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Development of an in-house HPLC method for the analysis of ecstasy-laced beverages

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Abstract

Background: Pills of ecstasy continue to be the major drug of abuse among nightclub patrons. This stimulant type of substance can be taken orally in many ways, one of which is by drink spiking where the tablet or its powder is deliberately added to some beverage before consumption.

Method: A high performance liquid chromatographic (HPLC) method was developed to quantify three analytes (methamphetamine, 3,4-methylenedioxymethamphetamine and ketamine) extracted from beverages associated with drink spiking. Ribena drink, Lipton tea drink and Pepsi soft drink were employed as the target sample matrices for validation studies.

Results: The HPLC method was found precise (RSD < 3.2%) and accurate (mean recovery between 97 and 107%) regardless of the matrix type used. The linearity curve for each analyte presented a good regression line with a coefficient of determination, $R^2 > 0.994$. The method was sensitive to detect at least 0.004 mg/mL spiked drugs and precise to quantify the lowest limit of analytes at 0.02 mg/mL.

Conclusion: Analysis of beverages which were contrivedly spiked with genuine ecstasy substances substantiated that the method is fit for the intended purpose.

Keywords: Ecstasy, Beverage, Drink spiking, HPLC

Background

Since the emergence of synthetic drugs, rampant use of ecstasy tablets in nightclubs has become a chronic issue for more than a decade. These tablets generally contain such amphetamine-type stimulants (ATS) as 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA) and amphetamine as the active ingredients (Cheng et al. 2003). The latter two are however less prevalent in locally seized materials. Ketamine (as an anesthetic and depressant (Kayama 1983)) instead is often employed in ecstasy preparations in lieu of these two compounds. Despite the presence of such a depressant, nearly all ecstasy formulations are able to induce euphoria in the users.

Recently in Malaysia, drug-laced beverages or candies were allegedly reported. Unsolicited addition of drug to a drink (or drink spiking) has been linked to 'date rape' (Greene et al. 2007). In the United Kingdom, sedative drugs were identified in deliberately spiked drinks (Scott-Ham and Burton 2005). ATS, opiates and cocaine were also found in urine samples from patients who claimed to have had spiked drinks (Hughes et al. 2007). Among other controlled substances, MDMA and cannabinoids were reported to be some of the illicit drugs used in drink spiking (Greene et al. 2007). Despite the widespread occurrence of drink spiking, there is (to the authors' knowledge) no published data reporting the common levels of drugs frequently found in beverages laced with ecstasy (especially in Malaysia).

For laboratory testing, ATS compounds embodied in ecstasy tablets are conveniently determined by gas chromatography (GC) and liquid chromatography (LC) (UNODC 2006). Illicit drugs including ATS that had been spiked in vape liquids were analyzed with GC coupled with a mass spectrometer (Chan and Harun 2016). Ultra performance liquid chromatography coupled with tandem mass spectrometry was employed to investigate beverage remains that contained drug residues possibly coming from oral fluids (Øiestad et al. 2014). Established

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methods pertaining to the analysis of beverages contaminated with illicit drugs are however limited. To cater for this need, an analytical method needs to be in place to quantify illicit drugs present in beverages that are spiked with ecstasy substances. The laboratory findings will eventually help the enforcement body justify whether drink spiking has taken place.

Viewing that MA, MDMA and ketamine are largely found in locally seized ecstasy tablets, so these three active compounds are given emphasis for drink spiking in this study. Since Ribena drink and Lipton tea drink had been submitted by the local authorities for analysis to incriminate date rape, this study thus seeks to develop an in-house high performance liquid chromatographic (HPLC) method for the analysis of the aforesaid beverages (as well as Pepsi soft drink) for MA, MDMA and ketamine present at sub-milligram levels.

Materials and method

Chemicals and reagents

Ketamine hydrochloride was purchased from Toronto Research Chemicals (North York, Canada). Methamphetamine hydrochloride and 3,4-methylenedioxymethamphetamine hydrochloride were the in-house reference materials prepared by the Department of Chemistry Malaysia (Petaling Jaya, Malaysia). Hexylamine was procured from Acros Organics (New Jersey, USA). Methanol, acetonitrile and phosphoric acid were commercially obtained from Merck (Darmstadt, Germany). Ultrapure water was generated from the ultrapure water system at 18.2 MΩcm.

