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DART-MS for rapid, preliminary screening of urine for DMAA

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Dimethylamylamine (DMAA) is a sympathomimetic amine found in weight-loss/workout supplements or used as an appetite suppressant. DMAA is a stimulant that is banned by the World Anti-Doping Agency (WADA). Adverse health effects as well as fatalities have been implicated with its use. Direct analysis in real time mass spectrometry (DART-MS) is an ambient ionization method that was employed to rapidly identify the presence of DMAA in various samples without any extraction or preparations whatsoever. DMAA was first identified in supplements, sampled directly in their solid forms. Furthermore, DMAA was detected directly in urine over 48 h as a means of indicating recent abuse of the substance. DART-MS analysis is instantaneous, and coupled with the high mass accuracy associated with the time-of-flight mass analyzer, results in unequivocal identification of the presence of DMAA. These features demonstrate DART-MS as an attractive potential alternative screening method for the presence of drugs and medications or for toxicological investigations. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: direct analysis in real time mass spectrometry; DART-MS; DMAA; urine testing; natural supplements; collision induced dissociation

Introduction

Dimethylamylamine (4-methylhexan-2-amine, DMAA) is a stimulant commonly found in athletic training or pre-workout supplements. A number of commercially available products containing DMAA have been marketed as exercise boosters, promoting weight loss, or acting as an appetite inhibitor. DMAA is an aliphatic amine, with structural and pharmacological similarities to other sympathomimetic amines like tuaminoheptane (1-methylhexylamine). DMAA and tuaminoheptane are structural isomers, central nervous system stimulants, and are both included on professional and amateur sports prohibition lists such as the World Anti-Doping Agency (WADA).[1–7] DMAA was originally developed in the 1940s by Eli Lilly, who commercially advertised it as the nasal decongestant Forthane.[2,4,8–10] Although Forthane was later removed from the market, DMAA eventually resurfaced as a dietary supplement in the 2000s, not long after the US ban of ephedrine.[9,11–13] For DMAA to qualify as a natural supplement, a single study was used to claim that it originated from geranium extracts, but this study is now widely debunked and no substantive evidence exists that DMAA occurs in nature.[3,14] In fact, in a study by Zhang *et al*., the presence of DMAA was not established in multiple geranium products, and the stereoisomeric compositions of DMAA in synthetic standards and commercial supplements were indistinguishable, confirming that DMAA cannot be considered a dietary supplement as defined by the Dietary Supplement Health and Education Act.[14]

DMAA does not currently have any recognized medical use and the substance is banned or restricted in a number of countries. Since its introduction as a nutritional supplement, DMAA use has been controversial. DMAA was detected in multiple routine athletic drug testing programmes in which it is prohibited, resulting in the athletes being banned from competition,[3,8,9,14] More importantly, DMAA has been implicated as contributing to severe adverse effects and/or death on numerous occasions.[2,15–17] Because the nature of DMAA as a natural supplement is in question, in 2012 the US Food and Drug Administration (FDA) sent letters to supplement companies requesting detailed safety information.[18] Subsequently, supplement manufacturers have begun to change their formulations, advertising, and product labels. However, products containing DMAA are still commonly available in stores and over the Internet, and more recently, DMAA has been found as a component of abused drug formulations, in particular 'legal high' designer drug mixtures in the USA.[2,19] Because DMAA was identified as present in multiple non-traumatic deaths in the US military,[15] in early 2012 products containing DMAA were removed from military bases and their use was prohibited pending further testing on their health effects. In early 2013, the FDA issued a warning, listing the substance as dangerous and it is now illegal for military personnel to consume any product that contains DMAA.[20–22] Regardless, due to the association of DMAA with military fatalities, sports doping, and abused drug mixtures, testing by such agencies may need to specifically include it in routine screenings.

A common sequence in routine screenings is to perform a rapid, preliminary assay, such as an immunoassay, followed by confirmatory mass spectrometry methods. For example, it is documented that branches of the US military employ three tests to report a positive result in urine screening, including two

