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Urgent first-step screening method for ketamine, phenobarbital, zopiclone, zolpidem, phenytoin and thiopental in adulterated soft drink

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Abstract: Date-rape drugs given to victims through drinks without their knowledge in drug-facilitated sexual assaults and thefts (money, property and body organs), are important threats for the public. Detection in beverage residues gains importance, since some of them can be quickly eliminated from the body, till the victim understands what he/she has experienced, goes to the hospital and gives a urine sample for analysis. Here, date-rape drugs; ketamine, thiopental, phenobarbital, zolpidem, phenytoin and zopiclone were analyzed simultaneously in 1.00mL caffeine-based carbonated beverage residue, through direct injection, using a modified, economical emergency first-step screening method with high performance liquid chromatography-diode array detector (elution time: 11 minutes). Screening power of the method was qualitatively observed in sour cherry juice, sweet soda and beer with some additional experiments. Caffeine in caffeine-based carbonated beverage could also be detected simultaneously. LODs and LOQs were between 0.02-1.79 and 0.08-5.60µgmL^{-1}. Repeatability and reproducibility values were <9.91%. HorRat values were between 0.184-0.500. As the first screening and quantitative study on the simultaneous analysis of these drugs in a beverage, it's important for solving the crimes committed using drugs in caffeine-based carbonated beverage residues found at the crime scene, when the use of these drugs is suspected after anamnesis and inspection.

Keywords: HPLC-DAD in emergency screening, date-rape and sexual assault, determination in beverage, drug facilitated crime, method validation.

INTRODUCTION

Drug facilitated crime such as drug-facilitated sexual assault (DFSA), robbery and organ theft is a serious problem concerning the whole world. Many victims are abused while they are under the influence of drugs (The White House Council on Women and Girls 2015). The number of recorded beverage-spiking events in England - rose significantly in 2021 (Blandamer *et al.,* 2023). Synergistic combinations are also used in such crimes, since they also cause loss of consciousness and sometimes death (Du Mont *et al.,* 2010, Negrusz *et al.,* 2005). Victims may consume the drinks spiked with incapacitating drugs which are odourless, colourless and tasteless, without noticing (Geraghty *et al.,* 2002). As well as the conventional hypnotic drugs as phenobarbital, thiopental and ketamine; untraditional agents such as phenytoin, zolpidem and zopiclone have gained usage recently in DFSA. Among these, zolpidem is increasingly reported in recent years (Kintz *et al.,* 2004a, b, Madea and Mußhoff 2009). A person using zolpidem may show complex behaviours during sleep, which may sometimes seem meaningless (Daley *et al.,* 2011). People under the effect of zolpidem may even commit crime. Phenytoin (antiepileptic and anticonvulsive) has been used in combination with barbiturates in forensic cases (Akvardar *et al.,* 2011, Du Mont *et al.,* 2010). Reports exist also on phenytoin use in DFSA (Akvardar *et al.,* 2011, Kantarci *et al.,* 2013).

However, the analysis of the beverage residues may give an idea of what the patient/victim may have ingested. Detecting these drugs in beverage residues may be an important evidence in the cases of elimination from the body fluids till the victim reaches to the hospital, is suspected of being drugged and till the time that the urine is taken. Fast and easy screening and quantitation methods in beverages found atcrime scenes are necessary for the determination of such drugs on time.

Few studies in the literature exist on multiple date-rape drug analyss in beverages. These are developed for a few drugs and based on mostly the benzodiazepines, GHB and derivatives and some single date-rape drugs such as fentanyl and class-selective amphetamine-type stimulants (Bishop *et al.,* 2004, Elliott and Burgess 2005, Famiglini *et al.,* 2015, Jürschik *et al.,* 2012, Lesar *et al.,* 2011, Meng *et al.,* 2020, Olsen *et al.,* 2005, Tan *et al.,* 2022, Ye *et al.,* 2021). The rest of the date-rape drugs which have the possibility of use alone or in combination are also needed to be determined. Honeychurch *et al.* (2008) developed an LC method for flunitrazepam and nitrazepam detection in beverages with dual-electrode detection by using carbon fiber electrode. Gautam *et al.* (2014) determined three benzodiazepines in alcoholic beverages by GC-MS. Yen *et al.* (2020) extracted the benzodiazepines using organic solvents and utilized carbon dots (hC-dots) to

