Forensic Science International 244 (2014) 42–49

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

DART–MS in-source collision induced dissociation and high mass accuracy for new psychoactive substance determinations

Rabi A. Musah [a], Robert B. Cody [b], Marek A. Domin [c], Ashton D. Lesiak [a], A. John Dane [b], Jason R.E. Shepard [a,*]

[a] Department of Chemistry, University at Albany, State University of New York (SUNY), 1400 Washington Ave., Albany, NY 12222, USA
[b] JEOL USA, Inc., 11 Dearborn Rd, Peabody, MA 01960, USA
[c] Mass Spectrometry Center, Merkert Chemistry Center, Boston College, 2609 Beacon Street, Chestnut Hill, MA 02467-3808, USA

ARTICLE INFO

Article history:
Received 20 March 2014
Received in revised form 28 July 2014
Accepted 31 July 2014
Available online 12 August 2014

Keywords:
DART–MS
Forensic
Bath salts
Designer drugs
Mass spectrometry
Collision induced dissociation

ABSTRACT

The influx of new psychoactive substances is a problem that is challenging the analytical capabilities of enforcement agencies. Cathinone designer drugs are less likely to be included in routine drug screens and typical drug formulations are commonly mixtures with continually shifting components. Ambient ionization mass spectrometry employs relatively mild conditions to desorb and ionize solid samples, imparting much less energy than that associated with conventional mass spectrometry methods. Direct analysis in real time mass spectrometry (DART–MS) is an ambient ionization method that was employed to rapidly screen cathinones, alone and in mixtures, readily enabling differentiation of the active drug(s) from various cutting agents. Accurate mass determinations provided preliminary identification of the various components of drug mixtures. The data generated in forensic mass spectrometry can be used for both elemental composition formulations and isotope abundance calculations for determination of unknown psychoactive substances, and we demonstrate how this data could be applied to the presence of new drugs as the active components shift in response to regulations. Isotope abundance calculations were used to develop a candidate pool of possible molecular formulas associated with cathinones as a specific class of designer drugs. Together, the combination of a time-of-flight (TOF) mass analyzer along with in-source collision-induced dissociation (CID) spectra were used to drastically narrow the pool of candidates to a single molecular formula. The $[M+H]^+$ and product ion peaks provided data for presumptive analysis of various substituted synthetic cathinones in a manner that is complementary to conventional GC–MS analysis of new psychoactive substances.

1. Introduction

A series of new psychoactive substances are now being manufactured and sold as alternatives to compounds such as ecstasy, methamphetamine, and marijuana [1–6]. One class of these designer drugs is cathinone "bath salts", which have a core β-ketophenethylamine structure upon which various substituents are appended to create novel variants purposefully designed to circumvent legal restrictions, while retaining psychoactive properties. Slight chemical modifications in the core structure take advantage of the vagueness of current controlled substance analog laws, which leads to the "marketing" of these compounds as legal alternatives to banned substances. These new substances are now widely available for sale on the Internet and have been linked to an increase in poison control center calls, emergency room visits, and fatalities. Recent research assessing the effects of a particular cathinone derivative methylenedioxypyrovalerone (MDPV), suggest that it has significantly greater potency than methamphetamine, poses a higher risk of abuse, and is more likely to have long-term toxicity effects or be fatal [7]. Contributing to the incidence of overdoses is that these substances are oftentimes found as mixtures of multiple cathinones, with ever changing formulations of the active ingredients [8–12]. The shifting of active component formulations, their varying concentration and purity, and the continual emergence of new variants all contribute to this problem. Although legislation has been continually modified to address the constant influx of new cathinone variants, controlling the access and abuse of these drugs remains a difficult challenge.

Current rapid, preliminary testing methods for cathinones are limited, with conventional color tests or immunoassays not fully

* Corresponding author. Tel.: +1 011 518 442 3634; fax: +1 518 442 3462.
E-mail address: jshepard@albany.edu (Jason R.E. Shepard).

http://dx.doi.org/10.1016/j.forsciint.2014.07.028
0379-0738/

developed or with limited effectiveness across this structural class of molecules, analogs, and emerging variants [8,13–16]. Furthermore, confirmatory testing techniques employed for detecting cathinones center around conventional GC–MS, which also can be problematic for a number of reasons [8,13,16]. Specifically, mass spectral fragmentation patterns of cathinones from GC–MS are of limited utility for the purposes of molecular characterization, as they often exhibit extensive fragmentation with weak or even absent parent peaks [10,17–19]. Generally, this extensive fragmentation means that mass spectra across this class of compounds are similar enough to impede the ability to distinguish between cathinones. Ultimately, the limitations associated with conventional methods for cathinone testing and analysis, the inclusion of multiple cathinones or adulterants in a single product, and the constantly changing ingredient profiles, all contribute to the sample testing backlogs that are a growing problem for U.S. crime labs and enforcement agencies [18,20]. With the continual expanding and changing field of designer drug abuse, high throughput, informative methods are needed to keep pace with ever increasing casework.

Advanced mass spectrometry techniques, specifically those employing higher resolution and high mass accuracy measurements, have recently gained traction in part because of their potential to help identify new psychoactive substances for which no reference standards are available, as well as their ability to compensate for some of the limitations associated with more conventional analytical techniques [21–23]. High mass accuracy measurements can be used to drastically narrow the list of potential candidate formulas used in drug class determination or identification of an unknown. In the analysis of suspected designer drug samples, the data provided by these high resolution methods can be searched against NIST or the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) libraries to further narrow the candidate pools or confirm an unknown substance's potential place within a drug class [24]. Once this preliminary information is established, it allows for further, more directed confirmatory analyses. An added advantage of these methods is that the information they yield is more detailed and/or complementary to that provided by conventional GC–MS methods. As compared to techniques such as immunoassay screening which provide information about the general chemical class of a drug that is present, methods such as high resolution DART–TOF–MS, when used as a preliminary screening tool, are not only just as rapid, but also provide high resolution $[M + H]^+$ and fragment ion information that is often not obtainable by the routine GC–MS protocols most often employed in crime labs.

In addition, high resolution data can be obtained using more recently developed ambient MS methods. Several ambient ionization methods, which include direct analysis in real time (DART), desorption electrospray ionization (DESI), and desorption atmospheric pressure photoionization (DAPPI), have demonstrated utility in forensic drug analysis applications, and have the added advantage that the analyses are instantaneous and performed directly on the solid or liquid sample [18,25–30]. In previous work, DART–MS was applied to cathinone analysis, demonstrating that DART–TOF–MS can be used to differentiate between structural isomers and closely related cathinone compounds [18]. However, although commercially available "street" samples can be found for sale as pure compounds, they are commonly observed to be combined with adulterants as binary mixtures or occasionally incorporated as mixtures of multiple cathinones [9,10,14]. Such street drugs are often "cut" with diluents to add bulk to the sample sold, thereby increasing profits for the drug dealer or manufacturer. Common cutting agents can include stimulants or anesthetics having their own central nervous system effects, such as benzocaine, lidocaine, and caffeine [10,20]. Indeed, work by Brandt and coworkers has shown that common cathinone mixture additives include these stimulants, among other compounds [9,10,14].