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preliminary immunoassays and one confirmatory test (gas chromatography–mass spectrometry (GC-MS)).[4] However, in terms of preliminary methods, unregulated sympathomimetic amines have triggered false positives for amphetamine immunoassay screening,[23–25] and DMAA in particular has been shown to cause false presumptive positive responses in urine, only to be correctly identified later by confirmatory MS methods.[4] With organizations like the US military and WADA performing hundreds of thousands to millions of immunoassays annually[4,26–28] and new substances continually being added to banned or controlled lists, rapid screening methods that can identify the presence of a broad spectrum of substances are of obvious value. Ambient MS methods have the potential to drastically simplify preliminary analyses by eliminating the need for sample preparations, while bolstering the information content by providing mass spectral data without requiring lengthy chromatography steps.[29–35] In this capacity, the rapidity of the test along with the high mass accuracy of the time-of-flight (TOF) analyzer would serve in a complementary fashion to traditional confirmatory GC-MS methods, which generally only provide integer mass information. Ambient methods combined with high accuracy instruments have demonstrated the potential to enhance analysis in sports doping, particularly in regards to emerging drugs.[6,36–43] Direct analysis in real time mass spectrometry (DART-MS) is an ambient ionization method that can test samples directly, streamlining testing of such large numbers of samples.[38,39,43] We demonstrate an ambient ionization method that provided instantaneous identification of DMAA directly in solid supplement formulations as well as in raw, unprocessed urine. DART-MS testing of urine has previously been demonstrated for amphetamines and over-the-counter medicines.[29,44] Herein, the high mass accuracy along with the fragmentation patterns provided by in-source collision induced dissociation (CID),[39,45–47] allowed differentiation from its banned isomer tuaminoheptane, as well as unequivocal identification of DMAA in urine over 48 h post-ingestion.

Solid nutritional supplements were tested directly as a solid powder or pill, without any preparation whatsoever, by simply holding the solid material between the ionization source and the inlet to the mass spectrometer (Figure 1). Urine samples were tested directly by DART-MS without any preparations to positively identify the presence of DMAA. For comparison, the raw urine samples were also processed using solid-phase extraction pipette tips prior to DART-MS analysis. DMAA was successfully detected in all sets of samples over two days, with extraction methods improving the signal intensity of the DMAA over background. Ultimately, DART-MS proved powerful in a rapid screening of DMAA directly in both solid supplements and urine samples, demonstrating the technique as a potential preliminary method of analysis of drugs, medications, pills, powders, and toxicology specimens.

Experimental

DART ionization

Positive ion mass spectra were acquired using a DART-SVP™ ion source (Ionsense, Saugus, MA, USA) in conjunction with an AccuTOF mass spectrometer (JEOL USA, Inc., Peabody, MA, USA). Powdered supplement samples were sampled directly by dipping the closed end of a melting point capillary into the solid sample and positioning the sample-coated tube between the DART ion source and the detector inlet. Supplements in pill form were simply held with tweezers in between the ion source and the inlet (Figure 1). Supplements in capsule form were opened to reveal the powdered supplement, which was tested as mentioned above. Raw urine samples or sample extracts were also sampled directly by dipping the closed end of a melting point capillary into the liquid sample and positioning the sample-coated tube between the DART ion source and the detector inlet.

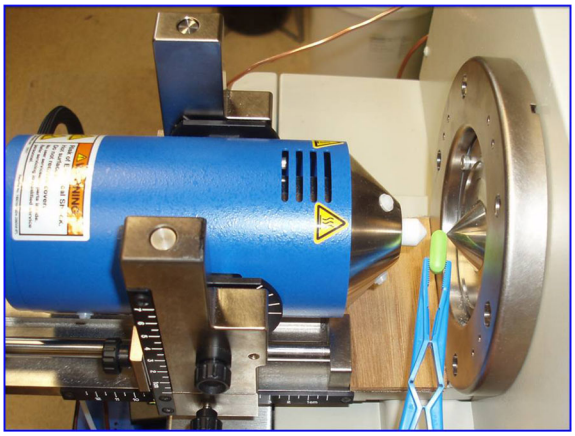

Figure 1. The utility of DART-MS analysis. Any type of samples (solid, liquid, or gas) can be tested by introducing the sample between the ion source (blue cylinder on left) and the spectrometer inlet (silver cone on right), without requiring any sample extraction or preparation. In terms of DMAA, we show DART-MS sampling of the supplement DNPX (a green pill held by tweezers), whose mass spectrum is shown in Figure 2a.

DART-MS parameters

An AccuTOF time-of-flight (TOF) mass spectrometer was used in positive ion mode for all mass measurements. The spectrometer resolving power was 6000 (FWHM definition), measured for protonated reserpine. A mass spectrum of poly(ethylene glycol) (PEG; Sigma, St Louis, MO, USA; average molecular weight 600) was obtained with each data acquisition. The PEG was set as an internal reference standard to enable exact mass measurements. All experiments were performed in duplicate, with the atmospheric pressure interface at orifice 1 operated at the following potentials: set at a constant value of 20 V or varied across 20, 30, 60, and 90 V to perform functional switching. The orifice 2 = 5 V, and ring lens = 3 V were constant for all measurements. The RF ion guide voltage was generally set to 400 V to allow detection of ions greater than *m/z* 40. All mass measurements were within the instrumentation specification of ±5 millimass (mmu) mass accuracy. The DART ion source was operated with helium gas (Airgas, Cambridge, MA, USA) at 350 °C, at a flow rate of 2 l/min, and a grid voltage of 530 V. TSSPro3 software (Shrader Analytical, Detroit, MI, USA) together with Mass Spec Tools programs (ChemSW Inc., Fairfield, CA, USA) were used for data processing. Data is presented in terms of relative abundance, based on the abundance relative to the base peak of each spectrum.