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detect nitro-containing benzodiazepines via quenching luminescence. Againtargeting only benzodiazepines; drug residues on plastic or ceramic cups, teaspoons, drinking glasses, etc. were extracted using dispersive liquid-liquid microextraction (dLLME) technique and analyzed via HPLC-HRMS/MS (Vincenti *et al.,* 2021). In another study, ketamine, flunitrazepam and diazepam were detected from dried spots of some beverages (LOD: 100ng/mL for ketamine, 25ng/mL for the other two) using LC-MS (Stelmaszczyk *et al.,* 2022). In another qualitative study, the detection of ketamine, nimetazepam, diazepam and xylazine was performed with attenuated total reflectance-fourier transform infrared **(**ATR-FTIR) spectroscopy, after vortex-assisted dispersive liquid-liquid microextraction (VAD-LLME) at pH =10 utilizing 300µL 1:1, v/v dichloromethane:ethanol solvent mixture (Teoh *et al.,* 2023). An MS-MS method with nano-extraction electrospray-ionization exists for cocaine in carbonated and non-carbonated caffeine-based beverages (Hu *et al.,* 2010). There is also a capillary electrophoresis method developed for the analysis of 8 benzodiazepines, GHB and GBL after micelle formation (Bishop *et al.,* 2004) and a DESI-MS method for flunitrazepam in alcoholic beverages (D'Aloise and Chen 2012). Very few LC-MS/MS (only for ketamine) (Albright *et al.,* 2012) and UPLC-MS/MS (for amphetamine and diazepam) (Øiestad *et al.,* 2014) studies exist in beverages. DART-MS method was developed for GHB analysis (Bennett and Steiner 2009) and an HPLC method for GHB and GHV, GBL and GVL (Mercer *et al.,* 2007). Validated methods in beverages are few and the drug scopes are very limited with one or two certain pharmaceutical groups with similar structures. In a validated method for tramadol, dextromethorphan, promethazine diphenhydramine and chlorpheniramine determination in lean cocktail samples, a dumbbell-shaped stir bar sorptive extraction device with a XAD-2 adsorbent was used (Nualdee *et al.,* 2022). LOQ values were between 1.0-1.5µg mL^{-1}. There is also a fast capillary electrophoresis method for scopolamine and butylscopolamine determination in carbonated caffeine-based beverage, energy drink, skol beats® and beer (Ribeiro *et al.,* 2022). We also have previously developed a validated analysis method for drug representatives from three different therapeutic groups (sertraline, zolpidem and fentanyl) in one drop of mixed fruit juice residue using LC-MS MS (Anilanmert and Yonar 2023).

In occurrence of any victimization/intoxication via beverages such as caffeine-based carbonated drink, in crimes such as rape, organ theft, etc. and even suicides, where date-rape drugs may be completely or partially eliminated from the blood or urine, the beverages consumed by the victim/patient become a useful evidence to find out what she/he might have been given. In this study, simultaneous determination of the date-rape drugs; ketamine, phenobarbital, thiopental, phenytoin, zolpidem and zopiclone was performed using an easy, fast and economical screening method in caffeine-based carbonated beverage residues found in crime scenes, using HPLC-DAD with no dilution or sample preparation step (except degassing). The chromatographic method was modified from our previously developed method for a different series of drugs in solution (Anilanmert *et al.,* 2018). The modified method was validated in 1.00mL caffeine-based carbonated beverage. No method exists in the literature for the simultaneous screening of representatives of these drug groups in beverages. As for the beverage matrix, we focused on caffeine-based carbonated beverage, but additional trials in other drinks performed here demonstrated qualitatively that; this method is promising in various beverage matrices such as lemonade, beer, fruit juice and sweet soda as well, although minor modifications may be required.

MATERIALS AND METHODS

Reagents and solutions

Zolpidem (ZOL) analytical grade working standard was kindly provided by both Sanofi Aventis (Turkey) and Dr. Aliashraf Seyffarshad from Legal Medicine Organization (Iran) and zopiclone (ZOPC) by Eczacıbasi (Turkey). Pfizer (Turkey) supplied ketamine HCl (KET) and phenytoin sodium (PHENY), Bayer (Turkey) supplied phenobarbital (PHENO) and Ibrahim Ethem Ulagay (Turkey) provided thiopental (THIO) as grant. Internal standard (Norketamine) was purchased from (Tocris, U.S.). Manufacturer of chromatography-grade methanol and acetonitrile were Merck (Darmstadt, Germany). Deionized water (18.2 MΩ) was produced via Elga water purification system (London, UK).

Instrumentation

Analyses were performed on a ThermoScientific Dionex Ultimate 3000 Rapid Separation LC-DAD system (software: Chromeleon 6.8, Thermofisher, U.S.A.). Ketamine, zopiclone, phenobarbital, zolpidem, phenytoin were detected at 215nm and thiopental at 237nm. An Onyx 100x4.6mm C18 monolithic column (Phenomenex, U.S.) was used for separation. A gradient program was applied using acetonitrile (A), methanol (B), 50mM pH=2.7 phosphate buffer (C) as the mobile phase, utilizing our previously developed method in pure solution for a different series of drugs (Anilanmert *et al.,* 2018). Different column temperatures and gradient programs were applied to decrease the elution time. The best separation in minimum time was achieved at 50°C column temperature and the following gradient program: 4.0 minutes ramp was applied from 3.0:7.0:90.0 (v:v:v) ratio of A:B:C (1.3mL min^{-1} flow rate) to 4.0:6.0:90.0 (v:v:v) ratio (1.5mL min^{-1}). Then a second ramp of 1.5 minutes to 9.0:11.0:80.0 (v:v:v) (1.8mL min^{-1}) and a third ramp of 3.5 minutes to 35.0:5.0:60.0 (v:v:v)(3.8mL min^{-1}) was applied. This composition was switched to

40.0:5.0:55.0 (v:v:v) (4.0mL min^{-1}) in 1.0 minute and finally to initial composition in another 1.0 minute.