Herein, DART–TOF–MS was not only used to identify cathinones within mixtures containing common adulterants, but it was also demonstrated that this method can serve as a means to characterize the individual components within complex MS profiles of drug mixtures. In-source collision induced dissociation (CID) was employed to demonstrate that the high mass accuracy measurements of constituents of these mixtures can provide informative $[M + H]^+$ values and specific molecular formulas related to both $[M + H]^+$ and product ions. In this capacity, seized samples could be triaged using rapid high resolution DART–TOF–MS to provide definitive information on the presence of novel cathinone components as well as to indicate the presence of various cutting agents. Solid samples were ionized directly without solubilization, extraction, derivatization, or coupling to chromatographic methods, which greatly reduced analysis time while providing important information that cannot be gleaned from traditional preliminary or confirmatory screening methods. The rapidity of the method may serve not only as a means to manage the backlog of forensic drug cases, but may also promote more effective regulation and response to the rapidly evolving synthetic drug production and distribution pipeline.

2. Experimental

2.1. DART–MS sample ionization

A DART-SVP™ ion source (Ionsense, Saugus, MA, U.S.A.) was used for ionization, combined with an AccuTOF™ mass spectrometer (JEOL USA, Inc., Peabody, MA, U.S.A.) to acquire all mass spectra. Samples were tested as previously described. Briefly, solid materials were sampled directly by dipping the closed end of a capillary melting point tube in the solid material and holding the tube between the heated helium stream from the DART ion source and the inlet of the mass spectrometer [18]. Samples were either held in the correct position manually, or by the use of the Dipit-tubes™ system (Ionsense, Saugus, MA U.S.A.). The Dipit-tube system consists of a multi-sample rack that moves capillary tubes laterally while placing them in the optimal position for sampling [18,31]. The automated rack moves perpendicular to the flow of ionizing gas to enable optimal positioning of samples and permit analysis of multiple samples within a single assay. Dipit-tubes with cathinone samples were positioned 1.8 cm apart in the rack and transported laterally through the helium stream at a speed of 1.0 mm/s while acquiring spectra. DART–MS analysis is not susceptible to sample carryover or contamination between samples, and no carryover or contamination is observed in any of our spectra.

2.2. DART–MS parameters

An AccuTOF mass spectrometer was run in positive ion mode for all measurements, with a resolving power of 6000 (FWHM definition) as measured for protonated reserpine. Poly(ethylene glycol) (PEG; average MW 600; Sigma-Aldrich, St. Louis, MO, U.S.A.) was measured with each data acquisition as a reference standard for exact mass determinations. Orifice 1 was varied from 20, 30, 60, and 90 V, while orifice 2 was operated at 5 V, and the ring lens voltage was 3 V. The RF ion guide voltage was generally set to 600 V to allow detection of ions above m/z 60. The DART ion source was operated with helium gas (Ultra high purity; Airgas, Cambridge, MA, U.S.A.) at 300 °C, a flow rate of 2 L/min, and a grid voltage of 530 V. The mass range was 60–600 Da. TSSPro3 software (Shrader Analytical, Detroit, MI, U.S.A.) together with Mass Spec Tools (MSTools) programs (ChemSW Inc., Fairfield, CA, U.S.A.) were

used for data processing. Specifically, data were averaged, background subtracted, and centroided to produce spectra that were then calibrated to PEG reference masses.

2.3. Synthetic cathinones

3,4-Dimethylethcathinone, 2,3-methylenedioxymethcathione, and 3,4-methylenedioxy-*N*-benzylcathione (Fig. 1) were obtained from Cayman Chemical (Ann Arbor, MI, U.S.A.). The caffeine, lidocaine and benzocaine cutting agents were acquired from Sigma-Aldrich (St. Louis, MO, U.S.A.). The components of each mixture were in equal proportions by mass, and were within the ranges of percentage of active drug reported in the literature for seized samples [10,11,19,32].

2.4. High mass accuracy measurements

All DART spectra were obtained in function switching mode, which allows multiple measurements within a single data file. The ability to perform multiple measurements within a single data file allows for a PEG measurement that serves as a reference for exact mass measurements and mass calibration with each dataset. PEG peaks extend out over the mass range of interest (600 Da), and the PEG mass spectrum is fitted with fourth degree polynomial order, with *R*-values below 1×10^{-10} to ensure all measurements are within the desired mass tolerance. Slight differences in relative abundance values for peaks that appear in both the spectra of pure cathinones as well as in the mixtures are a consequence of differences between desorption and ionization of the pure substance versus that of the mixture. Comparison of measured parent $[M + H]^+$ and product ions to calculated masses, formulas, and library comparisons, as well as the structural formula determinations for each sample shown in Table 5 were done with MSTools software (ChemSW, Inc., Fairfield, CA, U.S.A.). This program was used to make molecular formula determinations with a rank score within user-defined search restrictions. The score/ranking is based on the root-mean-square (RMS) mass accuracy values for all defined isotopes within a given tolerance, and it produces a match comparison between the calculated and theoretical values, among others. In this work, a mass tolerance of ±0.005 Da was used, along with saturation levels between −1 and 10, and elemental composition assessment parameters for the following elements: carbon (0–50); hydrogen (0–100); oxygen (0–10); and nitrogen (0–10). The output provides the difference between the measured *m*/*z* and the calculated exact mass values, as well as the number of elements of unsaturation corresponding to each candidate formula.