Urine testing

A healthy volunteer self-administered a nutritional supplement containing DMAA (Ripped Juice EX2, labelled as containing an undisclosed amount of Geranamine [1,3-dimethylpentylamine]). The supplement was tested via DART-MS to confirm the presence

of DMAA. A baseline urine sample was collected before initial administration and urine samples were collected at intervals after consumption of the supplement. Each urine sample was divided into 2-ml aliquots and stored without additives at −20 °C until analysis. Identical aliquots of urine samples were analyzed qualitatively in duplicate via three methods: (1) raw urine tested directly; (2) sample preparation using DPX WAX-1 tips following the manufacturer's protocol, starting with 400 μl urine; and (3) liquid-liquid extraction of urine with dichloromethane, starting with 2 ml urine concentrated into 1 ml final volume dichloromethane (DCM).

GC-MS parameters

Where applicable, sample analysis was confirmed by GC-MS (data not shown). An HP 6890 Series gas chromatograph equipped with an HP-5 30 m x 0.25 mm x 0.25 μm analytical column and helium as a carrier gas (2.0 ml/min; constant flow mode) was employed. The temperature was held at 40 °C for 1 min, ramped at 25 °C/min to 310 °C, and then held for 1.5 min. The GC was coupled to an HP 5972A mass selective detector in electron ionization (EI) mode at 70 eV. The transfer line was set at 310 °C. The acquisition range was *m/z* 40–400. Aliquots of urine samples (2 ml) were treated with 200 μl 5 M KOH and 200 mg of NaCl, and extracted with 1 ml of DCM. The samples were derivatized with 20 μl of cyclohexanone and allowed to evaporate to dryness. Samples were reconstituted in 200 μl of DCM and analyzed by GC-MS.

Materials

All supplements were purchased from local stores or from online vendors. DMAA was purchased from Cayman Chemical (Ann Arbor, MI, USA). Tuaminoheptane, urea, and creatinine standards were purchased from Sigma (St Louis, MO, USA). Synthetic urine was purchased from BioRad (Hercules, CA, USA).

Results and discussion

While athletic training or pre-workout supplements are heavily marketed as containing DMAA as an active ingredient, formulations also include several other components, including amino acids, extracts of natural components, caffeine, creatine monohydrate, and excipients. Such supplements were tested as either a pill or powder form. Both types of solid material were tested directly via DART-MS without any sample preparations, with Figure 1 showing the direct sampling of the supplement pill DNPX. Supplements tested include the specific products DNPX, Juice, Jack3d, and 1,3D-Bomb (all shown in Figure 2 and Table 1), as well as Dexaprine, Hyde, Hydroxystim, 1 More Rep, and Blox (data not shown), among others. The relatively simple mass spectra typical of the soft DART ionization process show a single $[M+H]^+$ peak representative of the individual ingredients specific to each formulation. In each case shown in Figure 2, a peak at *m/z* 116 is present, representing the $[M+H]^+$ species of DMAA. The high mass accuracy associated with the TOF mass analyzer unequivocally relates to the protonated formula weight of $C_7H_{18}N$, which has a calculated monoisotopic formula weight of 116.1439. Within the TOF instrument tolerance of ±5 millimass units (mmu), no other chemical formulas are possible for these measured mass values. The DART-MS spectra of the supplements also could identify various other peaks associated with the ingredients listed on the labels, most notably caffeine, β-alanine, and creatine (Table 1).

DART-MS analysis is instantaneous due to ionization of solid material without sample preparations and the lack of chromatographic separations associated with other mass spectrometric