Preparation of standard solutions

A stock mix solution including 1.0 $mgmL^{-1}$ KET, 2.0 $mgmL^{-1}$ ZOL, 4.0 $mgmL^{-1}$ PHENO and PHENY and 10 $mgmL^{-1}$ ZOPC and THIO was prepared in methanol, regarding the signal intensities in HPLC-DAD instrument. A second mix was prepared through 7.6 times dilution of the first stock mix solution. These mix solutions were spiked in appropriate volumes to 1.000mL beverage samples.

Sample analysis

Caffeine-based carbonated beverage, beer, lemonade, cherry juice, soda water and mixed fruit juice matrices were used. Beverage samples were purchased from the local market. The carbonated-beverages were degassed in ultrasonic bath. 100.0µL standard solution was spiked to each 1.000mL beverage sample, filtered using a 0.22µm filter, then injected to HPLC. All the spiked beverage chromatograms were compared with blank ones.

For validation of the method in caffeine-based carbonated beverage, 50.00µL I.S. (norketamine) and appropriate volumes of standard mix solutions which didn't exceed 52.7µL were spiked to each 1.000mL beverage, vortexed and filtered. 20.0µL portions of each sample were injected into HPLC-DAD without any further treatment. Blank samples were prepared by spiking 52.7µL methanol instead of the standard mix.

Method validation

The developed analytical method was validated in terms of selectivity, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) and accuracy.

Minimum 7 plots were studied for the calibration graphs in caffeine-based carbonated beverage, in the concentration ranges of 0.03-50.06µg mL^{-1} for KET, 1.21-500.60µg mL^{-1} for ZOPC, 0.24-200.25µg mL^{-1} for PHENO, 0.06-100.12µg mL^{-1} for ZOL, 0.12-200.25µg $mL^{-1}$for PHENY and 1.20-500.62µg mL^{-1} for THIO. Intermediate precision tests were performed in quality control (QC) samples of high, intermediate and low concentrations. QC levels studied for intermediate precision were 0.30, 6.03 and 50.06 µg mL^{-1} for KET, 0.60, 12.06 and 100.12 µg mL^{-1} for ZOL, 1.20, 24.13 and 200.25 µg mL^{-1} for PHENO and PHENY, 3.01, 60.32 and 500.62 µg mL^{-1} for THIO and ZOPC.

STATISTICAL ANALYSIS

LOQ was determined using Eurachem method (Eurachem 2014). LOD was calculated according to the following formula: LOD=3.3xs/slope, ($n \geq 6$) (Latha 2013, Mohamad *et al.,* 2020). RSD% values of ≥6 replicates for each concentration level were plotted versus concentration and the concentration that corresponded to 15% RSD was recorded as the LOQ. Precision was evaluated by means of repeatability and reproducibility (inter-day), using ANOVA and HorRat values at three concentration levels. One-way ANOVA was performed in MS Office Excel 2003 to calculate the variances pertaining to within- and between-days. The experimental F values obtained from ANOVA were compared with $F_{critical}$ values at 95% confidence level. Repeatability and intermediate precision (RSDr, %) were calculated using the mean sum of squares (within- and between-days) obtained from ANOVA at each concentration level. AOAC guidelines were used for HorRat calculation (AOAC 2016). PRSDr has been calculated from the Horwitz equation: PRSDr(%)=2C(-0.15). In this equation; C points out to the mass fraction of the spiked analyte.

RESULTS

In the modification of HPLC method, different proportions of buffer/methanol/acetonitrile mixture and different gradients were tried to achieve well resolved peaks of six drugs. The best result was again obtained with the composition that we had obtained in our previous study with a different combination of drugs (Anilanmert *et al.,* 2018). That was a good result promising that the method may also work for a wider scope of analytes in further studies. But since better-resolved peaks were needed, different column temperatures were scanned. 50°C column temperature were found to be the best for optimal resolution and retention. Caffeine-based carbonated beverage, beer, lemonade, cherry juice, soda water and mixed fruit juice samples were tried, to see if all analytes could be detected in each of these beverages. Validation of the method was performed in a commercial caffeine-based carbonated beverage sample by means of specificity/selectivity, linearity, LOD, LOQ, accuracy (recovery) and precision (repeatability, reproducibility).

Specificity and selectivity

All the spiked beverage sample chromatograms were compared with their blanks (fig. 1- 3). Wavelengths and the retention times where the analytes were observed are shown in able 1. The unknown blank peaks did not interfere with the signals of the drugs in sour cherry juice, caffeine-based carbonated beverage and sweet soda. The peak eluted at 4.75 min was confirmed as caffeine, utilizing a caffeine standard by diluting the matrix with water by a proportion of 1:1. The analyte peaks also did not interfere with each other in any of the beverages. The developed method was specific and selective for all drugs and beverages except zopiclone in lemonade and sweet soda.

