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Using ambient mass spectrometry and LC–MS/MS for the rapid detection and identification of multiple illicit street drugs

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ABSTRACT

In this study the recently developed technique of thermal desorption electrospray ionization/mass spectrometry (TD–ESI/MS) was applied to the rapid analysis of multiple controlled substances. With the reallocation of mass spectral resources [from a standard ESI source coupled with liquid chromatography (LC) to an ambient TD–ESI source], this direct-analysis technique allows the identification of a wider range of illicit drugs through a dual-working mode (pretreatment-free qualitative screening/conventional quantitative confirmation). Through 60-MRM (multiple reaction monitoring) analysis—in which the MS/MS process was programmed to sequentially scan 60 precursor ion/product ion transitions and, thereby, identify 30 compounds (two precursor/product ion transitions per compound)—of a four-component (drug) standard, the signal intensity ratios of each drug transition were comparable with those obtained through 8-MRM analysis, demonstrating the selectivity of TD–ESI/MS for the detection of multiple drugs. The consecutive analyses of tablets containing different active components occurred with no cross-contamination or interference from sample to sample, demonstrating the reliability of the TD–ESI/MS technique for rapid sampling (two samples min^{-1}). The active ingredients in seized drug materials could be detected even when they represented less than 2 mg g^{-1} of the total sample weight, demonstrating the sensitivity of TD–ESI/MS. Combining the ability to rapidly identify multiple drugs with the "plug-and-play" design of the interchangeable ion source, TD–ESI/MS has great potential for use as a pretreatment-free qualitative screening tool for laboratories currently using LC–MS/MS techniques to analyze illicit drugs.

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1. Introduction

Illicit drug use is a major public health issue globally. The last decade has seen a surge in the prevalence of drug abuse in most countries, and global seizure of illicit drugs has risen dramatically [1]. The drug matrices of these seized materials usually vary from liquids (e.g., tea, cola, soda, juice) to solids (e.g., tablets, powders, instant coffee, cigarettes). The numbers and concentrations of adulterants in these seized materials have also been diverse. Typically, separation of adulterants from the sample matrix of a controlled drug can be tedious. In Taiwan, the number of specimens seized for testing continues to increase each year, and has exceeded the handling capacity of public crime laboratories since 2014 [2]. Thus, the rapid identification and analysis of illicit drugs and other controlled compounds in materials confiscated from criminal suspects has become a critical challenge for forensic and crime laboratories in Taiwan.

When attempting to establish the identity of an illicit drug in a suspect material, the analytical approach must include the determination of at least two uncorrelated parameters, one of which should provide chemical structural information about the target compound [3]. Liquid chromatography–tandem mass spectrometry (LC–MS/MS), which can provide information relating to both the chromatographic separation and the mass spectral fragmentation, is one of the most common methods used in forensic laboratories because of its excellent sensitivity and specificity. In many previous studies, LC–MS/MS has been used to analyze illicit drug materials [4–7]. Nevertheless, the protocols developed to determine illicit compounds often require time-consuming sample preprocessing steps (e.g., extraction, filtration, concentration, and chromatographic separation), a burden for crime investigation. In fact, it is often necessary for forensic laboratories to promptly detect and identify illicit drugs with minimal sample preparation. One approach to lessen the typical laboratory workload is to increase the throughput of the analyses of the seized specimens.

Several new techniques for high-throughput detection of illicit drugs have emerged recently. Of particular interest are those approaches employing ambient ionization mass spectrometry (AMS), which allows samples to be investigated in the open air, often with limited sample pretreatment; these techniques include the use of an atmospheric pressure solids-analysis probe (ASAP) [8], laser diode thermal desorption (LDTD) [8], desorption atmospheric pressure photoionization (DAPPI) [9–12], direct analysis in real time (DART) [13–19], direct sample analysis (DSA) [20,21], desorption electrospray ionization (DESI) [22–26], and easy ambient sonic-spray ionization (EASI) [27,28]. Coupling of these ionization sources to mass spectrometers makes it possible to rapidly screen for illicit drugs on and in drug materials, with little or no prior sample cleanup. Various matrices (e.g., tablets, powders, spices, incense, black tar, bricks, blotter papers, drinks, leaves, blooms, resins) have been tested during the development of AMS methods for the rapid screening of illicit drugs [8–28]. Because various AMS methods can provide versatile information about the components in complex samples, Jagerdeo and Wriston analyzed a wide range of illicit drug samples, including spice packets and heroin black tar, by interfacing two AMS techniques, LDTD and ASAP, to high-resolution mass spectrometers [8]. Nevertheless, AMS cannot always differentiate adulterant mixtures, because of a lack of chromatographic separation, potentially limiting its applicability. Sabino and colleagues coupled thin layer chromatography with easy sonic-spray ambient ionization mass spectrometry (TLC/EASI–MS) to analyze seized samples of powder and crack cocaine containing a wide range of adulterants, including benzocaine, lidocaine, caffeine, and procaine [28]. Romão and colleagues used EASI–MS to directly analyze *meta*-chlorophenylpiperazine (m-CPP) tablets, and employed traveling wave ion mobility mass spectrometry (IMS) to differentiate m-CPP from its isomers [27]. Lian and colleagues used a DART ion source coupled with a time-of-flight mass spectrometer (DART–TOF–MS), as well as dopant-assisted positive photoionization ion mobility spectrometry (DAPP–IMS), to build a screening library of reduced mobility (K_0) and the masses of the precursor ion and the fragment ions for the identification of 53 abused drugs [19]. We are, however, unaware of any previous publications concerning the reallocation of AMS and LC–MS/MS resources toward more flexible analytical functions.

Thermal desorption–electrospray ionization mass spectrometry (TD–ESI/MS) is an ESI-based AMS technique that can characterize thermally stable compounds in liquids, semi-solids, and solids without the need for extraction or separation [29]. A TD–ESI/MS system consists of a direct sampling probe, a thermal desorption unit, an electrospray ionization device, and a mass analyzer. This technique has two advantages over traditional ambient ionization techniques. First, with the use of a sampling probe, TD–ESI/MS can analyze samples without the damage that would have been caused by cutting them into appropriate shapes and sizes for AMS analysis. Second, by integrating all of the connections into the source housing, as in a previous study [30], the TD–ESI body can connect with mass spectrometers in a plug-and-play manner, thereby making them conveniently interchangeable. Notably, a mass spectrometer featuring two readily interchangeable ion sources can provide the advantages of dual working modes through simple switching of the ion sources (see the Graphical Abstract). In previous studies, we demonstrated that both qualitative screening assays and quantitative confirmatory tests for illegal colorants on and in solid traditional Chinese pastries [31] and processed vegetables [32] can be performed by reallocating an ambient ionization source and an atmospheric pressure ionization source of a benchtop AMS system. This approach has not, however, been adopted previously for forensic analyses of illicit drug materials.