High performance liquid chromatography-photodiode array detector (HPLC-PDA)

A Waters e2695 Separations Module coupled with a Waters 2996 Photodiode Array Detector was employed for this study. A Kinetex 5u PFP 100A (150 × 4.6 mm) column was used to facilitate chromatographic separation. Table 1 summarizes the optimized HPLC operating conditions for this study.

Preparation of standard

A single stock mixture containing MA, MDMA and ketamine, each at 10 mg/mL was prepared in methanol and kept at -20 °C for storage. For daily calibration, 0.4 mL of the stock solution was transferred to a 5 mL volumetric flask. Methanol was added to the mark to obtain a working standard mixture with a final concentration of 0.8 mg/mL for each analyte.

Preparation of ion pairing solution

An ion pairing solution was prepared by pipetting 0.280 mL hexylamine and 5.319 mL phosphoric acid into a glass bottle containing 1 L ultrapure water. The solution was mixed well before use.

Table 1 Optimized conditions for HPLC

Parameter	Condition
Mobile phase 1 (Bottle B)	Acetonitrile
Mobile phase 2 (Bottle D)	Phosphoric acid + hexylamine in 1 L water
Mode of mobile phase	Isocratic 7:93 B:D
Flow rate	1 mL/min
Degasser	Normal
Column temperature	35 ± 5 °C
Sampler temperature	OFF
Injection volume	5 μL
Mode of data acquisition	3D data collection
Scan range	190 to 400 nm
Resolution	1.2
Sampling rate	1.0
UV max detection for quantification	192 nm
Total run time	21 min

Preparation of sample

1 mL of a well-shaken beverage sample was transferred into a 5 mL volumetric flask. Dilution was accomplished by adding methanol to the mark to extract the analyte(s). Then the sample was shaken vigorously for a few seconds. The solution was sonicated for 5 min and filtered through a nylon membrane (≤ 0.45 μm) preinstalled on a syringe filter. The filtrate was then stored in a vial pending analysis.

Method validation

Three beverage matrices used in this study were Ribena drink, Lipton tea drink and Pepsi soft drink which were purchased from a local store. These samples were employed throughout the course of validation guided by the general guidelines recommended by UNODC (2012).

Selectivity

Six compounds (amphetamine, MA, MDA, MDMA, ketamine and caffeine) can be co-present in any locally seized ecstasy tablets. Therefore, blank matrices of the three chosen beverages as well as their respective spiked samples containing the six co-present compounds were prepared and analyzed. Baselines and resolution of MA, MDMA and ketamine were assessed.

Precision

Seven aliquots were employed for precision studies: a working standard at 0.8 mg/mL was prepared; two portions of each beverage respectively containing the target