Finally, since the caffeine-based carbonated beverage was the most successful matrix where all the analytes could be

detected more satisfactorily, it was chosen for the quantitation and validation. The other matrices were left for future method development and validation studies.

Linearity and linear range

The linear ranges, linearities and the equations for each calibration graph are demonstrated in table 1. The calibration curve showed linearity over a wide range with excellent regression coefficients. The number of analysis results for each concentration level was n≥6. LOD and LOQ values are shown in table 1. Eurachem is the strictest and convenient method for LOQ determination, because RSD% limit for LOQ is 15%. LOD and LOQ were between 0.02-1.79 and 0.08-5.60µg mL^{-1}.

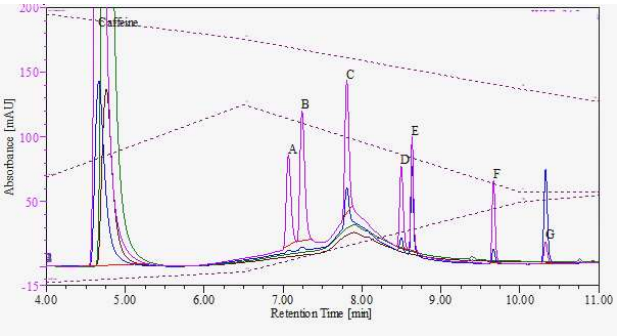

Fig. 1: 200.0µg mL^{-1} spiked caffeine-based carbonated beverage chromatograms and its blank chromatograms at 215 (green) and 237nm (claret-red), A: I.S, B: KET, C: ZOPC, D: PHENO, E: ZOL, F: PHENY, G: THIO (pink: 215nm, blue: 237nm).

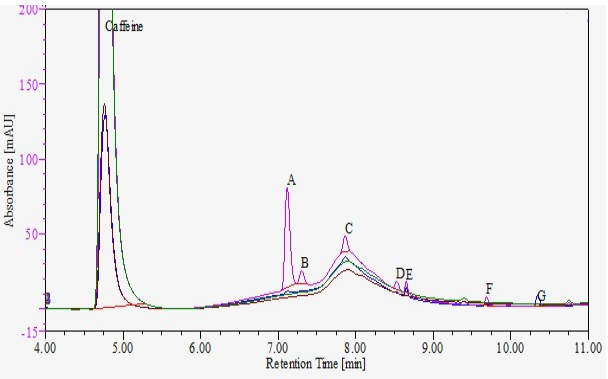

Fig. 2: Chromatograms of spiked caffeine-based carbonated beverage at low concentration levels and their blank. Colours of the chromatograms are defined as claret-red for blank at λ:237nm, green for blank atλ:215nm, blue for beverage at 10.0µg mL^{-1} (λ:215nm), pink at 10.0µg mL^{-1} (λ:237nm), A: I.S, B: KET, C: ZOPC, D: PHENO, E: ZOL, F: PHENY, G: THIO.

Repeatability and reproducibility

Repeatability and intermediate precision for each analyte were determined at three QC levels (table 2). Repeatability was found as ≤7.97% for n=6. Reproducibility was determined by means of intermediate precision at three QC levels regarding three days, utilizing ANOVA (table 2). For all analytes and QC levels, the intermediate precision values were ≤9.91%. HorRat values were assessed using PRSDr=predicted RSD (using Horwitz equation) and RSDr=experimentally measured RSD for each QC level. The calculated results determined whether the reproducibility found experimentally is acceptable or not. HorRat is one of the acceptability criteria recommended for various analysis methods of AOAC International, the European Union and other European organizations (e.g. European Committee for Standardization and Nordic Analytical Committee). HorRat values were found between 0.184-0.500 in this study.

DISCUSSION

A fast, easy method for date-rape drugs without dilution or sample preparation (except degassing), was successfully developed in beverages, using a modified HPLC method. Determination of these representative date-rape drugs from different therapeutic groups widens the scope of current drug screening in beverages. Monolithic column was chosen as the stationary phase due to its resistance to high flow rates which allows a shorter run-time. The final method provided a good chromatographic separation in 11 min elution time.

Only zopiclone couldn't be observed in lemonade and spiked sweet soda, because it was lost in the large matrix peak. If zopiclone is also suspected in the inspection of the victim/patient; in cases of lemonade and sweet soda, negative results should be confirmed with another method. The peaks of zopiclone are clearly discriminated from the baseline at 215nm, in spiked cherry juice and mixed fruit juice (fig. 3b and 3c), when compared with its blank, even if the intensity is low. The intensity of the analyte peak seems to be higher in the beer matrix (fig. 3e), where the reason is thought to be effect of alcohol. In the cherry juice sample, all the drugs were separated well and the continuous background noise between 6-9 min, didn't hinder the drug signals. In sweet soda, there was interference at 7.90min but this could be resolved by detecting at 237nm, the rest of the chromatogram was smooth. In caffeine-based carbonated beverage, there was no matrix peak interfering with drugs, additionally the peak eluted at 4.75 min was confirmed as caffeine, utilizing a caffeine standard by diluting the matrix with water by a proportion of 1:1. So that caffeine was also separated well from the other peaks, as well as the matrix peak. In mix fruit juice, there was an interference with zopiclone and a matrix background at 8.60 minutes, however this effect was overcome by the increasing concentration of calibration standards, a standard addition method will resolve the problem if zopiclone is suspected. The developed method was specific and selective for all the above-mentioned drugs and beverages except zopiclone in lemonade and sweet soda.