3. Results and discussion

The coupling of the Dipit-tube™ system with the DART–TOF–MS enabled semi-automated analysis that allowed samples, standards, and calibration standard spectra to be collected uniformly within a single data acquisition, without any concern for sample carryover or contamination [18,28,31]. Solid samples and standards were tested directly without the need for solubilization or other pre-analysis steps typically required for GC–MS. DART–MS spectra were obtained for pure cathinones, single cathinones mixed with adulterants/cutting agents, and multi-cathinone mixtures combined with cutting agents. All cathinones analyzed by DART–MS have been either found in illicit samples or are closely related in structure to cathinones such as methylone, that have been confiscated in drug seizures (Fig. 1) [10,11,19,32]. The DART–MS spectrum of 3,4-dimethylethcathinone (3,4-DMEC; Fig. 1a) is shown in Fig. 2a. The spectrum shows the expected single peak representative of the parent $[M + H]^+$ typical of the DART–MS soft ionization method, with high mass accuracy values within the instrument specifications (Table 1). The occurrence of the parent $[M + H]^+$ is a critical aspect of preliminary/presumptive analysis that is often absent in the GC–MS spectra of cathinones, making definitive mass spectrometry identifications problematic when using the latter method [10,15,17,18,33]. Binary

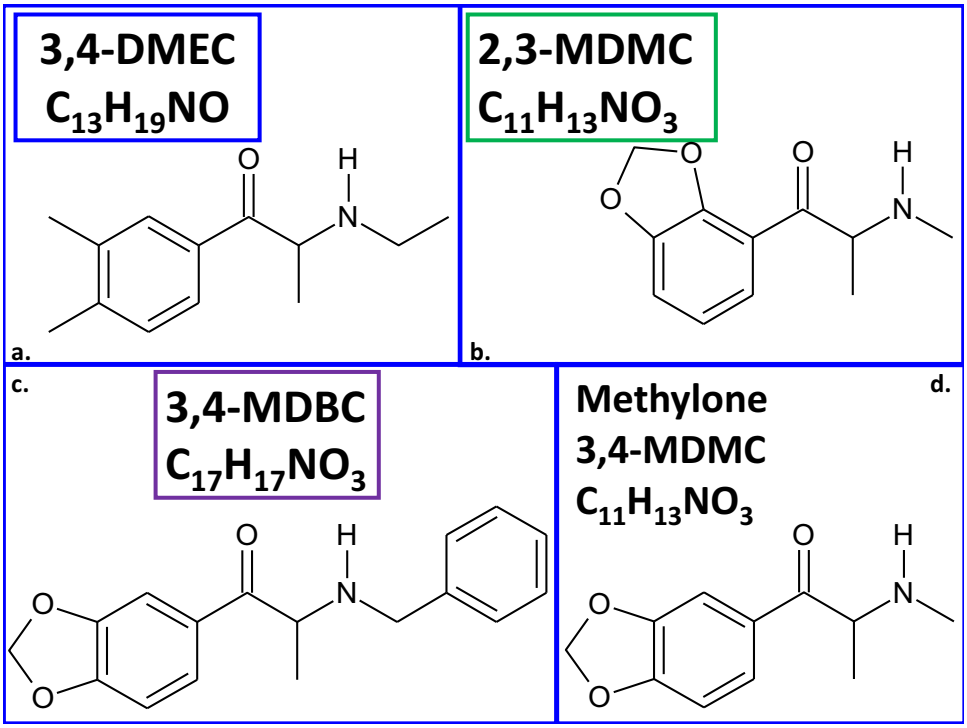

Fig. 1. The chemical structures and formulas of four cathinones. Panel (a) 3,4-dimethylethcathinone (3,4-DMEC); panel (b) 2,3-methylenedioxymethcathinone (2,3-MDMC); panel (c) 3,4-methylenedioxy-*N*-benzylcathinone (3,4-MDBC); and panel (d) methylone (3,4-MDMC).

Table 1
DART–TOF–MS data of the various cathinones, cutting agents, and mixtures shown in Figs. 2 and 3.

Standards (20 V)[a]	$[M+H]^+$	Calculated *m/z*	Measured *m/z*
3,4-Dimethylethcathinone	$C_{13}H_{20}NO$	206.1545	206.1543
Caffeine	$C_8H_{11}N_4O_2$	195.0882	195.0860
Mixtures (20 V)[a]	$[M+H]^+$	Calculated Mass	Measured *m/z*
3,4-Dimethylethcathinone	$C_{13}H_{20}NO$	206.1545	206.1524
Benzocaine	$C_9H_{12}NO_2$	166.0868	166.0872
3,4-Dimethylethcathinone	$C_{13}H_{20}NO$	206.1545	206.1530
Lidocaine	$C_{14}H_{23}N_2O$	235.1810	235.1775
3,4-Dimethylethcathinone	$C_{13}H_{20}NO$	206.1545	206.1555
Caffeine	$C_8H_{11}N_4O_2$	195.0882	195.0889
Standards (60 V)[b]	Formula	Calculated mass	Measured *m/z*
3,4-Dimethylethcathinone	$C_{13}H_{20}NO[M+H]^+$	206.1545	206.1515
	$C_{13}H_{18}N$	188.1439	188.1401
	$C_{12}H_{15}N$	173.1204	173.1206
	$C_{11}H_{14}N$	160.1126	160.1131
	$C_{10}H_{13}$	133.1014	133.1021
Caffeine	$C_8H_{11}N_4O_2[M+H]^+$	195.0882	195.0865
	$C_6H_8N_3O$	138.0667	138.0658
	$C_5H_6N_3O$	124.0511	124.0497
	$C_5H_8N_3O$	110.0718	110.0710

[a] Data were collected under soft ionization conditions (i.e. orifice 1 voltage = 20 V), resulting in little to no fragmentation.
[b] Data were collected under CID conditions (i.e. orifice 1 voltage = 60 V), which induced fragmentation while at the same time preserving the appearance of the $[M+H]^+$.

mixtures of the cathinone 3,4-DMEC and different adulterants, either benzocaine, lidocaine, or caffeine, were analyzed (Fig. 2b, c, and d, respectively, and Table 1). In each spectrum of a binary mixture, two peaks representative of the cathinone and its diluent were observed. As expected, because of the use of soft ionization conditions, no fragmentation occurred. However, more in-depth characterization of drug mixtures can be performed under in-source CID conditions. For comparison, in-source CID spectra were obtained for both 3,4-DMEC (Fig. 3a) and caffeine (Fig. 3b, Table 1). The two spectra are labeled with the $[M+H]^+$ and major product ions, with each molecular formula determined from the high mass accuracy values associated with each product ion. The mixture of these two substances was also analyzed under CID conditions (Fig. 3c, Table 2). While the CID spectrum of the mixture shows added complexity based on the simultaneous fragmentation of both the cathinone and caffeine, the high mass accuracy data of the $[M+H]^+$ and the product ions are perfectly aligned with the CID spectra of the two components of the mixture tested