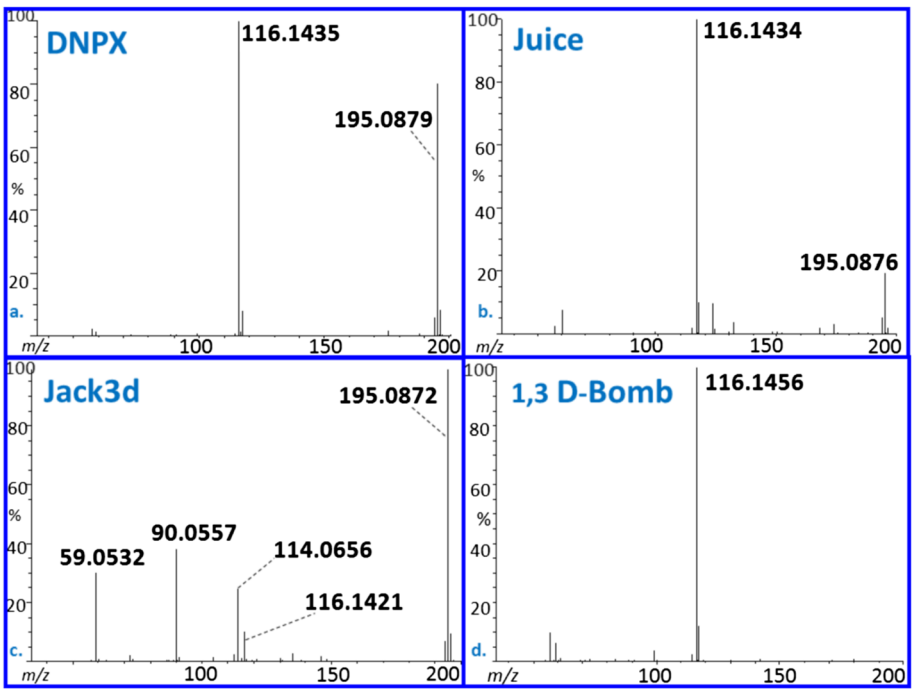

Figure 2. DART-MS spectra of four supplements containing DMAA. The measured masses associated with each $[M+H]^+$ are shown, also appearing in Table 1. DART-MS spectra for the supplements tested were (a) DNPX in pill form, (b) Juice [capsule] powder, (c) Jack3d powder, and (d) 1,3 D-Bomb powder.

Table 1. DART-MS data of supplements containing DMAA

Supplement	Formula	Measured (*m/z*)	Calculated mass	Difference (mmu)	Relative Abundance	Substance
Jack3d	C_3H_7O	59.0532	59.0497	3.5	30.3	n/a
	$C_3H_8NO_2$	90.0557	90.0555	0.2	38.2	β-Alanine
	$C_4H_8N_3O$	114.0656	114.0667	-1.1	24.6	Creatine
	$C_7H_{18}N$	116.1421	116.1439	-1.8	10.4	DMAA
	$C_8H_{11}N_4O_2$	195.0872	195.0882	-1.0	100.0	Caffeine
Juice	$C_7H_{18}N$	116.1434	116.1439	-0.5	100.0	DMAA
	$C_8H_{11}N_4O_2$	195.0876	195.0882	-0.6	34.5	Caffeine
DNPX	$C_7H_{18}N$	116.1435	116.1439	-0.4	100.0	DMAA
	$C_8H_{11}N_4O_2$	195.0879	195.0882	-0.3	79.7	Caffeine
1,3 D-Bomb	$C_7H_{18}N$	116.1456	116.1439	1.7	100.0	DMAA

methods. In theory, the lack of chromatography could present a problem for isomer identification and/or differentiation of DMAA with tuaminoheptane. The structural difference between the two compounds is that the alkyl portion of DMAA is branched, while tuaminoheptane is linear (Figure 3). However, DART-MS analysis can be set up to acquire multiple spectra under different parameters simultaneously without adding additional time to the assay. By setting the instrument acquisition program to collect data with different voltages at orifice 1, traditional soft ionization spectra can be acquired along with increased levels of fragmentation through in-source collision induced dissociation (CID). DART-MS CID spectra were obtained for both DMAA and tuaminoheptane (Figure 3, Table 2). Interestingly, the CID fragmentation patterns of the two compounds are distinct, enabling differentiation based on the appearance of *m/z* 114 at 60 V and 90 V measurements, which manifests as the base peak in tuaminoheptane, but is only of minor abundance in DMAA (~4%). This $[M - H]^+$ peak at *m/z* 114 is observed in relatively small abundance with election impact-MS of tuaminoheptane,[7] but is more prominent with the soft ionization associated with DART-MS. Differentiation of the two amine compounds with GC-MS has been cited as problematic and generally requires Schiff base derivatization, which adds time to the overall assay, extends chromatographic retention time, while resulting in identical fragment ions.[7,48]

Next, urine samples were collected after a volunteer ingested a DMAA-containing supplement (Ripped Juice EX2). Thevis *et al.* previously determined that the *N*-glucuronide of tuaminoheptane was not present to any appreciable extent, so the assumption is that DMAA would be similarly unchanged in urine.[7] Prior to ingestion, a blank urine sample was collected as a control, showing no discernible peak at *m/z* 116 (Figure 4). Post-ingestion, urine samples were collected at periodic intervals and tested via DART-MS over 48 h (Figure 5). To demonstrate the potential of DART-MS as a rapid screening method to test for the presence of DMAA use, urine samples were tested directly without any preparation, pre-concentration, or processing