Table 1: Wavelengths, retention times, LOD, LOQ values, linear ranges, regression values and the equations of the calibration graphs for each analyte determined in caffeine-based carbonated beverage sample. No analyte peak interacted with the caffeine peak at 4.75 minutes.

Drug	λ (nm)	Retention time (min)	LOD (μgmL^{-1})	LOQ (μgmL^{-1})	Linear range (µg/mL)	Equation of the calibration graph[a, b]
I.S.	215	7.12	-	-	-	-
KET	215	7.30	0.02	0.04	0.06-50.60	$y=6.02x10^{-2}(\pm2.64x10^{-3})x-8.03x10^{-2}(\pm4.71x10^{-2})$
ZOPC	215	7.87	0.48	0.89	1.21-500.60	$y=5.50x10^{-3}(\pm2.70x10^{-4})x-9.34x10^{-2}(\pm5.15x10^{-2})$
PHENOB	215	8.49	0.06	0.20	0.24-200.25	$y=6.70x10^{-3}(\pm3.00x10^{-4})x+4.33x10^{-2}(\pm2.34x10^{-2})$
ZOL	215	8.65	0.17	0.54	0.60-100.12	$y=1.43x10^{-2}(\pm7.32x10^{-4})x+6.02x10^{-2}(\pm2.80x10^{-2})$
PHENY	215	9.69	0.28	0.83	1.20-200.25	$y=6.08x10^{-3}(\pm3.16x10^{-4})x+5.19x10^{-2}(\pm2.61x10^{-2})$
THIO	237	10.33	0.21	1.65	3.01-500.62	$y=3.13x10^{-3}(\pm1.69x10^{-4})x+6.77x10^{-2}(\pm3.48x10^{-2})$

[a] $r^2> 0.99$, [b] $n\geq6$.

Table 2: Results of repeatability and reproducibility

QC Levels[c]	Drugs	Repeatability (RSD %) (within-day)	Intermediate precision (RSD_r %) (inter-day)	ANOVA $F_{EXPERIMENTAL}$ [a] (inter-day)	HorRat Ratio [b] (inter-day)
LOW	KET	4.11	4.65	2.81<4.84	0.190
	ZOPC	6.33	7.77	3.78<5.12	0.317
	PHENOB	8.24	9.91	3.67<3.68	0.500
	ZOL	5.07	5.35	0.89<4.49	0.270
	PHENY	7.97	8.13	1.31<4.67	0.410
	THIO	5.19	5.57	2.37<3.40	0.227
MID	KET	1.82	2.02	2.56<3.59	0.184
	ZOPC	2.89	3.32	3.31<3.52	0.427
	PHENOB	1.49	1.65	2.79<3.49	0.185
	ZOL	3.88	4.03	0.30<3.42	0.451
	PHENY	4.00	4.43	2.60<3.55	0.496
	THIO	2.72	2.87	2.08<3.40	0.369
HIGH	KET	2.18	2.22	0.66<3.42	0.250
	ZOPC	1.90	2.16	3.04<3.55	0.344
	PHENOB	1.87	2.01	2.47<3.40	0.279
	ZOL	1.46	1.65	3.17<3.47	0.229
	PHENY	1.55	1.77	3.18<3.55	0.246
	THIO	1.47	1.53	1.69<3.42	0.244

[a] $<F_{CRITICAL,\%95;}$ [b] acceptability range:0.25- 1.33; analysis per-day n≥3 ; [c]low, mid and high QC levels were 0.30, 6.03 and 50.06 for KET, 0.60, 12.06 and 100.12 µg mL^{-1} for ZOL, 1.20, 24.13 and 200.25 µg mL^{-1} for PHENO and PHENY, 3.01, 60.32 and 500.62 µg mL^{-1} for THIO and ZOPC. n_{days}=3 for all drugs in all QC levels, except for low QC level for KET, ZOPC, ZOL, PHENY:the results are given for 2 days.

LOD and LOQ values and the linearity ranges are compatible with the concentrations spiked in DFC cases and the validation results fit for the purpose. HorRat values found in this study were between 0.184-0.500. The original data developed from inter-laboratory studies assigned a HorRat value of 1.0 with acceptability limits of 0.5-2.0 (AOAC 2016). The corresponding within laboratory RSD% values were found to be typically 1/2 to 2/3 of the inter-laboratory values which correspond to 0.25-1.33. Lower values as in this study (table 2) are regarded by AOAC as excellent training. We obtain higher values in more complicated matrices. These lower results here can easily be explained by the use of less complicated matrices in this study.