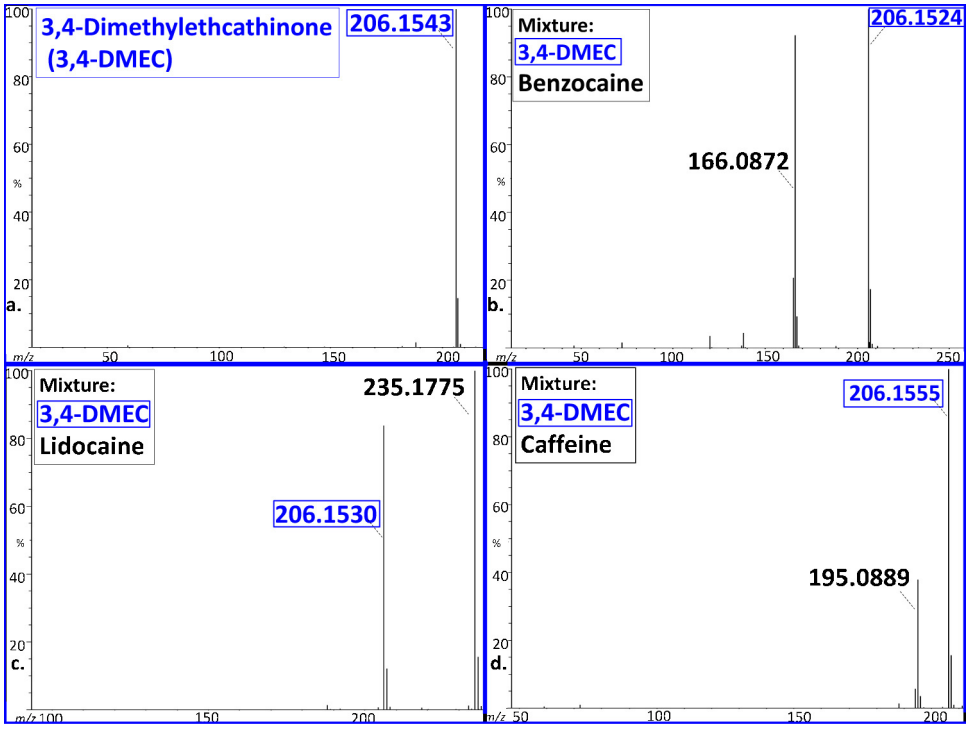

Fig. 2. DART-MS spectra of 3,4-dimethylethcathinone (3,4-DMEC) and mixtures of 3,4-DMEC with cutting agents acquired using an orifice 1 voltage of 20 V. Panel (a) DART–MS spectrum of 3,4-DMEC; panel (b) DART–MS spectrum of a mixture of 3,4-DMEC and benzocaine; panel (c) DART–MS spectrum of a mixture of 3,4-DMEC and lidocaine; and Panel (d) DART–MS spectrum of a mixture of 3,4-DMEC and caffeine. The $[M+H]^+$ peak(s) was/were readily apparent in each case, as a consequence of the soft ionization conditions. The data associated with the measured masses are shown in Table 1.

Table 2
DART–MS CID data used to identify 3,4-dimethylethcathinone (3,4-DMEC) and the cutting agent caffeine in the mixture (from the spectrum in Fig. 2c). Entries highlighted in blue indicate masses unique to 3,4-DMEC and the observation of which supported the presence of that substance in the mixture. Masses unique to the presence of caffeine in the mixture are not shaded.

Mixture					3,4-DMEC		Caffeine	
Measured (m/z)	Calculated (m/z)	Difference	Formula	Relative abundance	Measured (m/z)	Relative abundance	Measured (m/z)	Relative abundance
109.0634	109.0653	0.0019	C_7H_9O	1.9	–	–	109.0685	7.9
110.0704	110.0718	0.0014	$C_5H_8N_3$	3.2	–	–	110.0710	11.7
124.0498	124.0511	0.0013	$C_5H_6N_3O$	1.2	–	–	124.0497	4.4
133.0994	133.1017	0.0023	$C_{10}H_{13}$	6.7	133.1021	5.3	–	–
137.0562	137.0603	0.0041	$C_8H_9O_2$	1.4	–	–	137.0646	5.2
138.0659	138.0667	0.0008	$C_6H_8N_3O$	18.8	–	–	138.0658	76.2
159.1028	159.1048	0.0020	$C_8H_{15}O_3$	22.0	159.1037	16.1	–	–
160.1123	160.1126	0.0003	$C_{11}H_{14}N$	25.9	160.1131	19.8	–	–
161.0993	161.0966	−0.0027	$C_{11}H_{13}O$	19.9	161.0969	16.9	–	–
173.1206	173.1204	−0.0002	$C_{12}H_{15}N$	17.8	173.1206	13.3	–	–
174.1237	174.1283	0.0046	$C_{12}H_{16}N$	2.8	174.1238	2.2	–	–
188.1406	188.1439	0.0033	$C_{13}H_{18}N$	100.0	188.1401	100.0	–	–
189.1478	189.1517	0.0039	$C_{13}H_{19}N$	32.3	189.1471	28.2	–	–
195.0892	195.0882	−0.0010	$C_8H_{11}N_4O_2$	66.6	–	–	195.0865	100.0
206.1530	206.1545	0.0015	$C_{13}H_{20}NO$	82.7	206.1515	80.0	–	–

Differences in relative abundance values for peaks that appear in both the spectrum of the pure cathinones as well as in the mixture, are a consequence of differences between desorption and ionization of the pure substance versus that of the mixture.

separately, permitting confirmation of the presence of both species. It is apparent from the CID spectra that there is no overlap of the major peaks contributed by the two compounds.

For comparison, the chemical structures of four cathinones are shown in Fig. 1, illustrating the structural similarities within this class of abused drug. The variations between the structures of