In this present study, we performed qualitative screening and quantitative confirmation experiments using a commercial triple-quadrupole mass spectrometer featuring two plug-and-play ion sources: a TD–ESI source and a standard ESI source coupled to LC, respectively. When the ion source of the mass spectrometer was switched to a TD–ESI system, the selectivity of the method was evaluated by comparing the MS/MS results of a multiplex drug standard using two multiple reaction monitoring (MRM) methods. A short study was performed to assess the matrix effects, by detecting an illicit analyte in a drug sample containing multiple adulterants.

When the ion source of the mass spectrometer was switched to a standard LC–ESI system, the quantitative data for the controlled ingredients in seized illicit samples detected through TD–ESI/MS were used to demonstrate the sensitivity of TD–ESI/MS.

2. Materials and methods

2.1. Reagents and standards

Solvents for ESI (formic acid, MeOH) were purchased from Sigma–Aldrich (MO, USA) and Merck (Darmstadt, Germany), respectively. Distilled deionized water, purified through a PURELAB Classic UV system (ELGA, Marlow, UK), was used to prepare the electrospray solution and standard sample solutions. The illicit drug standards amphetamine, butylone, buprenorphine, cocaine, dibutylone, flurazepam, γ-hydroxybutyrate (GHB), heroin, JWH-018 [1-pentyl-3-(1-naphthoyl) indole], ketamine, lormetazepam, LSD (lysergic acid diethylamide), MDA (3,4-methylenedioxyamphetamine), MDMA (3,4-methylenedioxymethamphetamine), methamphetamine (MA), mephedrone, 5-MeO-DIPT (5-methoxydiisopropyltryptamine), morphine, nimetazepam, nitrazepam, PCP (phencyclidine), pethidine, pholcodine, PMA (*para*-methoxyamphetamine), THC (tetrahydrocannabinol), and zolpidem were purchased from Cerilliant (Austin, TX, USA). 2C–B (2,5-dimethoxy-4-bromophenethylamine) was obtained from TRC (Toronto, Canada), while MDPPP (3′,4′-methylenedioxy-α-pyrrolidinopropiophenone), 5-MeO-AMT (5-methoxy-α-methyltryptamine), and methedrone were purchased from Cayman Chemical (Ann Arbor, MI, USA). Seized drug samples were obtained from the Kaohsiung District Prosecutors Office, Ministry of Justice, Taiwan.

Stock solutions of all illicit drug standards were prepared individually in MeOH at 500 μg mL^{-1} and stored at approximately −20 °C in the dark. Spiking and calibration standard mixtures for LC–MS/MS analyses were prepared at various concentrations by combining aliquots of individual stock solutions, followed by dilution with MeOH.

2.2. Sample preparation

For TD–ESI–MS/MS analysis, no preparation steps were necessary for any of the seized drug samples, except those that were heterogeneous blends (i.e., cigarette: drug powder and tobacco blends; instant coffee: drug powder and coffee powder blends). Prior to TD–ESI–MS/MS analysis, a portion (0.2 g) of a tobacco blend or instant coffee granules was extracted/dissolved with/in MeOH (2 mL).

For LC–ESI–MS/MS analysis, seized drug samples were homogenized and dissolved in MeOH by sonicating for 5 min. The mixture was centrifuged (5 min, 2300*g*) and then the supernatants were decanted and dried under N_2. The residue was redissolved in solvent A (described in 2.3.2. *LC–MS/MS*; 0.5 mL). The mixture was vortex-mixed for 10 s and then filtered through a 0.22-μm filter into a small-volume autosampler vial. An aliquot (50 μL) of this sample was injected into the LC–MS/MS system. All control and fortified samples were prepared in the same manner.

2.3. Instrumentation

2.3.1. TD–ESI–MS/MS

The TD–ESI–MS/MS technique was set up in a same manner as described in previous publications [32]. In short, an interchangeable TD–ESI source, comprising a direct sampling probe, a thermal desorption unit, and an ESI interface, was coupled to a triple-quadrupole mass spectrometer (model 6420; Agilent, Santa Clara, CA, USA) in a plug-and-play mode (see the Graphical Abstract) for MS and MS/MS analyses. For solid samples, an acupuncture needle (diameter: 0.35 mm; length: 40 mm; Ching Ming Medical Device, Taipei, Taiwan) was used as a sampling probe to collect analytes from the samples. The needle was gripped by a stainless-steel pin clasp, which was embedded in an acrylic holder. For solid sampling, the needle was stabbed into the sample to a depth of 1 mm and then removed from the sample body. For liquid samples, a sampling probe featuring a commercially available stainless-steel inoculating loop (radius: 2 mm; Yu Shuan Technology, Kaohsiung, Taiwan) was used to collect the analytes from the samples. The sample solution (2 μL) was applied, using a micropipette, onto the ring at the end of the probe. After sampling, the probe was inserted promptly into the TD–ESI source; the desorption temperature was set at 280 °C, adjusted using a temperature controller (AT-502; ANLY, Taipei, Taiwan). A metal tube was attached to the heated oven to carry the heated N_2 prior to entering the desorption area. The preheated N_2 stream (5 psi) flowed from the top of the desorption unit to transfer the thermally desorbed analytes toward the ESI plume. The electrospray solution comprised MeOH, water, and formic acid (50/50/0.1, v/v/v). The ESI spray voltage was 3.5 kV for the positive mode. Between sample analyses, the sampling probe was cleaned by heating in the high-temperature flame of a hand-held gas torch for 3–4 s. The absence of any sample analyte residue was confirmed by checking the mass spectrum of the cleaned probe. The sequence of sampling, thermal desorption, ESI, detection, and probe cleaning was complete within approximately 30 s for each analysis. MS and MS/MS detection of illicit drugs in standard solutions and authentic materials were performed in positive-ion mode with a scan rate of 1800 Da s^{-1} to obtain spectra for the precursor and product ions. MS analyses were conducted in full-scan mode. MS/MS analyses were conducted in MRM mode, with the MRM channels and collision energies listed in Supplemental Table 1.