Furthermore, the method doesn't require sample preparation (except degassing), so minimum labor-work surely increases the precision. So it is suggested that the intermediate precision results in this study is acceptable.

Some screening methods for a few date-rape drugs exist in literature including sensors (Elliott and Burgess 2005, Garcia-Gutierrez and Lledo-Fernandez 2013, Meyers and Almirall 2004). Currently, sensors for instant determination of date-rape drugs are being developed; such as an electrochemical sensor with SPGEs (screen printed graphite electrodes) for flunitrazepam in alcoholic beverages (LOD=12µg mL^{-1}) (Garcia-Gutierrez and Lledo-Fernandez 2013) and a chemiluminescence based sensor for flunitrazepam using tris-(2, 2'-

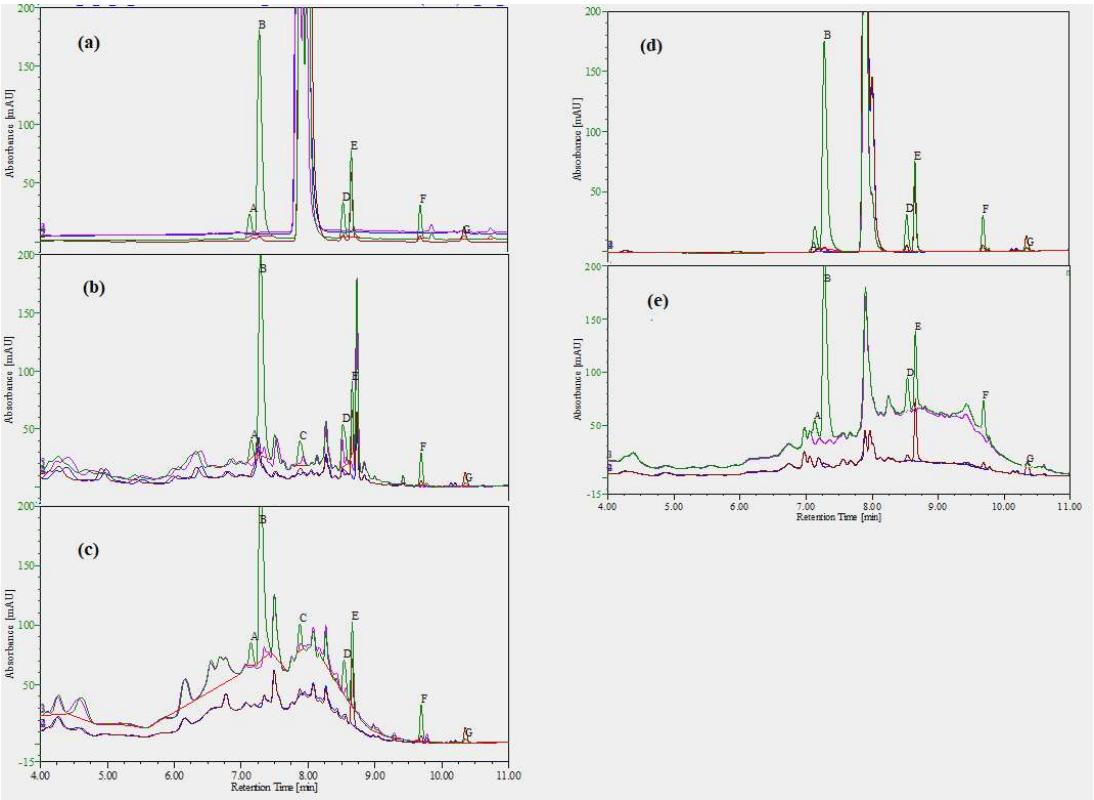

Fig. 3: Spiked beverage chromatograms of lemonade (a), mixed fruit juice (b), cherry juice (c), sweet soda (d), beer (e) in low concentration level and their blanks. Colours of the chromatograms; pink: blank (λ:215nm), blue: blank (λ:237nm), green: 10.0µg mL^{-1} (λ:215nm), claret-red: 10.0µg mL^{-1} (λ:237nm) A: I.S, B: KET, C: ZOPC, D: PHENO, E: ZOL, F: PHENY, G: THIO.

bipyridyl)ruthenium(II)-dichloride reagent, yet with a weak sensitivity at pH>4 (Lledo-Fernandez *et al.,* 2014). Those sensors are only for screening Olsen *et al.* (2005) developed an LC-MS method for 5 benzodiazepines, zopiclone, carisoprodol, morphine in beer and caffeine-based carbonated beverage samples. Another method with DART-MS was used for screening of GHB in beverages (Bennett and Steiner 2009). In both methods, no validation data was given. In this paper the developed method is validated for caffeine-based carbonated beverage. There are very few published researches on LC or UPLC with MS/MS detection for the determination of limited number of date-rape drugs in beverages. One is on ketamine (Albright *et al.,* 2012) and the other is on amphetamine and diazepam determination (Øiestad *et al.,* 2014). Majority of the developed methods for date-rape drug determination in beverages were studied in alcoholic beverages. Yet date-rape drugs could be used also outside of the bars/pubs/clubs, for different crime purposes.