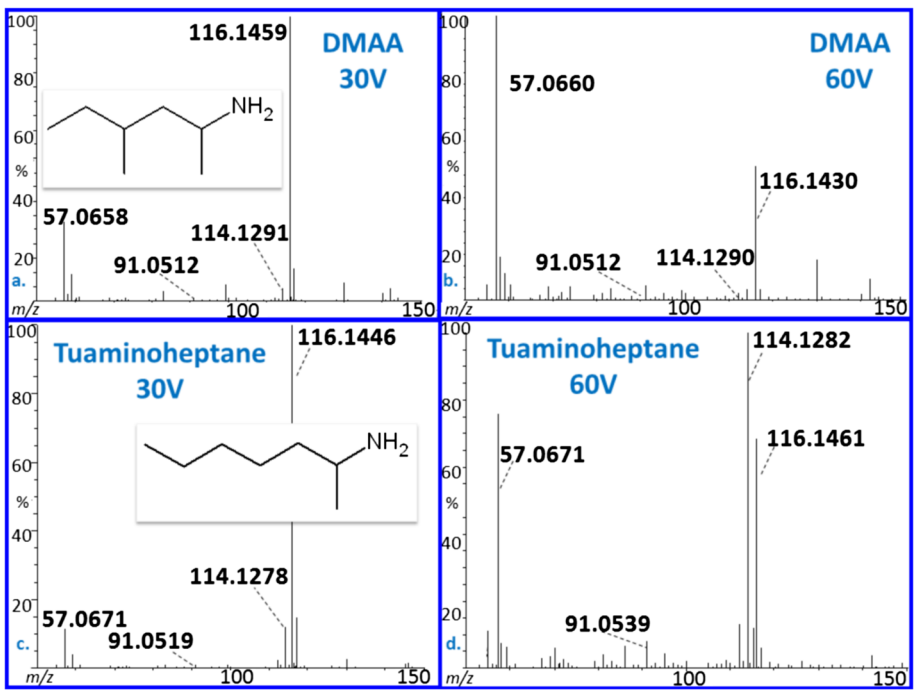

Figure 3. DART-MS spectra of DMAA and tuaminoheptane at different orifice 1 voltages to provide CID that was used for differentiation of the isomers. The extent of fragmentation increased with increasing orifice 1 voltages. The peak at *m/z* 114 was key in differentiation of the two substances. DMAA spectra are shown with the orifice 1 voltage was set at (a) 30 V, and (b) 60 V. Tuaminoheptane spectra are shown with the orifice 1 voltage was set at (c) 30 V, and (d) 60 V. The $[M+H]^+$ and relevant product ion peaks are labelled with high mass accuracy values, with data also appearing in Table 2.

Table 2. DART-MS CID data of DMAA and tuaminoheptane

Sample	Formula	Measured (*m/z*)	Calculated mass	Difference (mmu)	Relative Abundance
DMAA (30V)	C_4H_9	57.0658	57.0704	-4.6	27.6
	C_7H_7	91.0512	91.0548	-3.6	1.2
	$C_7H_{16}N$	114.1291	114.1283	0.8	4.6
	$C_7H_{18}N$	116.1459	116.1439	2.0	100.0
DMAA (60V)	C_4H_9	57.0660	57.0704	-4.4	100.0
	C_7H_7	91.0512	91.0548	-3.6	4.9
	$C_7H_{16}N$	114.1290	114.1283	0.7	3.7
	$C_7H_{18}N$	116.1430	116.1439	-0.9	47.3
Tuaminoheptane (30V)	C_4H_9	57.0671	57.0704	-3.3	11.8
	C_7H_7	91.0519	91.0548	-2.9	1.2
	$C_7H_{16}N$	114.1278	114.1283	-0.5	11.9
	$C_7H_{18}N$	116.1446	116.1439	0.7	100.0
Tuaminoheptane (60V)	C_4H_9	57.0671	57.0704	-3.3	75.9
	C_7H_7	91.0539	91.0548	-0.9	8.4
	$C_7H_{16}N$	114.1282	114.1283	-0.1	100.0
	$C_7H_{18}N$	116.1461	116.1439	2.2	68.7