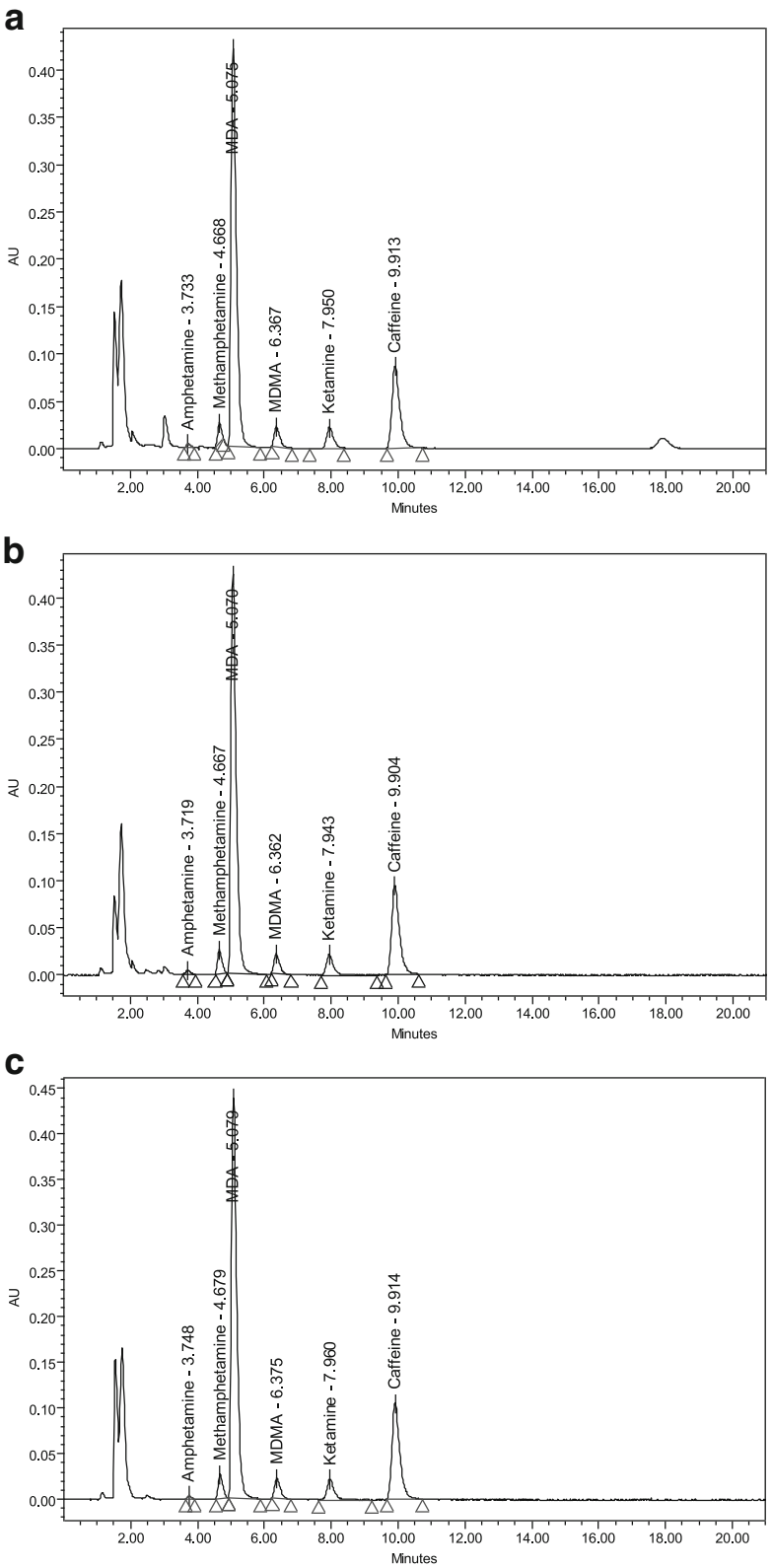

Fig. 1 Six compounds spiked in the matrices of (**a**) Ribena, (**b**) Lipton and (**c**) Pepsi

Table 2 Results for precision studies

Concentration (mg/mL	Level of analyte in matrix (mg/mL)						
	Standard	Ribena		Lipton		Pepsi	
	0.8	0.25	3	0.25	3	0.25	3
	Intra-day (%RSD)						
MA	0.63	0.46	1.23	1.37	0.97	1.31	1.06
MDMA	0.64	0.36	1.35	1.41	1.07	1.27	1.13
Ketamine	0.61	0.32	1.36	1.48	1.07	1.02	1.17
	Inter-day (%RSD)						
MA	2.79	2.86	2.14	2.14	1.51	2.73	2.41
MDMA	2.53	3.08	1.99	1.93	1.45	1.75	2.00
Ketamine	2.69	3.18	1.92	2.11	1.42	1.86	1.96

analytes at 0.25 mg/mL and 3 mg/mL were also prepared. Each prepared solution/aliquot was injected 8–10 times consecutively on the same day to study intra-day precision. For inter-day precision, the 0.8 mg/mL standard mixture was injected once over ten different days, while the prepared sample aliquots were each injected trice on six different days to obtain the daily average for the peak areas of each analyte. Percentage relative standard deviation (%RSD) was computed to assess consistency of the analyte's peak area.