2.3.2. LC–MS/MS

LC–MS/MS analysis [33] was performed using an interchangeable ESI source (model 6420; Agilent, CA, USA) coupled with an HPLC system (model 1200; Agilent, CA, USA). Chromatographic separation was achieved through a Zorbax C18 column (250 mm × 4.6 mm I.D., 3.5 μm; Agilent Technologies, CA, USA) at room temperature. The mobile phase comprised Solvent A and B. Solvent A consisted of 0.1% formic acid in MeCN; Solvent B was 0.1% formic acid in water. The two components of the mobile phase were filtered and degassed. The program of the mobile phase gradient composition began with a 2/98 Solvent A/Solvent B ratio, linearly ramping up to 20% of Solvent A over 2 min; this ratio was maintained for 7 min, then ramped down linearly to 2% of Solvent A over 3 min. The gradient was

changed to the starting conditions over 3 min, and then kept constant for 3 min to re-equilibrate the system. The total run time was 15 min. The autosampler was kept at RT and the injection volume was 50 μL. Identification and quantitation of the illicit drugs was completed in the ESI–TQ–MS system through MRM in positive-ion mode. The MS source conditions were set as follows: capillary voltage, 3.5 kV; sheath gas temperature, 300 °C; source temperature, 150 °C; sheath gas flow, 6 L min^{-1}; nebulizer pressure, 30 psi.

2.4. TD–ESI–MS/MS analysis

2.4.1. Repeatabilities and detection limits

Repeatabilities and limit of detections (LODs) were assessed by spiking real samples [mixtures of blank tobacco blends and blank instant coffee granules, 1:1 (w/w)] with the analytes at 50 μg g^{-1} and in decreasing concentrations, respectively. The methanolic extracts of these matrix standards were all analyzed in MRM mode. For the repeatability tests, 10 consecutive analyses of the analytes in the matrix were conducted to determine the repeatability. Extracted ion chromatograms (XICs) were recorded and relative standard deviations (RSDs) were calculated using the peak areas obtained from the XICs. LODs were determined when the S/N ratios of the major product ion signals were approximately 3:1 [34]. The S/N ratio was calculated from the height of the major product ion peak corresponding to the analyte concerned and the height of the peak-to-peak background noise [35].

2.4.2. Cross-contamination analysis

Four consecutive analyses of both licit drugs (two commercial acetaminophen tablets, 500 mg/tab, 400–410 mg g^{-1}) and illicit drugs (two seized nimetazepam tablets, 5 mg/tab, 12–14 mg g^{-1}) were conducted to obtain structural information on the active ingredients. Total ion chromatograms and MS spectra were recorded during TD–ESI–MS/MS sampling of four tablets.

2.4.3. Concurrent MRM transitions test

To evaluate the feasibility of multiplex analyses using the TD–ESI/MS technique, two MRM methods performed with different numbers of concurrent MRM transitions (see Supplemental Table 1)—that is, eight and sixty precursor ion/product ion transitions—were used to detect a four-component drug solution (containing MA, mephedrone, ketamine, and nimetazepam; Supplemental Table 1). This four-component drug standard was analyzed through TD–ESI/MS at a concentration of 10 μg mL^{-1}. Each component was identified simultaneously by two discrete transitions. The average intensity of each transition was calculated over five replicates.

2.4.4. Matrix effect test

To evaluate the feasibility of multiplex analyses using the TD–ESI/MS technique, a short study was performed to assess the matrix effects in multiplex analysis by comparing the signal intensities of 10 μg mL^{-1} THC with those of 10 μg mL^{-1} THC in a matrix of 12 other compounds (MDMA, pethidine, mephedrone, amphetamine, nitrazepam, ketamine, cocaine, lormetazepam, pholcodine, buprenorphine, codeine, and caffeine).

2.5. LC–MS/MS analysis

To ensure the system's suitability and stability, a quality control (QC) sample, prepared by combining equal aliquots of all the samples, was injected at regular intervals throughout the analytical run. Blank samples (i.e., injection of the reconstitution solvent) were also run to check the presence of artifacts or contaminant peaks. For this validated method, the limits of quantification (LOQs) at S/N ratios of 10 for nimetazepam, nitrazepam, ketamine, butylone, ethylone, heroin, mephedrone, MDA, MDMA, and MA in the corresponding matrices listed in Table 3 were 2/2 (tablet/instant coffee), 1, 2/1 (powder/cigarette), 2, 2, 2, 2, 2, 2, and 9 ng g^{-1}, respectively. Any drug identified in the sample was interpreted as positive if the levels were above the LOQ.

3. Results and discussion

The high variety of compounds that require investigation through MS has led to the development and use of various ion sources. While AMS is one of the most widely used techniques for direct identification of illicit drugs, the reallocation of AMS ion sources for more flexible analytical functions has not been reported previously for the analyses of illicit drugs in seized materials. As a proof of concept, in this present study a mass spectrometer featuring two rapidly interchangeable ion sources was used to characterize, through qualitative screening analyses and quantitative confirmatory analyses, illicit drugs in seized real samples.

3.1. TD–ESI–MS/MS analysis

3.1.1. Repeatabilities and detection limits

The stabilities of drug ion signals, detected using TD–ESI/MS, in real sample spiked with studied drugs (50 μg g^{-1}) were analyzed. The methanolic extracts of these matrix standards were repeatedly analyzed using TD–ESI–MS/MS. The RSDs of the stability tests were all under 10.5%. Because of the ambient nature of the TD–ESI/MS technique, background noise peaks were usually difficult to eliminate in the MS mode (but not in the case of the MS/MS mode). MS/MS could produce characteristic product ions of an illicit drug that were readily identifiable at its LOD. To evaluate the sensitivity of the use of TD–ESI/MS to detect drugs in real sample (e.g., samples of instant coffee and cigarette), the LODs of drugs in MeOH-extracted samples (mixtures of tobacco blends and instant coffee granules) were determined. Table 1 demonstrates that the LODs of the studied drugs were at the sub-microgram-per-gram (μg g^{-1}) level; thus, this approach is sufficiently sensitive for criminal seizure applications, because the concentrations of drugs in confiscated materials reported previously [36] or in the present study or documented by the Kaohsiung City Police forensic laboratory system (data not shown) are usually much higher than the LODs. The LODs of aminoindane-group drugs, including amphetamine, MA, MDMA, MDA, and PMA, were at 1 ng g^{-1}, while opiate group drugs, including morphine and buprenorphine, had LODs of 60 ng g^{-1}. Differences in the volatility, thermal stability, proton affinity, and matrix effect of the studied drugs may have accounted for the varying LODs.