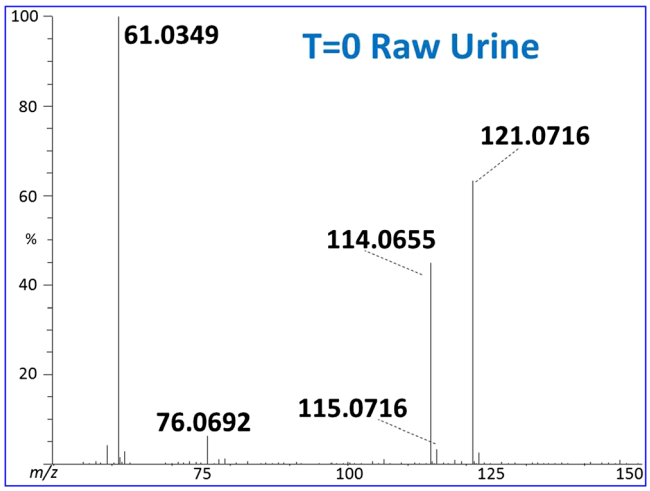

Figure 4. DART-MS control data of urine prior to ingestion of a DMAA-containing supplement.

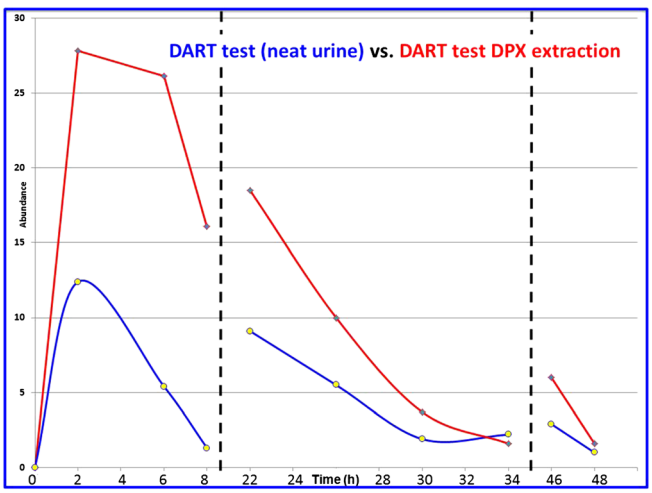

Figure 5. The relative abundance profile comparison for DMAA testing in urine over 48 h. Urine tested directly without sample preparation is shown in blue, while the same samples processed with DPX solid phase extraction tips is shown in red. Breaks in the graph represent overnight hours when samples were not collected and increases in abundance at these points are presumed to be indicative of continued metabolism and accumulation of DMAA during overnight hours.

whatsoever. Raw urine was sampled directly by dipping the closed end of a capillary tube into the urine and holding the liquid droplet between the ion source and mass spectrometer inlet. Within 2 h of ingesting the supplement, the presence of DMAA was a readily apparent in the urine, with a relative abundance of 19% (Figure 6a). Over the two-day sampling period at every time point tested, the urine samples tested positive for the presence of DMAA with, with ≥1% relative abundance in the mass spectra. The DART-MS spectrum in Figure 6a is typical of what was observed in our studies, with major peaks associated with DMAA and matrix components identified and confirmed by the high mass accuracy of the TOF mass analyzer (Table 3). It has been proposed that some of these substances contribute to matrix effects in urine that can suppress ionization of target analytes,[44,49,50] although suppression did not present itself as a problem for these studies. Accordingly, two methods were explored to process the urine samples to counter matrix effects and improve the detectability. First, a rapid sample cleanup method was performed using DPX WAX-1 disposable pipette extraction tips.[51] These DPX tips include a solid-phase weak anion exchange resin that binds drugs and metabolites allowing biological interferences to be washed away, with the drug recovered in an elution solvent (acetonitrile) which was then subjected to DART-MS testing. Second, a traditional liquid-liquid extraction was performed, extracting the urine with dichloromethane (DCM), with the DCM extract then analyzed directly by DART-MS. A series of comparison DART-MS spectra of the same DMAA-containing urine sample at 24 h post-ingestion were obtained as shown in Figures 6b–6d (data shown in Table 3). Both processing methods resulted in an increase in the relative abundance of DMAA over testing the urine directly. Specifically, in comparison of neat urine testing versus DPX tip extractions for samples from 24 h post-ingestion, the abundances of DMAA increase 3-fold while the creatinine peak abundance decreased ~60% relative to urea (Figures 6b and 6d, respectively). Liquid-liquid extraction in DCM