In this study, date-rape drugs from different therapeutic classes were determined in caffeine-based carbonated beverage. Some preliminary trials were performed to observe the screening capability of the method in fruit juices, lemonade and sweet soda and an alcoholic beverage (beer).

While there are also a few analysis studies on limited number of drugs as; cocaine, ketamine, ketamine with benzodiazepines and amphetamine with diazepam (Albright *et al.,* 2012, Hu *et al.,* 2010, Øiestad *et al.,* 2014) in the literature, studies in beverages are mostly focused on GHB, its derivatives and benzodiazepines. There is a need to widen the scope of drugs from different therapeutic groups at least through using the group representatives. No simultaneous determination method was encountered for KET, ZOPC, PHENO, ZOL, PHENY and THIO in beverages, in the literature. This study will contribute to the area, as an emergency screening method in hospitals and forensic laboratories, in case of suspect of these drugs after the anamnesis or inspection of the victim. The caffeine-based carbonated beverage, which is one of the most consumed beverages in the world, was the most successful matrix for this method, thus it was chosen for quantitation and validation. The other matrices require future method modification studies. It is also foreseen regarding the chromatograms obtained in various beverages that; through slight modifications in the method, these analytes can be detected in those beverages in urgent cases where a screening for a preliminary evaluation is needed. The method is ready to be used in analysis of caffeine-based carbonated beverage residues in emergency cases.

CONCLUSION

A fast and economical emergency first-step screening method in caffeine-based carbonated beverage with a good chromatographic separation was developed using HPLC-DAD for screening of 6 date-rape drugs, most of which belong to different therapeutic groups. The method required no extraction/dilution and was validated in caffeine-based carbonated beverage, which is among the most preferred beverages in the world. Caffeine could also be detected without any interaction. Although method was validated in only caffeine-based carbonated beverage, we have demonstrated that it can work in other beverages as lemonade, beer, fruit juice and sweet soda, if minor modifications can be performed in lemonade and sweet soda. This method is important both for routine emergency screening analysis from the point of being the first study in the simultaneous determination of the above-mentioned drugs in beverages and for finding out clues on the suspected intoxicant drugs at hospitals, in crimes related with these drugs and detecting the reasons of deaths.

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REFERENCES

Akvardar Y, Arıkan Z, Berkman K, Dilbaz N, Oral G, Ulug B, Uzbay IT, Akgür SA, Bilici M, Gürol DT, Mete L, Gürel ŞC and Zorlu N (2011). Handbook of diagnosis and treatment, Ankara: TR Ministry of Health, Headquarters of Health Services. Pozitif Matbaa, pp.879-253.

Albright JA, Stevens SA and Beussman DJ (2012). Detecting ketamine in beverage residues: Application in date rape detection. *Drug Test. Anal.,* **4**(5): 337-341

Anilanmert B, Narin İ, Aydin İ and Acikkol M (2018). Fast chromatographic screening method for 7 drugs of potential threat in drug facilitated crimes. *Pak. J. Pharm. Sci.,* **31**(1): 231-235.

Anilanmert B and Yonar FC (2023). Speedy gonzales analysis method in one drop of beverage, for crimes committed with drugs from three different groups. *Anal. Sci.*, **39**(6): 843-849

AOAC (2016). Appendix F: Guidelines for standard method performance requirements. *AOAC Off. Methods Anal.* 9.

Bennett MJ and Steiner RR (2009). Detection of gamma-hydroxybutyric acid in various drink matrices via AccuTOF-DART. *J. Forensic Sci.*, **54**(2): 370-375.

Bishop SC, Lerch M and McCord BR (2004). Micellar electrokinetic chromatographic screening method for common sexual assault drugs administered in beverages.*Forensic Sci. Int.,* **141**(1): 7-15.

Blandamer T, Daniels J, Dear J, Birse F, Carlton E, Burgess K and Roberts T (2023). Drink and injection spiking: how to approach an increase in presentations? *Emerg. Med. J.* , **40**(4): 308-312

D'Aloise P and Chen H (2012). Rapid determination of flunitrazepam in alcoholic beverages by desorption electrospray ionization-mass spectrometry. *Sci. Justice,* **52**(1): 2-8.

Daley C, Mcniel DE and Binder RL (2011). 'I did what?' Zolpidem and the courts. *J. Am. Acad. Psychiatry Law,* **39**(4): 535-542.

Du Mont J, MacDonald S, Rotbard N, Bainbridge D, Asllani E, Smith N and Cohen MM (2010). Drug-facilitated sexual assault in Ontario, Canada: Toxicological and DNA findings. *J. Forensic Leg. Med.,* **17**(6): 333-338.