Table 1 – Data from MS and MS/MS analyses of illicit drugs.

Analyte	Mass (Da)	MS (*m*/z)	MS/MS (*m*/z)	LOD (ng g^{-1})
GHB	104.1	105	**87**[a], 55, 43	20
Amphetamine	135.2	136	119, **91**[a]	1
MA	149.2	150	119, **91**[a]	1
PMA	165.2	166	**149**[a], 121	1
Mephedrone	177.2	178	**160**[a], 145, 119	2
MDA	179.2	180	**163**[a], 135	1
Methedrone	193.2	194	**176**[a], 161,58	2
MDMA	193.2	194	**163**[a], 105, 77	1
5-MeO-AMT	204.2	205	**188**[a], 147	2
Butylone	221.2	222	204, **174**[a], 72	2
Dibutylone	235.2	236	**161**[a], 149, 86	2
Ketamine	237.7	238	220, 207, 179, 163, 152, **125**[a]	1
PCP	243.3	244	**86**[a], 159	1
MDPPP	247.2	248	**147**[a], 98, 70	1
Pethidine	247.3	248	220, **174**[a], 70	1
2C–B	260.1	261	**244**[a], 229	2
5-MeO-DIPT	274.4	275	174, **114**[a], 102	2
Nitrazepam	281.3	282	**236** [a], 180, 152	10
Morphine	285.3	286	165, **152**[a], 128	60
Nimetazepam	295.3	296	**250**[a], 221, 165	5
Cocaine	303.3	304	**182**[a], 82	5
Zolpidem	307.3	308	263, **235**[a], 92	2
THC	314.4	315	259, **193**[a], 123, 93	20
LSD	323.4	324	281, **223**[a], 207, 180	20
Lormetazepam	335.2	336	**290**[a], 318	20
JWH-018	341.4	342	214, **155**[a]	2
Heroin	369.4	370	328, 268, 211, 193, **165**[a], 152	20
Flurazepam	387.8	388	317, **315**[a]	6
Pholcodine	398.4	399	381, **114**[a]	10
Buprenorphine	467.6	468	396, 101, **55**[a]	60

[a] Numbers in bold indicate major product ions of respective illicit drugs.

Table 2 – Comparison of ion intensities of a THC standard and THC within a matrix of 12 compounds.

Transition (*m*/z)	10-ppm THC		10-ppm THC within matrix		Relative intensity (%)
	Avg Area (cps)	RSD (%)	Avg Area (cps)	RSD (%)	
315/193	1,195,043	2.8	128,354	5.1	10.7
315/259	555,083	4.0	58,533	6.2	10.5

3.1.2. *MS and MS/MS detection of illicit drugs in standard solutions and in soft drinks*

Thirty illicit drugs having various chemical structures (aminoindanes, phencyclidine, phenethylamines, piperazines, synthetic cannabinoids, synthetic cathinones, and tryptamines) and a wide range of molecular weight (from 104 to 467 Da) were tested in the study. First, these drugs were analyzed using TD–ESI/MS. The TD–ESI mass spectra of all of the illicit drug standards were analyzed in positive-ion mode. The mass spectral data of these drugs are listed in Table 1. The signal of the protonated molecule ($[M + H]^+$) was the base ion of each drug. Because the analytes were ionized through an ESI mechanism, the mass spectra obtained using TD–ESI/MS were nearly identical to those obtained using conventional standard ESI/MS [37]. Notably, the MS/MS data obtained using TD–ESI could be compared directly with the laboratory-generated MS/MS library using ESI. That is, the development of a TD–ESI-specific library does not appear to be necessary. Direct comparison of TD–ESI- and ESI- generated MS/MS spectra, however, might not be possible in all situations. Differences observed using TD–ESI and ESI ion-generation processes might result in different ion structures and internal energies, giving rise to significant MS/MS spectral differences. Future studied into the TD–ESI mechanism should provide insight into such phenomena.

To test the capability of TD–ESI–MS/MS to directly detect illicit drugs in soft drinks, we analyzed samples of black tea, orange juice, cola, and soda without sample pretreatment. Four model illicit drugs—MA, mephedrone, ketamine, and nimetazepam—were spiked into selected soft drinks (concentration of each drug in a drink: 50 μg mL^{-1}). Our experimental results indicated that all of the illicit drugs were readily protonated in the TD–ESI source to form positive ions, allowing us to employ ESI in the positive-ion mode. Fig. 1 displays the mass spectra of the residual illicit drugs detected in the soft drinks; the MRM mode was used for the MS/MS analyses presented in the insets. Because all of the drinks had complex matrices, MS/MS was necessary to identify the analytes detected from them. MS/MS spectra and their product ions were used to assist with the identification of the drug

Table 3 – Compounds detected in illicit drug seizures, using TD-ESI-MS/MS and LC-ESI-MS/MS.

Sample	Matrix	Compound	MW (Da)	TD–ESI–MS/MS	LC–ESI–MS/MS	Composition[a] (mg g^{-1})
Item 1	Tablet	Nimetazepam	295.3	Y	Y	11.2
Item 2	Tablet	Nimetazepam	295.3	Y	Y	13.6
Item 3	Tablet	Nitrazepam	281.3	Y	Y	23.1
Item 4	Powder	Ketamine	237.7	Y	Y	16.3
		Butylone	221.2	Y[b]	Y	1.5
		Ethylone	221.2	Y[b]	Y	1.7
Item 5	Powder	Butylone	221.2	Y[b]	Y	142.5
		Ethylone	221.2	Y[b]	Y	145.0
Item 6	Powder	Ketamine	237.7	Y	Y	931.6
Item 7	Cigarette	Ketamine	237.7	Y	Y	27.3
Item 8	Cigarette	Heroin	369.4	Y	Y	12.6
Item 9	Instant coffee	Mephedrone Nimetazepam	177.2295.3	Y	Y	2.3
				T	Y	<0.1
Item 10	Instant coffee	MDA	179.2	Y	Y	6.7
		Nimetazepam	295.3	Y	Y	3.5
Item 11	Instant coffee	MDMA	193.2	Y	Y	1.6
		MA	149.2	Y	Y	3.3
		Mephedrone	177.2	Y	Y	2.7

Y = detected; N = not detected; T = trace; MW = molecular weight; MA = methamphetamine.
[a] Analyzed using LC–ESI–MS/MS.
[b] Might represent a mixture of structural isomers.

