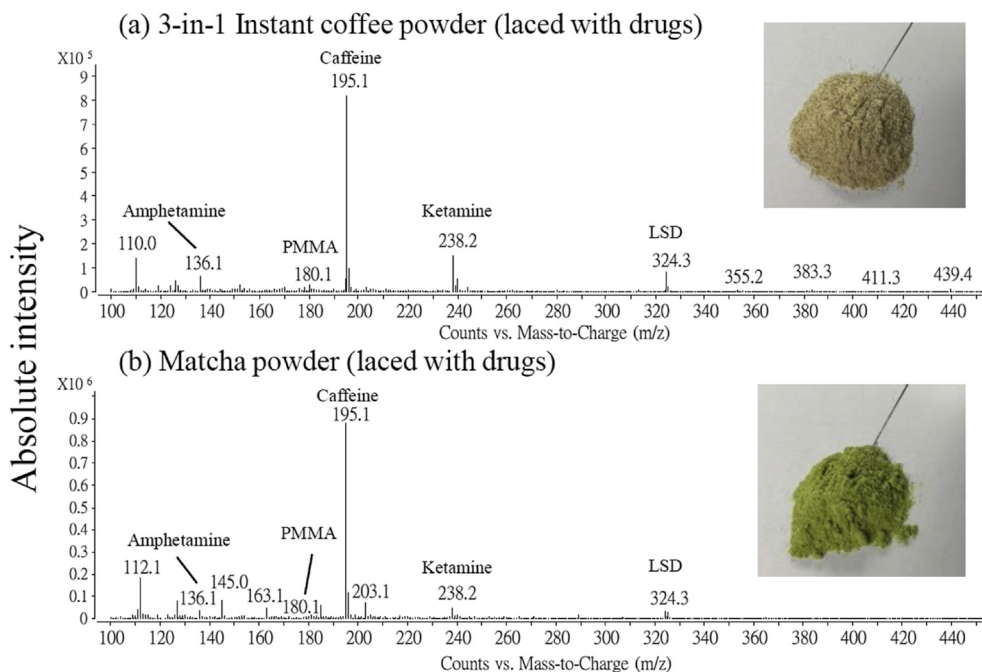


**Fig. 1** – TD-ESI mass spectra for (a, d) fruit and vegetable juice, (b, e) whole fat milk, and (c, f) sugar free green tea laced (a–c) without or (d–f) with illicit drugs (MA, mephedrone, MDMA, methylone, 2C-C, nitrazepam, and FM2; each at 100 ppm), respectively.

**Table 2 – The LODs of TD-ESI/MS for illicit drugs in three drinks analyzed with and without solvent extraction.**

Compound	Sugar-free green tea				Whole fat milk				Fruit and vegetable juice			
	Direct analysis		Solvent extraction before analysis		Direct analysis		Solvent extraction before analysis		Direct analysis		Solvent extraction before analysis	
	LOD (ppb)	S/N ratio*	LOD (ppb)	S/N ratio*	LOD (ppb)	S/N ratio*	LOD (ppb)	S/N ratio*	LOD (ppb)	S/N ratio*	LOD (ppb)	S/N ratio*
FM2	100	5.0	5	7.0	100	34.1	1	3.2	100	6.3	1	3.0
Nitrazepam	100	12.6	5	3.2	100	6.7	1	6.3	100	3.7	1	7.5
MDMA	100	21.9	1	3.3	100	18.9	5	13.6	100	21.5	10	13.6
Mephedrone	100	7.7	1	4.2	100	24.5	1	8.9	100	11.1	10	8.6
Methamphetamine	100	7.2	1	5.9	100	15.6	5	3.0	100	6.6	10	5.5
Methylone	100	13.9	1	4.7	100	56.6	1	9.7	100	23.0	10	3.5
2C-C	100	10.8	5	4.9	100	6.5	5	4.0	100	4.8	10	9.4

\* The S/N ratio of each analyte was determined based on the peak of first MRM transition shown in Table 1.