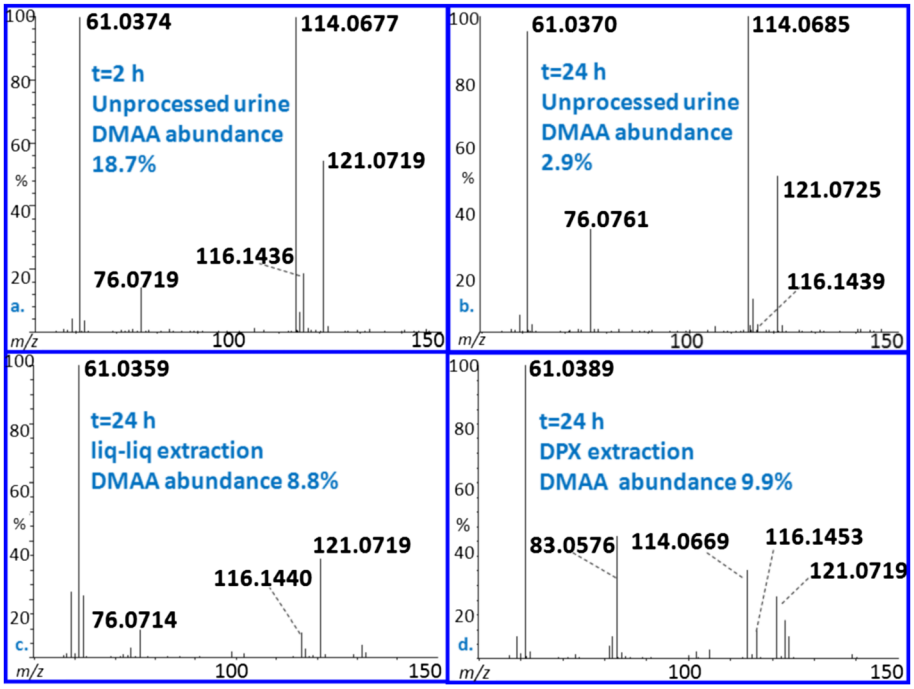

Figure 6. DART-MS spectra of urine containing DMAA. In each case, both DMAA and common urine metabolites are identified, as shown in Table 3. (a) DART-MS spectrum of unprocessed urine at 2 h, representing a maximum relative abundance of DMAA in urine. (b) DART-MS spectra of unprocessed urine at 24 h with a relative abundance of DMAA of 2.9%, used for comparison with (c) 24-h urine sample tested after liquid-liquid extraction with a relative abundance of DMAA of 8.8%, and (d) 24 h urine sample tested after DPX extraction with a relative abundance of DMAA of 9.9%.

appeared to completely remove the presence of creatinine, but did not result in an increase in DMAA signal over the DPX extraction.

Current limitations and future direction

Perrenoud *et al.* demonstrated that for confirmatory testing and a 40 mg dose, detection of the excreted, unchanged DMAA was possible over 80 h with LC-MS/MS.[48] While we discuss DART-MS in a rapid preliminary capacity, within 48 h we demonstrate that detection of DMAA from urine was possible via DART-MS without any sample preparation or chromatography. However, the DPX tip extraction method is relatively quick and provided more robust detectability of DMAA, in terms of signal/relative abundance and presumably could lead to a longer detection interval post-ingestion. This phenomenon is understandable because other components in urine such as urea and creatinine are in relatively large concentrations as compared to trace levels of drugs or metabolites. Any interference with the ionization of the drug would result in decreased intensity, so any sample preparation such as the DPX extraction method that selectively removes these substances has potential to benefit the assay. For preliminary screening methods, WADA cites the minimum required performance levels (limit of detection) for stimulants such as DMAA to be 500 ng/ml.[52] DMAA was detected by DART-MS at this level based on serial dilutions of DMAA in synthetic urine within 5 mmu (Table 4). Our acceptance criteria for the limit of detection was determined to be where the measured mass of the $[M+H]^+$ of DMAA no longer fell within the instrument manufacturer's specification of ±5.0 mmu of the calculated value or was no longer visible in the spectrum.

Urine analysis can be challenging due to its extensive background matrix composition, containing proteins, acids, bases, and various other compounds having similar chemistry to the analyte of interest. Indeed, the annotation of the human urinary metabolome is ongoing, showing metabolic profiles containing hundreds to thousands of constituents.[53,54] Even with accurate mass measurements, smaller peaks within such a complex matrix would be challenging to reliably identify from the background unless included in routine screening. However, Perrenoud, *et al.* determined that urinary concentrations roughly thirty times this level (~15,000 ng/ml), would be representative of recent ingestion of efficacious doses prior to urine collection.[48] The DMAA peaks in our spectra were substantial relative to the background matrix in support of this determination. Our spectra were relatively simple, with the major components of urine, specifically urea and creatinine, dominating the spectra. Regardless, matrix effects did not preclude the detection of DMAA under the conditions and time periods studied. If lower detection limits or a longer detection interval post-ingestion are desired, parameters such as the gas flow rate and gas temperature could be adjusted, but doing so could quite possibly result in the appearance of additional matrix components or greater abundances, which could lead to more complications.