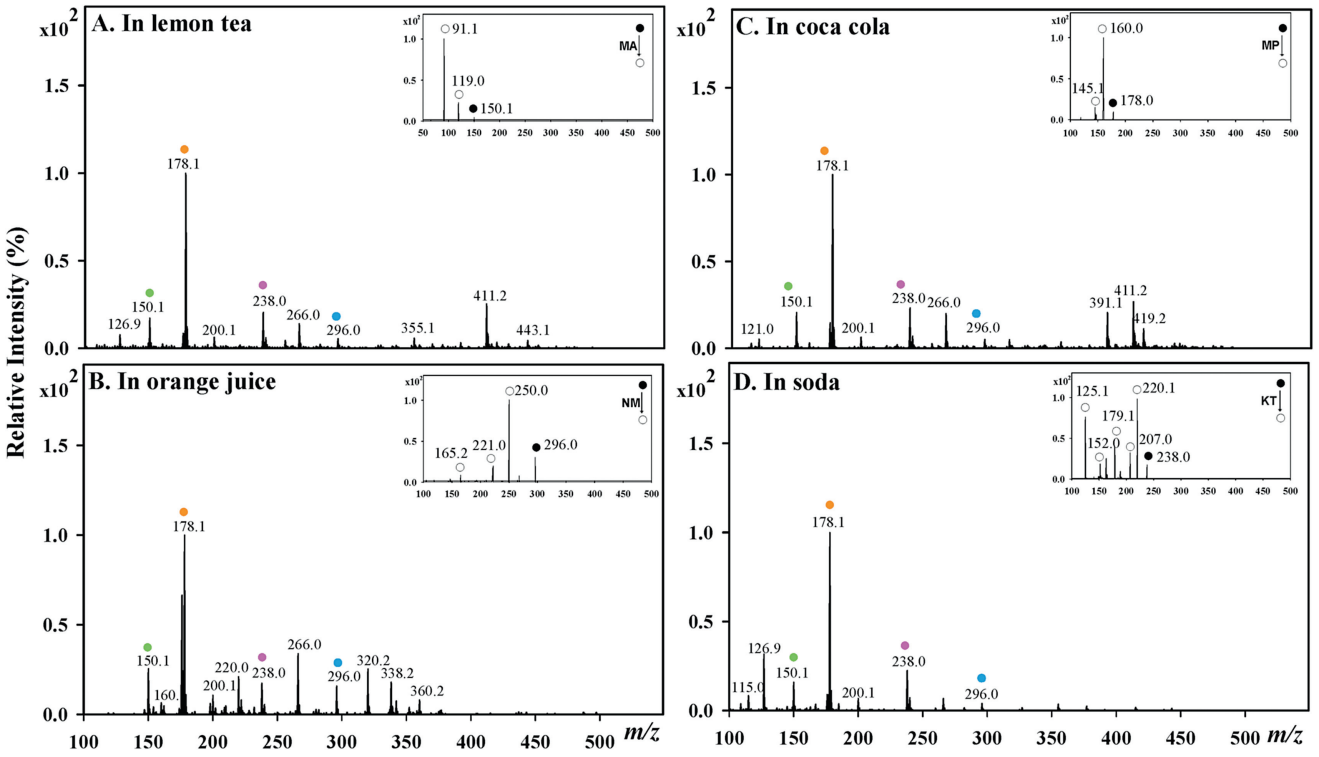

Fig. 1 – TD–ESI mass spectra of a drug mixture in lemon tea, orange juice, coca-cola, and soda. The drug mixture contained methamphetamine (MA; green spot), nimetazepam (NM; blue spot), mephedrone (MP; orange spot), and ketamine (KT; pink spot). The concentration of each drug was 50 μg mL^{-1}.

molecules in the complex liquids. The MS/MS data of the four illicit drugs in the drinks were concordant with the data obtained from the authentic compounds dissolved in MeOH. Notably, many other peaks were present in the mass spectra. Thus, TD–ESI can directly detect multiple components from a drink matrix without sample pretreatment, opening up the possibility of similarly using DART for the rapid detection of multiple components in liquid samples [18].

3.1.3. *Cross-contamination analysis*

The concentrations of active ingredients in confiscated drug samples are typically high; therefore, sensitivity is usually not

an issue in their analysis. Nevertheless, because of high analyte concentrations, memory effects and instrument contamination can be problematic—especially for traditional ambient ionization techniques that use a high-speed gas and/or solvent flow, resulting in disintegration of the samples and the loss of dust from their surfaces during the desorption process [11]. In forensic analysis, false-positive findings due to memory effects might potentially lead to false accusations. To ensure reliable real-time sampling, the TD–ESI/MS method should be free of interference or cross-contamination from previously tested samples. For example, when using the TD–ESI technique to screen multiple tablets, a cause for concern might be interference or cross-contamination arising from previously run tablets. Fig. 2 presents TD–ESI–MS/MS data obtained from consecutive screenings of two different types of tablets. The first and third tablets contained nimetazepam (MW 295 Da), while the second and fourth tablets contained the non-opioid analgesic acetaminophen (MW 151 Da). The MS data obtained for the nimetazepam tablets (left panels) feature a molecular ion at *m*/*z* 296. For the other two tablets, a molecular ion corresponding to acetaminophen was present. No interference or cross-contamination was observed in any of the spectra, demonstrating that (i) any memory effects from previous samples were readily removed from the probe by heating it in a high-temperature flame and (ii) the TD–ESI/MS technique can be used reliably during the rapid analysis of significantly different samples. The additional peak at *m*/*z* 325 is tentatively assigned to 2-propenyl ester octadecanoic acid (allyl stearate), a commonly used tablet binder ($C_{21}H_{40}O_2$; MW 324 Da); the peak at *m*/*z* 365 is probably due to lactose ($[\text{lactose} + Na]^+$, of *m*/*z* 365), a commonly used tablet excipient; the peak at *m*/*z* 183 is probably due to sugar alcohol ($[C_6H_{14}O_6 + H]^+$, of *m*/*z* 183) [38], a common in-source fragment of starch, a commonly used tablet excipient.