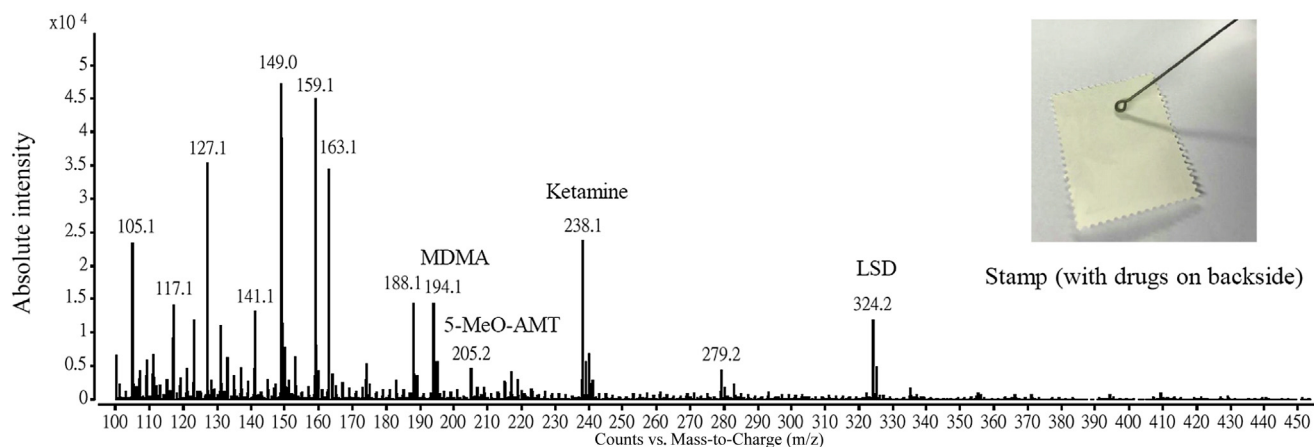


**Fig. 2 – TD-ESI mass spectra for (a) 3-in-1 instant coffee powder and (b) instant matcha powder laced with amphetamine, PMMA, ketamine, and LSD (each at 100 ppm).**

**Table 3 – The LODs of TD-ESI/MS for illicit drugs in two instant powders analyzed with and without solvent extraction.**

Compound	3-in-1 instant coffee powder				Instant matcha powder			
	Direct analysis		Solvent extraction before analysis		Direct analysis		Solvent extraction before analysis	
	LOD (ppb)	S/N ratio*	LOD (ppb)	S/N ratio*	LOD (ppb)	S/N ratio*	LOD (ppb)	S/N ratio*
Ketamine	1000	5.2	100	4.7	500	26.3	100	6.4
Amphetamine	1000	6.8	500	6.5	100	4.9	100	7.7
PMMA	1000	3.1	500	6.6	500	18.2	100	3.7
LSD	500	8.0	100	13.3	500	26.2	50	5.1

\* The S/N ratio of each analyte was determined based on the peak of first MRM transition shown in Table 1.