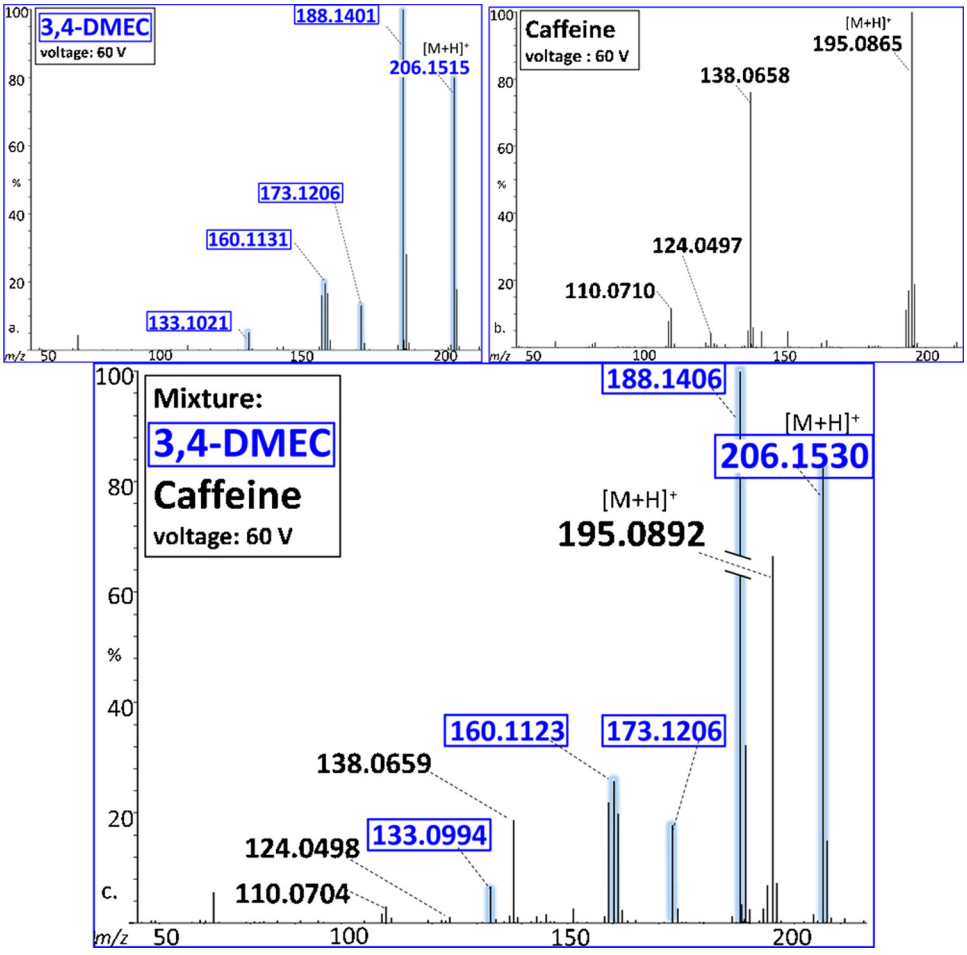

Fig. 3. DART–MS spectra of the cathinone 3,4-dimethylethcathinone (3,4-DMEC), the cutting agent caffeine, and a mixture of the two compounds. The spectra show in-source collision-induced dissociation (CID) at 60 V. Panel (a) DART–MS CID spectrum of 3,4-DMEC; panel (b) DART–MS CID spectrum of caffeine; panel (c) DART–MS CID spectrum of 3,4-DMEC mixed with caffeine. The $[M + H]^+$ peaks associated with the cathinone and the caffeine were readily apparent in the mixture. All relevant CID peaks for 3,4-DMEC are also apparent in the mixture spectrum. The measured masses associated with the spectral data are shown in Tables 1 and 2.

these cathinones is informative, as each cathinone features the typical β-ketophenethylamine core scaffold, but with varying substituents at different positions within the molecular framework. These substituents are indicative of how chemical modifications are introduced by manufacturers to create analogs or variants that evade detection, circumvent legal regulations, and/or complicate sample analysis. Methylone (3,4-MDMC, Fig. 1d), is a widely abused cathinone commonly found as an active ingredient in seized bath salt mixtures [14,34,35]. The other three cathinones are methylone structural variants (i.e. 3,4-DMEC, 2,3-methylenedioxymethcathinone (2,3-MDMC), and 3,4-methylenedioxy-*N*-benzylcathinone (3,4-MDBC), shown in Fig. 1a, b, and c, respectively). In relation to the structure of methylone, 3,4-DMEC has a dimethyl substitution in place of the 1,3-dioxole ring, the 2,3-MDMC has a shift of the methylenedioxy ring from the 3,4-position to the 2,3-position, and the 3,4-MDBC has an *N*-benzyl rather than a methyl substituent. DART CID spectra of 2,3-MDMC and 3,4-MDBC are shown in Fig. 4a and b, respectively. Importantly, since the formula weights of these cathinones are different, they would be readily distinguished via DART–MS based on their prominent $[M + H]^+$ peaks and the associated high mass accuracy data. Furthermore, the CID spectra of these cathinones each exhibit 5–6 prominent product ion peaks that, along with the high mass accuracy measurements, support their tentative identification and demonstrate this technique as a rapid, efficient, informative means to distinguish between them.

As mixtures comprised of multiple cathinones have also been observed in samples seized by enforcement agencies [9,10,20], various combinations of these cathinones and adulterants were also tested via DART–MS. Although it was previously demonstrated that cathinone isomers exhibit similar fragmentation patterns [18], the DART CID spectra in Fig. 4 demonstrate that their structural dissimilarity is also enough to result in product ions that can be readily distinguished despite their presence within more complex mixtures. The two cathinones 2,3-MDMC and 3,4-MDBC were separately added in equal proportions by mass to the 3,4-DMEC/caffeine mixture from which Fig. 3c was derived. The DART–MS CID mixture spectrum of 2,3-MDMC, 3,4-DMEC, and caffeine (Fig. 4c) shows the three corresponding $[M + H]^+$ values and the accompanying product ion peaks from the mixture. A comparison between the CID spectra of 2,3-MDMC alone (Fig. 4a) and 2,3-MDMC in the mixture (Fig. 4c) illustrates that both the $[M + H]^+$ peaks and product ion peaks characteristic of the 2,3-MDMC CID spectrum remain discernible and prominent despite the added complexity of the mixture spectrum (relevant data in Table 3). Similarly, the DART–MS CID spectrum of the mixture of 3,4-MDBC, 3,4-DMEC, and caffeine shows uniqueness at the level of both the $[M + H]^+$ and the product ion peaks, making identification of 3,4-MDBC relatively straightforward upon comparison of its spectrum alone (Fig. 4b) to that of the mixture (Fig. 4d). The data identifying the $[M + H]^+$ values and the key product ion peaks, along with the high mass accuracy values are shown in Table 4.

4. High mass accuracy for preliminary determination of unknowns

Obtaining specific elemental formula data on unknowns would provide valuable information for the chemical analysis of unknowns, particularly when reference standards are not available for comparison, which is often the case. The low voltage DART–MS spectra can be employed for the determination of the number of components in the mixture. Importantly, the high mass accuracy data imparts a significant amount of information in-and-of itself, in terms of the classes of compounds present, while the CID spectra provide more chemical detail and structural confirmation. The ability to rapidly obtain such information can serve to triage an unknown white powder presumed to be a new psychoactive substance toward more directed confirmatory methods. For example, consider a situation where the three cathinones 3,4-DMEC, 2,3-MDMC, and 3,4-MDBC each were present as unknowns. The measured $[M + H]^+$ value of 206.1530 (3,4-DMEC) when