3.1.4. *Feasibility of multiplex analysis: concurrent MRM transitions test and matrix effect test*

Because of the need for rapid screening, an AMS method involving a large suite of drugs was necessary to ensure that all of the target drugs would be detectable simultaneously. Methods for the identification and quantification of analytes using LC–MS/MS have usually involved the MRM monitoring of two product ions from the same precursor ion, resulting in four identification points that meet the requirements of 2002/657/EC [39]. It is well established that the number of concurrent MRM transitions and the matrix effect are two important factors influencing the MRM signal intensities in multiplex analyses using MS/MS instruments. The dwell time is the time spent acquiring the targeted MRM transition during each cycle. Increasing the number of MRM transitions by maintaining the dwell time would extend the cycle time and, thus, result in poor analytical results, because of an insufficient number of data points across the MRM peak. Therefore, an increase in multiplexing resulting in more concurrent MRM transitions would decrease the analytical accuracy and reproducibility. To ensure that the signal intensity ratios remained unaffected during multiplex analyses, a four-component drug standard was analyzed using two MRM methods, involving eight and sixty precursor ion/product ion transitions. The results of this experiment are displayed in Fig. 3, where the average area of each transition is compared for both methods. Despite small variations (as might be expected), the ion intensities were not greatly affected by increasing the number of transitions programmed into the method.

Using AMS without separation would subject a sample to ionization effects (e.g., ion suppression and matrix effects) that chromatography would otherwise eliminate; these effect can influence signal intensities greatly. To investigate this possibility, THC was chosen for testing because it is a compound that is largely affected by matrix effects, including ion suppression and ion enhancement [40]. In the present study, the signal intensities were determined from 10 $\mu g\ mL^{-1}$ THC and those from 10 $\mu g\ mL^{-1}$ THC in a matrix of 12 other compounds. Table 2 lists the precision and the ion suppression effects caused by the matrix of 12 compounds. The precisions (RSD < 10.7%) for both the THC standard solution and the THC matrix solution were similar to those in the stability tests (RSD < 10.5%). Despite the presence of the matrix, THC remained detectable far above its LOD. In addition, the loss in ion intensities did not affect the ratios between the ion pairs and, thus, did not hinder qualification. To assist in quantification, an isotopically labeled internal standard may be added, to compensate for matrix effects, because the isotopic transitions would be affected to the same extent, helping to ensure that quantification would not be compromised [40]. Nevertheless, the use of an isotopically labeled internal standard would not adequately quantify all compounds; several studies have found that the matrix effect is not always minimized [41–43].

3.2. *Application*

Users abuse drugs in varying ways; some drugs are taken orally, while others are smoked, injected, or snorted. Abused drugs are found in many forms, including tablets, powders, sugars, jellies, instant coffee, and tobacco cigarettes. Earlier studies with TD–ESI/MS included the analyses of gastric lavage fluid samples and whole blood specimens containing controlled psychotropic drugs (e.g., FM2, MDMA, LSD, cocaine, amphetamine, ketamine) [44]. New examples of seized illicit drug materials that have been analyzed using TD–ESI/MS are provided in Table 3. Most of the results obtained with TD–ESI/MS are comparable with the results obtained using LC–MS/MS, also provided in Table 3.

3.2.1. *Detection of drug tablets*

Previous studies have demonstrated the ability of TD–ESI/MS to characterize the active ingredients in various pharmaceutical products formulated as, for example, clear liquids, syrups, ointments, and a tablet containing sildenafil citrate (Viagra) [29]. "Erimin" (nimetazepam) tablets are a common drug of abuse in Asia. Most of the tablets seized have been illicit in nature. Commercial tablets containing nimetazepam as the active ingredient are used as therapeutic agents for insomnia. The chemical composition of the confiscated illicit tablets often differs from that of the commercial version. In these illicit tablets, nimetazepam has often been detected along with nitrazepam. In some seized samples, nitrazepam