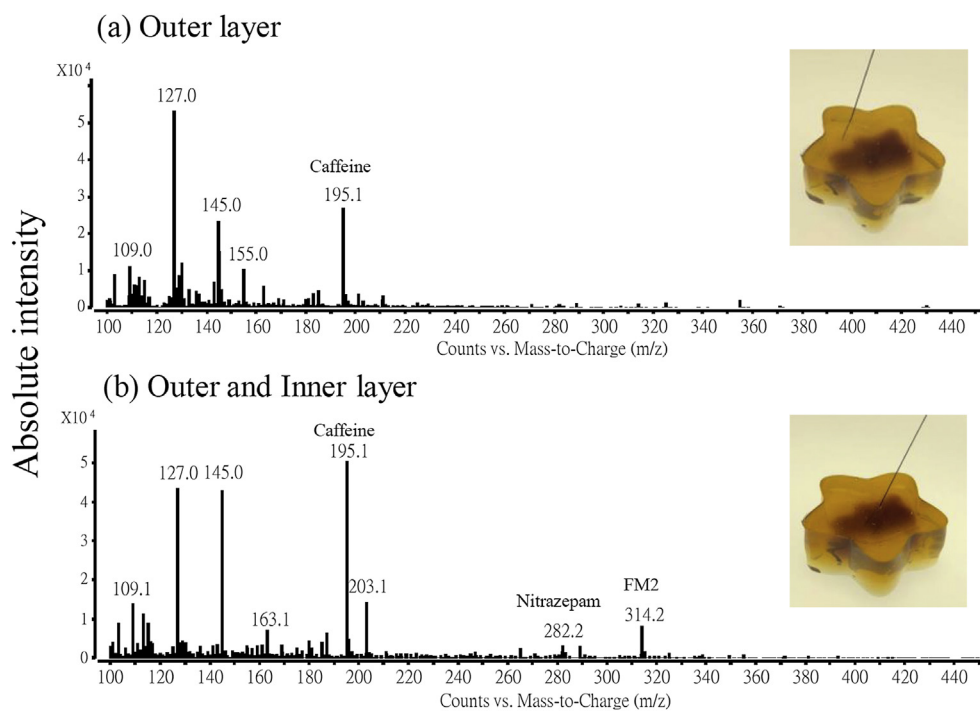
**Fig. 3** – TD-ESI mass spectrum for a stamp with ketamine, MDMA, 5-MeO-AMT, and LSD applied on the backside of stamp surface (each 5.2 ng/mm<sup>2</sup>).

**Table 4** – The LODs of TD-ESI/MS for illicit drugs on a stamp characterized with and without solvent extraction.

Compound	Direct analysis		Solvent extraction before analysis	
	LOD (ppb)	S/N ratio*	LOD (ppb)	S/N ratio*
Ketamine	6.5 pg/mm <sup>2</sup>	3.2	1.3 pg/mm <sup>2</sup>	4.8
MDMA	1.3 pg/mm <sup>2</sup>	5.0	1.3 pg/mm <sup>2</sup>	3.3
5-MeO-AMT	1.3 pg/mm <sup>2</sup>	6.9	6.5 pg/mm <sup>2</sup>	10.0
LSD	1.3 pg/mm <sup>2</sup>	9.9	1.3 pg/mm <sup>2</sup>	12.6

\* The S/N ratio of each analyte was determined based on the peak of first MRM transition shown in Table 1.

deposited on blotting paper to avoid suspicion and facilitate transportation; the blotting paper can then be placed under the tongue to orally administer the drug. In this study, solutions of MDMA, ketamine, 5-MeO-AMT, and LSD were deposited on the backside of a stamp to simulate a “blotter” form sample. The concentration of each illicit drug deposited on the stamp paper was between 1.3 pg/mm<sup>2</sup> and 5.2 ng/mm<sup>2</sup>. To collect drug molecules for detection, an inoculating loop was swept across the back of the stamp over an area of approximately 200 mm<sup>2</sup> (see inset in Fig. 3). Fig. 3 shows the TD-ESI mass spectrum for the stamp with drugs deposited on its back surface. The protonated ions [M+H]<sup>+</sup> of illicit drugs



**Fig. 4** – TD-ESI mass spectra for a jelly candy. To collect the sample, an acupuncture needle was (a) inserted into the outer star-shaped layer and (b) pierced through the outer layer to insert into the inner gummy bear.

including MDMA ( $m/z$  194), ketamine ( $m/z$  238), 5-MeO-AMT ( $m/z$  205), and LSD ( $m/z$  324) were all detected. In addition, phthalate-related ions ( $m/z$  149 and 279) and the fragment ions of MDMA ( $m/z$  105 and 163) and 5-MeO-AMT ( $m/z$  188) were also detected (Fig. 3). When the mass analyzer was operated in MRM mode, the LODs of these illicit drugs detected on stamp backside surface by TD-ESI/MS/MS LODs was 1.3 pg/mm<sup>2</sup> for MDMA, LSD, and 5-MeO-AMT, and 6.5 pg/mm<sup>2</sup> for ketamine (Table 4). Since a much higher concentration of the illicit drugs would be encountered in real cases, TD-ESI/MS/MS is a useful tool to examine the suspected blotting paper samples.