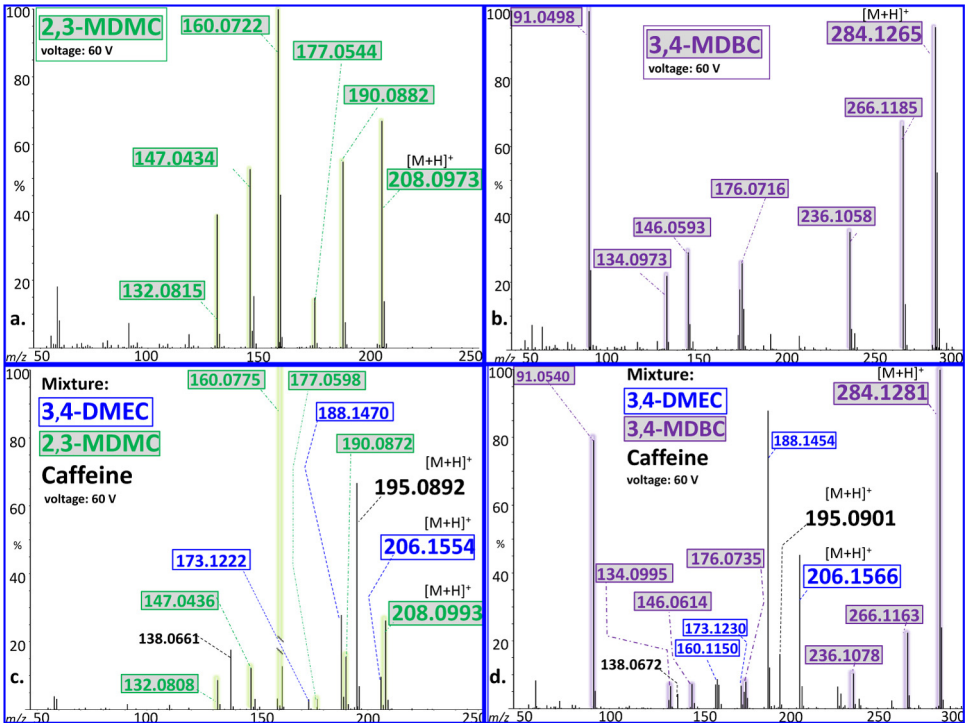

Fig. 4. DART–MS CID spectra of the two synthetic cathinones 2,3-MDMC and 3,4-MDBC alone and in mixtures. Panel (a) DART–MS CID spectrum of 2,3-MDMC; panel (b) DART–MS CID spectrum of 3,4-MDBC; panel (c) a mixture of the two cathinones 3,4-DMEC, 2,3-MDMC, and the adulterant caffeine; panel (d) a mixture of the two cathinones 3,4-DMEC, 3,4-MDBC, and the adulterant caffeine. The mass spectral data for these mixtures are shown in Tables 3 and 4.

Table 3
DART–MS CID data used to identify the two cathinones in the mixture whose spectrum is shown in Fig. 4c. Entries from Fig. 4 highlighted in blue indicate masses unique to 3,4-dimethylethcathinone (3,4-DMEC) and the observation of which supported the presence of that substance in the mixture, while entries from Fig. 4 highlighted in green indicate masses unique to 2,3-methylenedioxymethcathinone (2,3-MDMC). Entries in Fig. 4 shown in white indicate peaks for caffeine.

Mixture					3,4-DMEC		2,3-MDBC	
Measured (m/z)	Calculated (m/z)	Difference	Formula	Relative abundance	Measured (m/z)	Relative abundance	Measured (m/z)	Relative abundance
132.0808	132.0813	0.0005	$C_9H_{10}N$	8.8	–	–	132.0815	39.6
138.0661	138.0667	0.0006	$C_6H_8N_3O$	17.9	–	–	–	–
147.0436	147.0446	0.0010	$C_9H_7O_2$	12.4	–	–	147.0434	52.9
160.0775	160.0762	−0.0013	$C_{10}H_{10}NO$	100.0	–	–	160.0722	100.0
173.1222	173.1204	−0.0018	$C_{12}H_{15}N$	3.2	173.1206	13.3	–	–
177.0598	177.0551	−0.0047	$C_{10}H_9O_3$	3.3	–	–	177.0544	15.0
188.1470	188.1439	−0.0031	$C_{12}H_{17}N$	28.0	188.1406	100.0	–	–
190.0872	190.0868	−0.0004	$C_{11}H_{12}NO_2$	15.6	–	–	190.0882	55.1
195.0892	195.0882	−0.0010	$C_{13}H_{19}N$	67.3	–	–	–	–
206.1554	206.1545	−0.0009	$C_{12}H_{18}NO$	9.8	206.1530	80.0	–	–
208.0993	208.0974	−0.0019	$C_{11}H_{17}NO_3$	26.4	206.1515	80.0	208.67.1	67.1

Differences in relative abundance values for peaks that appear in both the spectrum of the pure cathinones as well as in the mixture, are a consequence of differences between desorption and ionization of the pure substance versus that of the mixture.

Table 4
DART–MS CID data used to identify the two cathinones in the mixture whose spectrum is shown in Fig. 4d. Entries from Fig. 4d highlighted in blue indicate masses unique to 3,4-dimethylethcathinone (3,4-DMEC) and the observation of which supported the presence of that substance in the mixture, while entries from Fig. 4d highlighted in purple indicate masses unique to 3,4-methylenedioxy-*N*-benzylcathinone (3,4-MDBC). Entries in white indicate peaks for caffeine.

Mixture					3,4-DMEC		3,4-MDBC	
Measured (m/z)	Calculated (m/z)	Difference	Formula	Relative abundance	Measured (m/z)	Relative abundance	Measured (m/z)	Relative abundance
91.0540	91.0547	0.0007	C_7H_7	79.0	–	–	91.0498	100.0
134.0995	134.0970	−0.0025	$C_9H_{12}N$	6.7	–	–	134.0973	21.7
138.0672	138.0667	−0.0005	$C_6H_8N_3O$	4.4	–	–	–	–
146.0614	146.0606	−0.0008	C_9H_8NO	7.4	–	–	146.0593	28.5
160.1150	160.1126	−0.0024	$C_{11}H_{14}N$	8.7	160.1131	19.8	–	–
173.1230	173.1204	−0.0026	$C_{12}H_{15}N$	6.8	173.1206	13.3	–	–
176.0735	176.0712	−0.0023	$C_{10}H_{10}NO_2$	7.9	–	–	176.0716	25.4
188.1454	188.1439	−0.0015	$C_{13}H_{18}N$	87.8	188.1401	100.0	–	–
195.0901	195.0882	−0.0019	$C_8H_{11}N_4O_2$	16.4	–	–	–	–
206.1566	206.1545	−0.0021	$C_{13}H_{20}NO$	45.3	206.1530	80.0	–	–
236.1078	236.1075	−0.0003	$C_{16}H_{14}NO$	10.4	–	–	236.1058	34.5
266.1163	266.1181	0.0018	$C_{17}H_{16}NO_2$	22.5	–	–	266.1185	65.3
284.1284	284.1287	0.0003	$C_{17}H_{18}NO_3$	100.0	–	–	284.1265	94.5