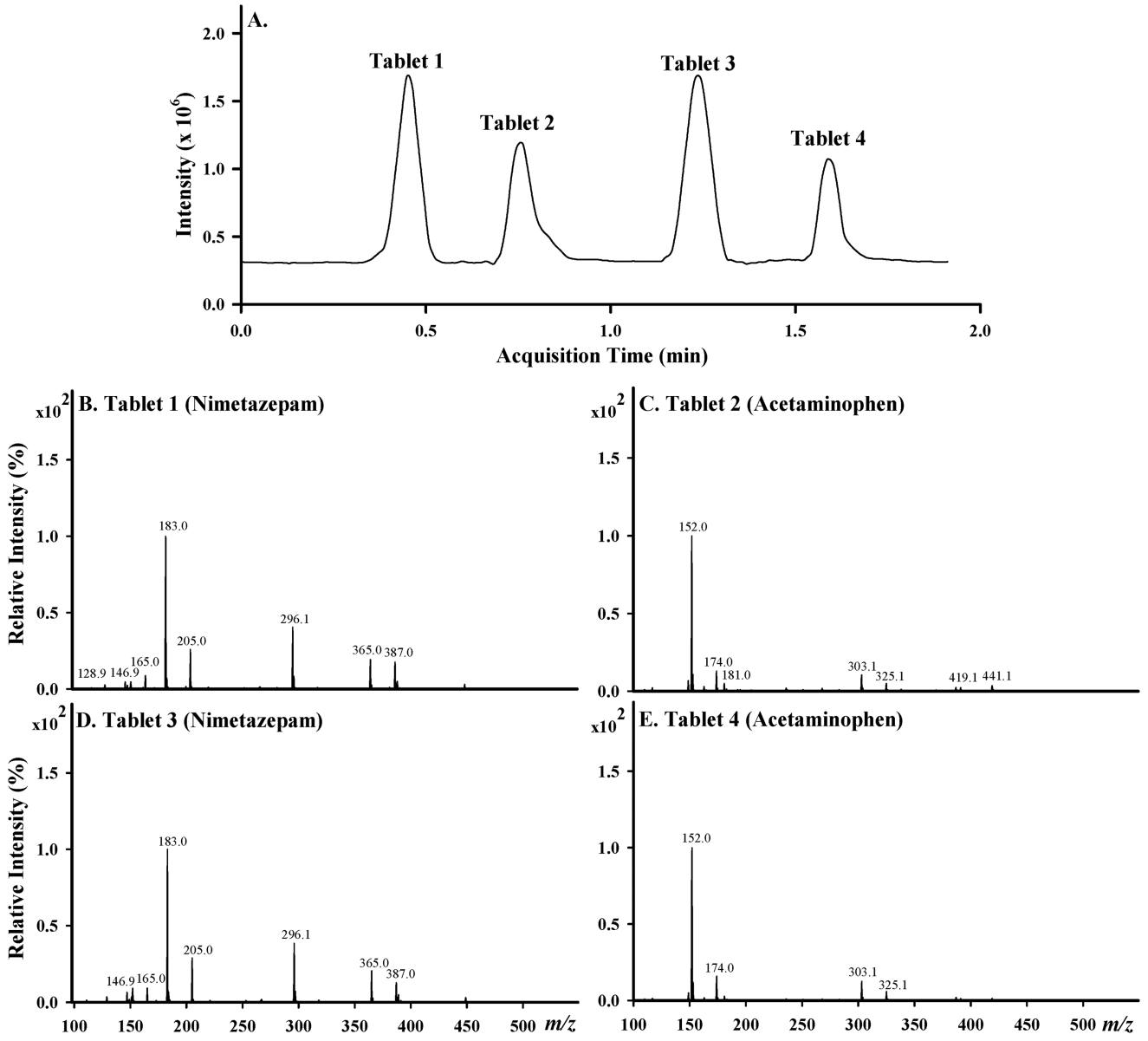

Fig. 2 – Rapid sampling of nimetazepam and acetaminophen tablets using TD–ESI–MS/MS: (A) Total ion chromatogram obtained during TD–ESI–MS/MS sampling of four tablets, (B) MS spectrum of tablet 1, (C) MS spectrum of tablet 2, (D) MS spectrum of tablet 3, and (E) MS spectrum of tablet 4.

has been detected alone. Other adulterants, including carbamazepine, melatonin, and caffeine, have also been encountered [45]. In this present study, three seized illicit tablets (Items 1–3 in Table 3) were characterized through TD–ESI–MS/MS to contain nimetazepam (ion transitions: *m/z* 296 → 250 and 296 → 221) or nitrazepam (ion transitions: *m/z* 282 → 236 and 282 → 180). Drug contents ranging from 11.2 to 23.1 mg g^{-1} were determined analytically through LC–ESI–MS/MS in our laboratory, but even higher amounts have been reported elsewhere [46]. Notably, spatial distribution would play an important role when applying AMS techniques (including TD–ESI–MS/MS) to tablet samples containing contents in the range of a few percentage points, because microcrystals of the illicit ingredients might not be distributed uniformly on the surfaces of the tablets.

3.2.2. *Detection of drug powders*

A number of different illicit drugs usually come in powder form. Some common examples of controlled psychotropic substances that are obtained as white powders are stimulants (e.g., cocaine, amphetamine, MDMA, mephedrone), sedatives (e.g., ketamine, GHB, PCP), and hallucinogens (e.g., 2C–B, α-methyltryptamine). When receiving an illicit white powder, the analyst can never be fully sure of its contents. Because of the desorption/ionization processes required for some AMS methods, powdered samples must be compressed into disks, pressed into an adhesive, or dissolved in a solvent to prevent puffing of the powder [10]. Here, to allow direct detection of these powders without sample preparation, an acupuncture needle (sampling probe) was used to contact with the sample surface to remove a sample, and then the

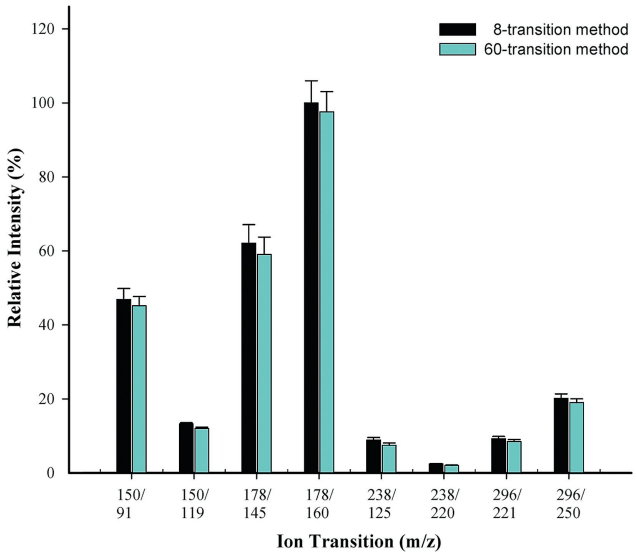

Fig. 3 – Average intensity of each precursor ion/product ion transition from a four-component standard using a method containing eight transitions compared with a method containing sixty transitions.

probe with the sample was placed into the TD–ESI thermal desorption unit for analysis. For all of the compounds analyzed through TD–ESI–MS/MS, the LODs were far below the level of 1 ng per sampling, highlighting the possibility of detection through contact with a sample's powder surface, instead of sampling the bulk powder itself. This type of approach would minimize the risk of exposure to the analyst by sampling only nanogram quantities of powder materials from the outside of a specimen bag or container and, therefore, reducing the likelihood of coming into contact with a bulk powder material.

From the set of 11 seized samples that were analyzed, two powder samples were identified through TD–ESI–MS/MS as illicit butylone powders (ion transitions: *m*/z 222 → 174 and 222 → 204). A special aspect when analyzing butylone is isomerism: an isomer of butylone, ethylone, might be present, having the same empirical formula and sharing identical prominent fragments at *m*/z 72, 174, and 204. In the absence of chromatography, a positive screening result from TD–ESI–MS/MS would not give any structural information to distinguish butylone from its positional isomer ethylone. It is usually necessary for chromatographic separation to be performed if one isomer is controlled and another is not. In this case, however, butylone and ethylone are included on lists of controlled psychotropic substances in most countries. These seized powders were confirmed positive for their associated isomers through LC–ESI–MS/MS analyses performed in the Kaohsiung City Police forensic laboratory system prior to our quantitative analyses (Table 3).