### 3.4. Analysis of illicit drugs in jelly candies

Gummy bears, which are made of gelatin, have been laced with MDMA to avoid suspicion and facilitate oral administration. The drug laced jelly candies were covered with normal gelatin to avoid suspicion and examination. Therefore, we made a double-layered jelly candy with a light brown star-shaped layer enveloping a purple gummy bear (see the inset in Fig. 4). The inner gummy bear was made by mixing of gelatin powder, grape juice, and illicit drugs (FM2 and nitrazepam) together, whereas the outer star shape was made by mixing gelatin powder and green tea solution without drugs. Only caffeine ( $m/z$  195) and dehydrated monosaccharide ions ( $m/z$  127 and 145) were detected in the outer layer of the candy (Fig. 4a). On the other hand, illicit drugs including FM2 ( $m/z$  314) and nitrazepam ( $m/z$  282), sugar-related ions ( $m/z$  127, 145, and 163), and caffeine ( $m/z$  195) were detected as the sampling needle pierced through the outer layer and insert into the gummy bear (Fig. 4b).

The LODs for the detection of aforementioned drugs with TD-ESI/MS/MS were determined by examining candies laced with different concentrations of illicit drugs, which were found to be 5 ppm for FM2 and 1 ppm for nitrazepam. The double-layered jelly candies were also bifurcated with a knife to expose the drug-laced inner layer so that a sampling needle was directly inserted into the inner layer to collect analytes. Under these conditions, FM2 and nitrazepam both had a LOD of 1 ppm.

### 3.5. Solvent extraction prior TD-ESI/MS analysis

Tables 2–4 show the results of fast solvent extraction followed by TD-ESI/MS analysis. The LODs for the illicit drugs extracted from the drinks were between 1 and 10 ppb, which were lower than those obtained by direct analysis of the liquid samples (Table 2). The LODs for the illicit drugs extracted from the powders were between 50 and 500 ppb (Table 3), which were similar to those obtained by direct analysis of the powder samples. Similarly, the LODs for the illicit drugs extracted from the solid stamp were between 1.3 and 6.5 pg/mm<sup>2</sup> (Table 4), which were similar to those obtained by direct analysis of the solid sample. These results demonstrate that direct TD-ESI/MS sample analysis is faster than solvent extraction followed by TD-ESI/MS analysis while yielding a similar sensitivity.

## 4. Conclusion

Thermal desorption electrospray ionization mass spectrometry (TD-ESI/MS) combined with probe sampling was utilized to rapidly characterize illicit drugs in suspicious samples including drinks, instant 3-in-1 coffee powders, jelly candies, and stamps. Rapid and direct analysis of drug-laced samples was done without pretreatment while requiring very small sample amounts. The results of TD-ESI/MS analysis indicate LODs for illicit drugs at 100 ppb for drinks, 100–1000 ppb for instant powders, 1–5 ppm for jelly candies, and 1.3–6.5 ng/mm<sup>2</sup> for stamps. The LODs of this approach are low enough to enable the sensitive analysis of real samples that are adulterated with illegal drugs at a concentration above 1% ( $w/w$ ). Since the entire analytical process took less than 30 s, probe sampling combined with TD-ESI/MS is a promising approach for the rapid and high-throughput screening of illegal drugs in suspicious samples. TD-ESI/MS can also be combined with solvent extraction to improve the LODs of illicit drugs in more complex sample matrices like biological fluids. Furthermore, the use of isotopically labeled internal standard will improve the precision of TD-ESI/MS for drug quantification, allowing this technique more reliable for legal judgment.

## Acknowledgement

This work was partially supported by the Research Center for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

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