Differences in relative abundance values for peaks that appear in both the spectrum of the pure cathinones as well as in the mixture, are a consequence of differences between desorption and ionization of the pure substance versus that of the mixture.

combined with elemental abundance profiling results in two candidate formulas: $C_8H_{20}N_3O_3$ and $C_{13}N_{20}NO$. If a cathinone is suspected, the first formula does not have a sufficient number of carbons to correspond to the core β-ketophenethylamine structure or the correct level of unsaturation, but the second formula meets both these criteria and is the correct formula of the cathinone (Table 5). The same process can be applied to the 2,3-MDMC (observed $[M+H]^+$ of 208.0993), where an elemental composition assessment also results in the two candidate formulas $C_7H_{10}N_7O$ and $C_{11}H_{14}NO_3$, the second of which is the correct formula (Table 5). Again, the C_7 candidate formula does not accommodate the core β-ketophenethylamine backbone associated with cathinones, while the C_{11} candidate meets this structural criterion. Finally, the elemental composition determination of 3,4-MDBC, (observed $[M+H]^+$ of 284.1281), yields four possible candidate formula weights (Table 5), with the larger number of candidates being a consequence of its increased mass. The first three candidate formulas ($C_6H_{18}N_7O_6$, $C_{12}H_{18}N_3O_5$, and $C_{13}H_{14}N_7O$) are again non-viable as potential cathinones, and the fourth candidate formula is correct. Together, the TOF mass measurements and the CID fragmentation provide key information not available with GC–MS. Ultimately, for analysis of the multitude and increasingly varied number of cathinones, amphetamines, and other related new

Table 5
Elemental composition candidate search results based on the high mass accuracy $[M+H]^+$ m/z values from Figs. 2c and 4c and d. Provided in the output are the differences between the measured m/z and the calculated values, as well as the number of elements of unsaturation corresponding to each formula. In each case, the correct molecular formula is shaded in green.

Calculated m/z	Difference	Unsaturation	Composition
Elemental composition, m/z 206.1530			
206.1505	0.0025	0.5	$C_8H_{20}N_3O_3$
206.1545	−0.0015	4.5	$C_{13}H_{20}NO$
Elemental composition, m/z 208.0993			
208.0945	0.0046	6.5	$C_7H_{10}N_7O$
208.0974	0.0019	5.5	$C_{11}H_{14}NO_3$
Elemental composition, m/z 284.1281			
284.1314	−0.0038	1.5	$C_6H_{18}N_7O_6$
284.1246	0.0035	5.5	$C_{12}H_{18}N_3O_5$
284.1260	0.0021	10.5	$C_{13}H_{14}N_7O$
284.1287	−0.0006	9.5	$C_{17}H_{18}NO_3$

Search restrictions used in the molecular formula determinations included a ±0.005 Da tolerance. Elemental composition assessment parameters included the following upper limits for the indicated elements: carbon (0–50); hydrogen (0–100); oxygen (0–10); and nitrogen (0–10). Saturation levels between −1 and 10 were also used.

psychoactive substances, the high mass accuracy, high information content provided in these TOF measurements is not available with low resolution GC–MS instrumentation. Because analysis by both GC–MS and DART–TOF–MS yields important complementary information, a more comprehensive and thus more complete dataset that enhances the ability to more definitively characterize unknowns is made possible. Conceptually, this idea of employing two MS techniques for analysis can be likened to the complementary information furnished by the combined use of ^{1}H and ^{13}C NMR. Many of the known cathinone analogs are isomers which vary only in the relative position of the aromatic ring substituent, such as is the case for 2,3-MDMC and 3,4-MDMC. Therefore, the combination of GC–MS, which would yield isomer retention time and minimal structural information [35,36], and DART–TOF–MS with CID, which would provide detailed structural information including both the molecular ion and product fragments with high mass accuracy, would be extremely powerful in providing a complete picture of the constituents of a sample of interest.

5. Conclusions

Rapid, preliminary analysis of bath salt designer drugs contained within mixtures that include cathinones and cutting agents is demonstrated using DART–MS and the high mass accuracy of a TOF mass analyzer. This ambient ionization method allows rapid analysis without the sample pre-preparation or derivatization steps often required for analysis by conventional mass spectrometry methods, and the high mass accuracy narrows possible drug candidate molecular formulas to provide presumptive positive analysis, which can signify the need for additional testing directed at specific drug classes. This methodology has the potential to reduce sample testing backlogs associated with cathinone bath salts, streamline processing for more efficient use of time, and ultimately conserve resources for identification of new and emerging designer drugs.

Acknowledgements

The authors gratefully acknowledge the University at Albany, State University of New York, USA, for financial support of this project. This work was also funded in part by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice Award no. 2013-DN-BX-K041. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