3.2.3. *Detection of drug-laced cigarettes*

Smoking drugs is one of the most common forms of illicit drug intake. The most commonly smoked drugs after tobacco include ketamine, heroin, marijuana, cocaine, opium, MA, and PCP [47]. The popularity of this intake method is largely due to the fact that smoking a psychotropic substance is the fastest way to deliver a high concentration of the drug into the bloodstream and transport it from the lungs to the brain, producing an intense rush of euphoria. The Ministry of Health and Welfare in Taiwan has reported a rapid rise in the abuse of drug-laced cigarettes over the last decade; indeed, smoking cigarettes containing ketamine, known as a "K-cig," is the most common method of ketamine use in Taiwan [48]. Previous investigations have examined the pyrolysis products produced by smoking drugs, including ketamine, heroin, MA, cocaine, and phencyclidine (PCP), along with tobacco [49–53]. In those studies, LC–UV, GC–MS/MS, micro gas chromatography (μGC), infrared spectrometry, MS, and proton magnetic resonance spectrometry were employed to examine the pyrolysis products obtained when smoking illicit drugs with tobacco. We are, however, unaware of any previous publications concerning the use of AMS for the direct detection of illicit psychotropic substances in drug-laced cigarettes.

There are two common ways to smoke a drug with a tobacco cigarette: the powdered drug can be rolled into a cigarette and smoked with tobacco or the cigarette can be dipped into a liquid drug and smoked. In this present study, two seized samples of tobacco cigarettes were identified through TD–ESI–MS/MS as containing illicit blends of ketamine and heroin tobacco (ion transitions: *m*/z 238 → 125 and 238 → 220 for ketamine; *m*/z 370 → 165 and 370 → 152 for heroin). These seized cigarettes were further confirmed positive, containing 27.3 mg g^{-1} ketamine and 12.6 mg g^{-1} heroin, through LC–ESI–MS/MS analysis in our laboratory (Table 3). To the best of our knowledge, no similar results have been reported previously.

3.2.4. *Detection of drug-laced instant coffee*

Recently, the age of people abusing drugs in Taiwan has decreased, accompanied by an increase in the types of distribution methods. For example, ketamine, nimetazepam, amphetamine, MA, MDMA, and new synthetic cathinone drugs have frequently been found in instant coffee packets and tea bags confiscated from criminal suspects [54]. Drug dealers can purchase instant coffee packets readily in a market; the packets are then opened and resealed after mixing the drug powder with the instant coffee mixture. Coffee laced with potent psychotropic substances has claimed the lives of several people in Taiwan in the last two years [55]. The following emerging drugs of abuse, mixed in "instant dissolve" forms of three coffee packets seized by police, were detected through TD–ESI–MS/MS: mephedrone (ion transitions: *m*/z 178 → 160 and 178 → 145), nimetazepam (ion transitions: *m*/z 296 → 250 and 296 → 221), MDA (ion transitions: *m*/z 180 → 163 and 180 → 135), MDMA (ion transitions: *m*/z 194 → 163 and 194 → 105), and MA (ion transitions: *m*/z 150 → 91 and 150 → 119) (Table 3). Where specified as trace amounts, although MS data were obtained, the MRM and fragmentation data (MS/MS) could not be exploited because of the low quantities of the protonated molecules present (<100 counts). Although the LC–ESI–MS/MS results demonstrate that these drug-laced coffee packets contained only low dosages of the controlled psychotropic substances, consumers might still intake excessive amounts of these drugs if binge-drinking the coffee.

3.3. *Analytical figures of merit*

Supplemental Table 2 compares the figures of merit of the proposed method and previously published methods using AMS for the analysis of illicit drugs in real samples [8–28]. First, our proposed method, involving the reallocation of AMS and LC–MS/MS interchangeable resources, provides more flexible analytical functions, including both qualitative screening assays and quantitative confirmatory analyses, relative to those of the conventional AMS methods involving fixed resources. New psychoactive substances (NPS) are a range of drugs that have been designed to mimic established illicit drugs. In recent years, NPS have often appeared on the market disguised as cigarettes or snack foods (e.g. candy, jellies, plum powders, instant coffee, milk tea) [48]. Notably, most of the other AMS methods listed in Supplemental Table 2 have been used to analyze illicit drugs only in traditional dosage forms (e.g. tablets, powder, spice); our present method is the only one that has been used to analyze tobacco cigarettes and a snack food (i.e., instant coffee).

4. Conclusion

During recent years, the application of AMS to the qualitative analysis and screening of seized drug samples has demonstrated its applicability to the rapid chemical profiling of illicit samples. Although traditional AMS usually has few advantages over LC–MS/MS (commonly employed for drug abuse control) when applied for accurate quantitative analysis, our present method, designed with an interchangeable ion source, is an alternative for pretreatment-free qualitative screening in laboratories that employ LC–MS/MS techniques frequently. Here, we reallocated an ambient ionization source (i.e., TD–ESI source) and an atmospheric pressure ionization source (i.e., standard ESI source coupled to LC) of a benchtop MS system to provide analytical functions of greater flexibility, including qualitative screening assays and quantitative confirmatory tests, for analyses of illicit drugs in seized drug samples. AMS using interchangeable TD–ESI sources has three advantages over conventional AMS and conventional preliminary screening methods when analyzing seized drug samples: (i) when compared with most conventional AMS systems, the present approach is more analytically flexible, because the ion sources for the screening assay and confirmatory test can be rapidly interchanged in plug-and-play style; (ii) unlike other AMS qualitative screening methods, there is no need to compress or cut the solid samples into acceptable shapes and sizes, because of the nature of the sampling probe used in the present system; (iii) relative to conventional qualitative screening methods (e.g., spectroscopy, colorimetric spot tests, TLC), the detection specificity of the present system is high because it uses tandem mass spectrometry. A great potential exists to expand this approach to encompass a variety of common illicit drug compounds that are routinely determined through LC–MS/MS, for future intelligence, for rapid qualitative analysis, and for chemical profiling.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfda.2018.11.003.

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