Ultimately, preliminary testing methods are evaluated on their ease of use, cost, throughput, and numerous performance characteristics, such as limits of detection cut-offs and specificity/cross-reactivity.[23,55,56] Although the data presented represents a limited number of samples, DART-MS analysis has been employed for other more diversified compounds, with broadly similar results. While these data represent an interesting alternative method for urine screening, numerous factors would affect such testing and would need to be studied in detail and validated

Table 3. DART-MS data from functional switching experiments used for the comparison of signal strength in relation to the presence of DMAA in urine samples tested with and without sample prepration

Sample	Name	Formula	Measured (*m/z*)	Calculated mass	Difference (mmu)	Relative abundance
t=2	Urea	CH_5N_2O	61.0374	61.0402	-2.8	99.9
unprocessed	Trimethylamine *N*-oxide	$C_3H_{10}NO$	76.0719	76.0762	-4.3	14.2
	Creatinine	$C_4H_8N_3O$	114.0677	114.0667	1.0	100.0
	DMAA	$C_7H_{18}N$	116.1436	116.1439	-0.3	18.7
	Urea Dimer	$C_2H_9N_4O_2$	121.0719	121.0725	-0.6	54.3
t=24	Urea	CH_5N_2O	61.0370	61.0402	-3.2	95.5
unprocessed	Trimethylamine *N*-oxide	$C_3H_{10}NO$	76.0761	76.0762	-0.1	32.8
	Creatinine	$C_4H_8N_3O$	114.0685	114.0667	1.8	100.0
	DMAA	$C_7H_{18}N$	116.1439	116.1439	0.0	2.9
	Urea Dimer	$C_2H_9N_4O_2$	121.0725	121.0725	0.0	49.5
t=24	Urea	CH_5N_2O	61.0359	61.0402	-4.3	100.0
liq-liq extraction	Trimethylamine *N*-oxide	$C_3H_{10}NO$	76.0714	76.0762	-4.8	9.8
	Creatinine	$C_4H_8N_3O$	-	114.0667	-	-
	DMAA	$C_7H_{18}N$	116.1440	116.1439	0.1	8.8
	Urea Dimer	$C_2H_9N_4O_2$	121.0719	121.0725	-0.6	34.0
t=24	Urea	CH_5N_2O	61.0389	61.0402	-1.3	100.0
DPX extraction	Acetonitrile Dimer	$C_4H_7N_2$	83.0576	83.0609	-3.3	42.0
	Creatinine	$C_4H_8N_3O$	114.0669	114.0667	0.2	30.3
	DMAA	$C_7H_{18}N$	116.1453	116.1439	1.4	9.9
	Urea Dimer	$C_2H_9N_4O_2$	121.0719	121.0725	-0.6	21.3

Table 4. DART-MS limit of detection data from synthetic urine doped with DMAA at decreasing concentrations. High mass accuracy values are within ±5 mmu based on the calculated value of 116.1439

DMAA Concentration in blank urine (mg/ml)	Measured *m/z*	Difference (mmu)	Relative Abundance
0.5	116.1445	0.6	100.0
0.1	116.1436	-0.3	17.9
0.05	116.1441	0.2	17.6
0.01	116.1457	1.8	1.7
0.005	116.1446	0.7	1.3
0.001	116.1452	1.3	0.7
0.0005	116.1438	-0.1	0.2

before adoption of such as technique, including the ionization potential of the analyte and how the concentration of matrix components could affect DART ionization. Organizations or government agencies performing such drug tests often employ both preliminary and confirmatory screening methods. DART-MS analysis is instantaneous and the high mass accuracy associated with TOF mass measurements can be much more informative and less prone to false positives than other preliminary methods such as immunoassays or colour tests. In addition, DART-MS screening can be more comprehensive in a single assay, targeting a wider range of analytes without being restricted to the few drug classes associated with immunoassays that are currently each tested individually. Organizations performing large volumes of such screenings per year may find such instrumentation could save substantial resources in terms of both time and cost over time.

Conclusion

DART-MS is demonstrated as a rapid, informative method for preliminary drug screening. This technique was employed for rapid screening of DMAA in supplements, as well as urine without sample preparations over 48 h. Newer, simpler cleanup methods are also now available that can extend the detectability in this or other detection formats. The economies of scale are such that this method can provide substantial cost- and time-savings for labs that test millions of samples annually with disposable and/or consumable methods, such as colour tests or immunoassays. The high throughput nature of the method provides instantaneous results with minimal sample consumption. The high mass accuracy measurements provide important qualitative information related to molecular formula and structure not possible with low resolution instruments. In this sense, DART-MS can be thought of as complementary to other mass spectrometry methods, such as GC-MS, that are commonly used in confirmatory drug testing methods.

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