References

[1] S. Dresen, N. Ferreiros, M. Putz, F. Westphal, R. Zimmermann, V. Auwarrter, Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds, J. Mass Spectrom. 45 (2010) 1186–1194.
[2] L. Fattore, W. Fratta, Beyond THC: the new generation of cannabinoid designer drugs, Front. Behav. Neurosci. 5 (2011) 1–12.
[3] J.M. Prosser, L.S. Nelson, The toxicology of Bath Salts: a review of synthetic cathinones, J. Med. Toxicol. 8 (2011) 33–42.
[4] C.D. Rosenbaum, S.P. Carreiro, K.M. Babu, Here today, gone tomorrow. . .and back again? A review of herbal Marijuana alternatives (K2, Spice), synthetic cathinones (bath salts), kratom, *Salvia divinorum*, methoxetamine, and piperazines, J. Med. Toxicol. 8 (2012) 15–32.
[5] N. Uchiyama, R. Kikura-Hanajiri, J. Ogata, Y. Goda, Chemical analysis of synthetic cannabinoids as designer drugs in herbal products, Forensic Sci. Int. 198 (2010) 31–38.
[6] I. Vardakou, C. Pistos, C. Spiliopoulou, Spice drugs as a new trend: mode of action, identification and legislation, Toxicol. Lett. 197 (2010) 157–162.
[7] S.M. Aarde, P.K. Huang, K.M. Creehan, T.J. Dickerson, M.A. Taffe, The novel recreational drug 3,4-methylenedioxypyrovalerone (MDPV) is a potent psychomotor stimulant: self-administration and locomotor activity in rats, Neuropharmacology 71 (2013) 130–140.
[8] M. Aromatario, E. Bottoni, M. Santoni, C. Ciallella, New "lethal highs": a case of a deadly cocktail of GHB and mephedrone, Forensic Sci. Int. 223 (2012) e38–e41.
[9] S.D. Brandt, S. Freeman, H.R. Sumnall, F. Measham, J. Cole, Analysis of NRG 'legal highs' in the UK: identification and formation of novel cathinones, Drug Test. Anal. 3 (2011) 569–575.
[10] S.D. Brandt, H.R. Sumnall, F. Measham, J. Cole, Analyses of second-generation 'legal highs' in the UK: initial findings, Drug Test. Anal. 2 (2010) 377–382.
[11] S. Davies, D.M. Wood, G. Smith, J. Button, J. Ramsey, R. Archer, D.W. Holt, P.I. Dargan, Purchasing 'legal highs' on the Internet—is there consistency in what you get? QJM 103 (2010) 489–493.
[12] D. Zuba, Identification of cathinones and other active components of 'legal highs' by mass spectrometric methods, TrAC, Trends Anal. Chem. 32 (2012) 15–30.
[13] L. Lindsay, M.L. White, Herbal marijuana alternatives and bath salts—"barely legal" toxic highs, Clin. Pediatr. Emergency Med. 13 (2012) 283–291.
[14] J.M. Pearson, T.L. Hargraves, L.S. Hair, C.J. Massucci, C. Clinton Frazee, U. Garg, B.R. Pietak, Three fatal intoxications due to methylone, J. Anal. Toxicol. 36 (2012) 444–451.
[15] J.D. Power, S.D. McDermott, B. Talbot, J.E. O'Brien, P. Kavanagh, The analysis of amphetamine-like cathinone derivatives using positive electrospray ionization with in-source collision-induced dissociation, Rapid Commun. Mass Spectrom. 26 (2012) 2601–2611.
[16] E.A. Ross, G.M. Reisfield, M.C. Watson, C.W. Chronister, B.A. Goldberger, Psychoactive "bath salts" intoxication with methylenedioxypyrovalerone, Am. J. Med. 125 (2012) 854–858.
[17] R.P. Archer, Fluoromethcathinone, a new substance of abuse, Forensic Sci. Int. 185 (2009) 10–20.
[18] A.D. Lesiak, R.A. Musah, R.B. Cody, M.A. Domin, A.J. Dane, J.R.E. Shepard, Direct analysis in real time mass spectrometry (DART–MS) of "bath salt" cathinone drug mixtures, Analyst 138 (2013) 3424–3432.
[19] F. Westphal, T. Junge, U. Girreser, W. Greibl, C. Doering, Mass, NMR and IR spectroscopic characterization of pentedrone and pentylone and identification of their isocathinone by-products, Forensic Sci. Int. 217 (2012) 157–167.
[20] A.B. Hall, S.L. Coy, E.G. Nazarov, P. Vouros, Rapid separation and characterization of cocaine and cocaine cutting agents by differential mobility spectrometry–mass spectrometry, J. Forensic Sci. 57 (2012) 750–756.
[21] E. Fornal, A. Stachniuk, A. Wojtyla, LC–Q/TOF mass spectrometry data driven identification and spectroscopic characterisation of a new 3,4-methylenedioxy-*N*-benzyl cathinone (BMDP), J. Pharm. Biomed. Anal. 72 (2013) 139–144.
[22] A.H. Grange, G.W. Sovocool, Determination of elemental compositions by high resolution mass spectrometry without mass calibrants, Rapid Commun. Mass Spectrom. 13 (1999) 673–686.
[23] I. Ojanperä, M. Kolmonen, A. Pelander, Current use of high-resolution mass spectrometry in drug screening relevant to clinical and forensic toxicology and doping control, Anal. Bioanal. Chem. 403 (2012) 1203–1220.
[24] Scientific Working Group for the Analysis of Seized Drugs Recommendations. ⟨http://www.swgdrug.org/⟩, accessed on 3/1/2014.
[25] R.B. Cody, J.A. Laramee, H.D. Durst, Versatile new ion source for the analysis of materials in open air under ambient conditions, Anal. Chem. 77 (2005) 2297–2302.
[26] R.G. Cooks, Z. Ouyang, Z. Takats, J.M. Wiseman, Ambient mass spectrometry, Science 311 (2006) 1566–1570.
[27] T.J. Kauppila, V. Arvola, M. Haapala, J. Pól, L. Aalberg, V. Saarela, S. Franssila, T. Kotiaho, R. Kostiainen, Direct analysis of illicit drugs by desorption atmospheric pressure photoionization, Rapid Commun. Mass Spectrom. 22 (2008) 979–985.
[28] R.A. Musah, M.A. Domin, R.B. Cody, A.D. Lesiak, A. John Dane, J.R.E. Shepard, Direct analysis in real time mass spectrometry with collision-induced dissociation for structural analysis of synthetic cannabinoids, Rapid Commun. Mass Spectrom. 26 (2012) 2335–2342.
[29] R.R. Steiner, R.L. Larson, Validation of the direct analysis in real time source for use in forensic drug screening, J. Forensic Sci. 54 (2009) 617–622.
[30] A.D. Lesiak, K.J. Adams, M.A. Domin, C. Henck, J.R.E. Shepard, DART–MS for rapid, preliminary screening of urine for DMAA, Drug Test. Anal. 6 (2014) 788–796.
[31] S. Yu, E. Crawford, J. Tice, B. Musselman, J.-T. Wu, Bioanalysis without sample cleanup or chromatography: the evaluation and initial implementation of direct analysis in real time ionization mass spectrometry for the quantification of drugs in biological matrixes, Anal. Chem. 81 (2008) 193–202.
[32] S.D. McDermott, J.D. Power, P. Kavanagh, J. O'Brien, The analysis of substituted cathinones. Part 2: An investigation into the phenylacetone based isomers of 4-methylmethcathinone and *N*-ethylcathinone, Forensic Sci. Int. 212 (2011) 13–21.
[33] A.T. Navare, J.G. Mayoral, M. Nouzova, F.G. Noriega, F.M. Fernández, Rapid direct analysis in real time (DART) mass spectrometric detection of juvenile hormone III and its terpene precursors, Anal. Bioanal. Chem. 398 (2010) 3005–3013.
[34] S. Gibbons, M. Zloh, An analysis of the 'legal high' mephedrone, Bioorg. Med. Chem. Lett. 20 (2010) 4135–4139.
[35] P. Kavanagh, J. O'Brien, J. Fox, C. O'Donnell, R. Christie, J.D. Power, S.D. McDermott, The analysis of substituted cathinones. Part 3. Synthesis and characterisation of 2,3-methylenedioxy substituted cathinones, Forensic Sci. Int. 216 (2012) 19–28.
[36] S. Mohr, J.A. Weiß, J. Spreitz, M.G. Schmid, Chiral separation of new cathinone- and amphetamine-related designer drugs by gas chromatography–mass spectrometry using trifluoroacetyl-l-prolyl chloride as chiral derivatization reagent, J. Chromatogr. A 1269 (2012) 352–359.