Limits of detection (LOD) and quantification (LOQ)

Three standard mixtures respectively at 0.0008, 0.0015, and 0.0030 mg/mL were prepared in methanol to determine the level at which three signal-to-noise ratio (S/N) with an undistorted ultraviolet (UV) spectrum could be obtained for each analyte. At the same time, the precision achieved by these low level mixtures was also evaluated. Based on the findings derived from the drugs prepared in methanol, it was then decided that each beverage should be spiked with 0.004 and 0.02 mg/mL analytes (equivalent to 0.0008 and 0.004 mg/mL respectively as though after dilution) to test for the LOD and LOQ respectively. For the LOD, the chromatogram was examined to ensure at least 3 S/N was obtained for each peak. For the LOQ, it was confirmed based on the precision of the peak area.

Linearity test

A series of dilutions were prepared in methanol to cover the three analytes at 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.2 mg/mL in separate mixtures. This series was meant to include 0.0625 to 6 mg/mL analytes in beverage as though before dilution. The series was analyzed for six times on the same day. An area versus concentration curve was plotted for each compound.

Accuracy by recovery

Each beverage was respectively spiked with the target analytes at 0.25 mg/mL (low) and 3 mg/mL (high) prior to sample preparation. Each prepared sample was transferred into two separate vials. Each vial was analyzed in

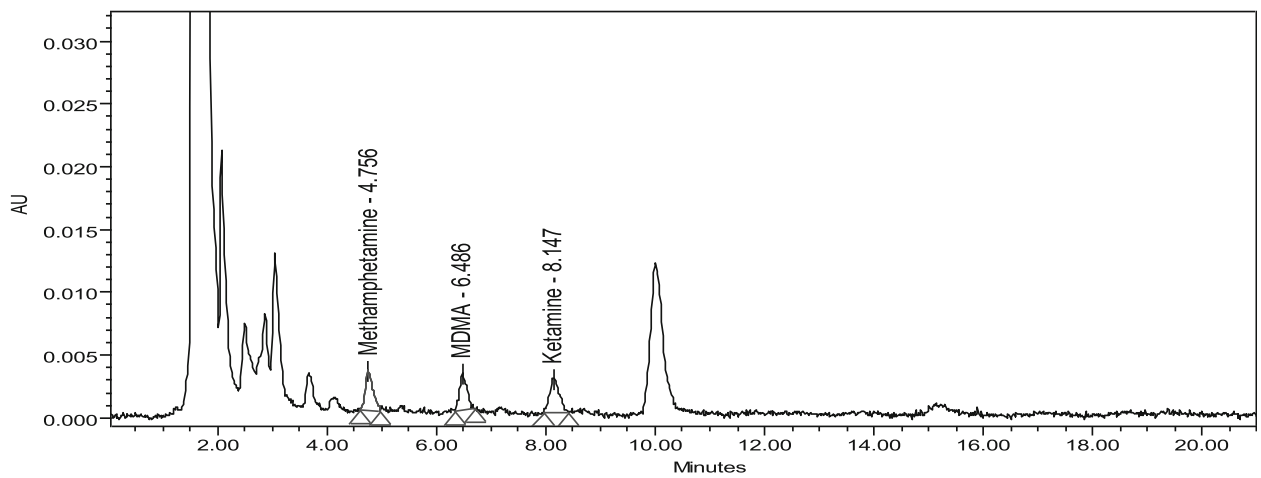

Fig. 2 Peak intensities of 0.004 mg/mL analytes in relation to the background matrix of Lipton

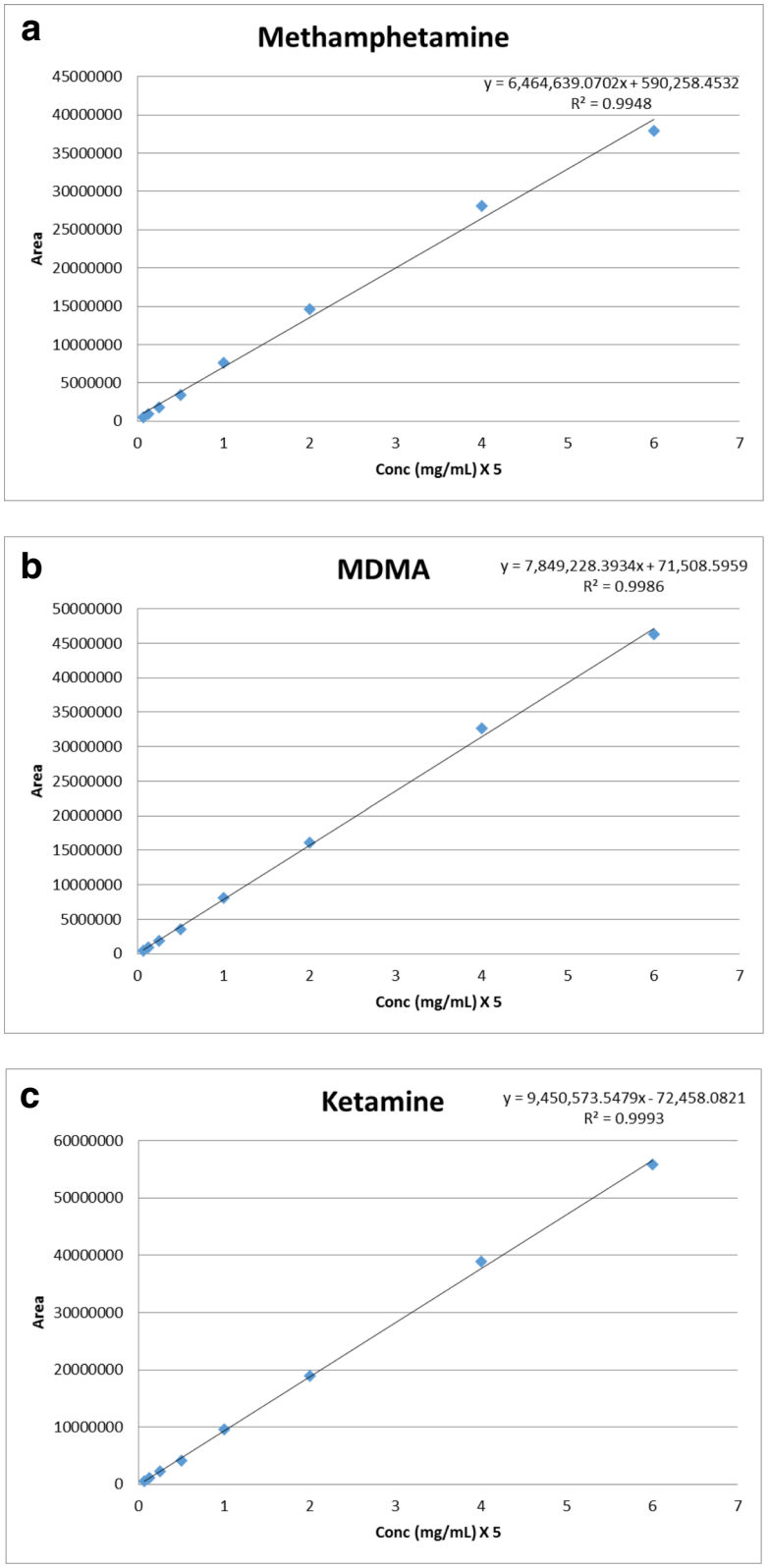

Fig. 3 Linearity curves for (**a**) MA, (**b**) MDMA and (**c**) ketamine

triplicate. The procedure was repeated to obtain data over three days.

Results and discussion

System optimization

A routinely adopted C18 stationary phase in various column dimensions was tested using buffers prepared from orthophosphoric acid, formate, acetate and phosphate salts at different pH values. Separation of the three target compounds was, in fact, achievable with 30 mM phosphate buffer at pH 7.2 but it was only ideal for analytes at low concentrations. Resolution suffered when a high amount of drug substance was injected, where MA and MDMA peaks coalesced. In contrast, a pentafluorophenyl (PFP) column was able to offer a better base-to-base separation with sufficient resolution between all the target peaks when hexylamine was employed as the major mobile phase in this study.

The use of any available internal standards (e.g. nortriptyline) was impossible as the HPLC conditions seemed insensitive to retain them. Hence, it was decided to utilize the analyte's peak area alone for accurate quantification. This decision is not unjustifiable since external standard calibration is still plausible in certain situations (UNODC 2012).

Selectivity

A selectivity test anticipates to obtain a chromatogram having specific elution times for all known compounds. Hence, this test was performed to evaluate the target retention times (RTs) of MA, MDMA and ketamine in the presence of other frequently found compounds (amphetamine, MDA and caffeine). The method was found selective towards the three target analytes that eluted at specific RTs (Fig. 1).

Then, inherent interference introduced by the three beverages was checked. Results obtained from blank matrices displayed that the target RTs of MDMA and ketamine respectively at 6.3 and 7.9 min were totally free of matrix interference. But a slightly raised baseline near the RT of MA at 4.6 min was observed from the Ribena matrix, whereas the Lipton and Pepsi matrices showed a readily present caffeine peak at 9.9 min as well as an unknown peak (if zooming in on the chromatogram) eluting before MA. They however did not seem to pose significant threats to the analytical outcome as the target RTs were not significantly interfered.

Table 3 % Recovery obtained from a three day analysis

	MA		MDMA		Ketamine	
	Low	High	Low	High	Low	High
Ribena	110.3	102.7	95.7	98.7	94.3	98.7
Lipton	110.7	103.3	95.7	99.3	94.7	98.7
Pepsi	113.7	101.7	98.3	97.7	97.3	97.3
Mean	107.05 ± 5.94		97.48 ± 3.29%		96.77 ± 3.49%	

Precision

A good method should display a narrowly spread dataset to imply good stability. Commonly, a minimum of 6 replicates must be employed for precision studies. Since the 0.8 mg/mL mixed standard solution was chosen as a routine calibrator, so more injections (e.g. 10 replicates) were required to confirm its consistency. However for the samples, the replicates (e.g. 8 injections for intra-day or trice on 6 inter- days) were decided based on the possible number of injections required for routine practice.

According to Table 2, all the target analytes achieved the intra-day precision value < 1.5% and inter-day precision < 3.2%. Hence, the peak areas were sufficiently precise and fit for the purpose although internal standard was not used for correction of errors commonly arising from sample injection and solvent evaporation.

Limits of detection (LOD) and quantification (LOQ)

The three prepared levels of mixed standards (0.0008, 0.0015 and 0.0030 mg/mL) approximated five times dilution from the target LOD levels at 0.004, 0.0075 and 0.015 mg/mL (the level in the target beverages prior to dilution) respectively. Analysis showed that the lowest level at 0.0008 mg/mL was able to show > 3 S/N. This in turn infers that the diluted aliquot should contain a final concentration at this level. Thus, the three beverages were spiked with 0.004 mg/mL analytes and diluted five folds to obtain 0.0008 mg/mL in the final aliquots. A similarly good S/N ratio (Fig. 2) and intact UV spectrum were again attained by each peak regardless of the matrix type. Hence, the LOD of this method was decided to be at 0.004 mg/mL.

Table 4 Drugs (after 5 times dilution) in mg/mL recovered from beverages in comparison with its amount in solid substances

	Ecstasy A				Ecstasy B				Ecstasy C			
	S	R	L	P	S	R	L	P	S	R	L	P
MA	–	–	–	–	0.0401	0.0388	0.0380	0.0375	0.0380	0.0405	0.0385	0.0384
MDMA	0.3937	0.3879	0.3817	0.3863	0.0115	0.0114	0.0113	0.0111	0.6127	0.5897	0.5917	0.5946
Ketamine	–	–	–	–	0.0975	0.0868	0.0858	0.0857	–	–	–	–

'-' = below LOQ
S solid substance, *R* Ribena, *L* Lipton, P Pepsi

From the three serial dilutions prepared in methanol, a good RSD (< 4%) was achieved at 0.0030 mg/mL. This again means that stable readings can be obtained if the treated sample has an area response corresponding to that level. Subsequently, it was decided to spike 0.02 mg/mL analytes in each beverage prior to five times dilution to serve as the LOQ. Each prepared aliquot was then injected seven times. All the target peak areas displayed an RSD < 5%, indicating a good level of consistency for quantification.

Linearity

Linearity testing aims to assess whether the instrument's detector responds in a linear manner to the analyte's concentration. To this end, a calibration curve for the tested range from 0.0625 to 6 mg/mL in beverage was constructed for each analyte (Fig. 3). The instrument presented ideal linear curves for all the analytes with a coefficient of determination, $R^2 > 0.994$, which is apparently more than the required value, 0.99 stipulated in the method validation guidelines for narcotic analysis (UNODC 2009). In other words, all the curves demonstrated a sufficient level of goodness of fit for the intended use.

In narcotic analysis, one-point calibration is widely adopted for routine analysis. In this study, 0.8 mg/mL (equivalent to 4 mg/mL in beverage before dilution) was chosen for this purpose. As a result, this level had to be examined for its suitability by constructing a multiple point calibration curve for the range, 0 to 4 mg/mL through the origin using the linearity data obtained from 0.0125 to 0.8 mg/mL. The curves displayed an R^2 value ≥0.999 for all the three compounds. This implies that the one-point calibration curve can ideally approximate the linear calibration pattern when 0.8 mg/mL is chosen as the routine calibration point.

Accuracy by recovery

A method is accurate if it is able to recover 100% of what is spiked into the sample. The accuracy of the current method was checked with two levels of spiking at 0.25 (low) and 3 mg/mL (high) in each blank beverage. Table 3 summarizes the recovery results from the three-day analysis.

At the low spiking level, the matrices demonstrated overestimation on the recovered values of MA. This was probably due to the sized up signal contributed by the tiny peak that eluted near MA. Such an enhancement was significantly obscured by a high amount of MA observed at the high spiking level. For MDMA and ketamine, the recovery performance was sufficiently good at both low and high levels although some underestimation was observed. On the whole, the recovery data were satisfactory for the intended purpose.

It must also be noted that good recovery is commonly feasible if the calibration point is set close to the amount to be measured. That said, the 0.8 mg/mL calibration point should not be fixed but adjusted on a case-by-case basis.

Sample analysis

A corresponding study was performed using three different types of ecstasy tablets. A specified amount of a finely ground ecstasy powder was weighed into four 5 mL volumetric flasks. One of the four flasks was added with methanol to the mark whereas the remaining three were respectively added with 1 mL of each beverage matrix and mixed well before they were diluted with methanol to the mark. Results obtained from the three beverages were compared with that of the powdery substance. Table 4 shows that most of the analytes recovered from the beverages tended to show amounts that are slightly lower than their corresponding amounts in solid substances. This was tolerable since such an underestimation (and not overestimation) would still deliver justice to the criminal.

Conclusion

A simple and straightforward HPLC method was developed for simultaneous determination of MA, MDMA and ketamine present in beverages that had been laced with ecstasy substances. Sample preparation only required each sample to be diluted with methanol (e.g. 1 part sample:4 parts methanol) prior to analysis. The method was found to be precise and accurate. Sample analysis indicated that nearly all of the measured values for the analytes in beverages are slightly underestimated. This however does not compromise justice and thus the method is still fit for the intended purpose.

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Availability of data and materials

Data are available but not put in the public domain. It is because we do not wish that irrelevant parties (e.g. defence lawyers) can access and misuse the data.

Authors' contributions

KWC: preparing the manuscript, performing instrumental analysis and data interpretation. SHR: doing sample preparation and instrumental analysis, contributing ideas. Both authors read and approved the final manuscript.

Authors' information

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Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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