Elliott S and Burgess V (2005). The presence of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone 11(GBL) in alcoholic and non-alcoholic beverages. *Forensic Sci. Int.,* **151**(2-3): 289-292.

Eurachem (2014). The fitness for purpose of analytical methods. a laboratory guide to method validation and related topics. 2nd Ed., Available from http://www.eurachem.org.

Famiglini G, Capriotti F, Palma P, Termopoli V and Cappiello A (2015). The rapid measurement of benzodiazepines in a milk-based alcoholic beverage using QuEChERS extraction and GC-MS analysis. *J. Anal. Toxicol.,* **39**(4): 306-312.

Garcia-Gutierrez E and Lledo-Fernandez C (2013). Electroanalytical sensing of Flunitrazepam based on screen printed graphene electrodes. *Chemosensors,* **1**(3): 68-77.

Gautam L, Sharratt SD and Cole MD (2014). Drug facilitated sexual assault: Detection and stability of benzodiazepines in spiked drinks using gas chromatography-mass spectrometry. *PLoS One* **9**(2): e89031.

Geraghty, Edward, Wu, Ching, McGann W (2002). Effective screening for 'club drugs' with dual mode ion trap mobility spectrometry. *Int. J. Ion Mobility Spectrom.* **5**(3): 41-44.

Honeychurch KC and Hart JP (2008). Determination of flunitrazepam and nitrazepam in beverage samples by liquid chromatography with dual electrode detection using a carbon fiber veil electrode. *J. Solid State Electrochem.,* **12**(10): 1317-1324.

Hu B, Peng X, Yang S, Gu H, Chen H, Huan Y, Zhang T and Qiao X (2010). Fast quantitative detection of cocaine in beverages using nanoextractive electrospray ionization tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.,* **21**(2): 290-293.

Jürschik S, Agarwal B, Kassebacher T, Sulzer P, Mayhew

CA and Märk TD (2012). Rapid and facile detection of four date rape drugs in different beverages utilizing proton transfer reaction mass spectrometry (PTR-MS). *J. Mass Spectrom.*, **47**(9): 1092-1097

Kantarci MN, Kandemir E, Berber G, Ozcan ME and Sahin E (2013). Anti-Epileptic facilitated sexual assault: A case of incest. *Klin. Psikofarmakol. B.*, **23**(3): 254-256.

Kintz P, Villain M and Ludes B (2004a). Testing for zolpidem in oral fluid by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **811**(1): 59-63.

Kintz P, Villain M and Ludes B (2004b). Testing for the undetectable in drug-facilitated sexual assault using hair analyzed by tandem mass spectrometry as evidence. *Ther. Drug Monit.*, **26(2**): 211-214

Latha KP, Ramachandran D (2013). Method development and validation for the simultaneous estimation of vildagliptin and metformin in tablet dosage form by RP-HPLC. *Int. J. Pharm. Sci.,* **5**(1): 459-463.

Lee Henry C, Palmbach T and Miller MT (2001). Henry Lee's crime scene handbook. San Diego, California: Elselvier, Academic Press. 1st Ed: p.189

Lesar CT, Decatur J, Lukasiewicz E and Champeil E (2011). Report on the analysis of common beverages spiked with gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) using NMR and the PURGE solvent-suppression technique. *Forensic Sci. Int.,* **212**(1-3): e40-5

Lledo-Fernandez C, Pollard P and Romerosa A (2014). The date rape drug-flunitrazepam-electroanalytical sensing using electrogenerated chemiluminescence. *Int. J. Electrochem. Sci.,* **9**(1): 227-237.

Madea B and Mußhoff F (2009). K-o-mittel: Häufigkeit, wirkungsweise, beweismittelsicherung. *Deutsches Arzteblatt*, **106**(20): 341-347.

Meng L, Chen S, Zhu B, Zhang J, Mei Y, Cao J and Zheng K (2020). Application of dispersive liquid-liquid microextraction and GC-MS/MS for the determination of GHB in beverages and hair. *J. Chromatogr. B.,* **1144**: 122058

Mercer JW, Oldfield LS, Hoffman KN, Shakleya DM and Bell SC (2007). Comparative analysis of gamma-hydroxybutyrate and gamma-hydroxyvalerate using GC/MS and HPLC. *J. Forensic Sci.,* **52**(2): 383-388.

Meyers JE and Almirall JR (2004). A study of the effectiveness of commercially available drink test coasters for the detection of 'date rape' drugs in beverages. *J. Anal. Toxicol.,* **28**(8): 685-688.

Mohamad AM andac CA and Andac SC (2020). Cross referencing 2D-LC determination of intact gliptins in Urine. *J. Chrom. Sci.,* **58**(10): 907-914.

Negrusz A, Juhascik M and Gaensslen RE (2005). Estimate of the incidence of drug-facilitated sexual assault in the U.S. NCJ Number **212000**: 20, 86, 89

Nualdee K, Buain R, Janchawee B, Sukree W, Thammakhet-Buranachai C, Kanatharana P, Chaisiwamongkhol K, Prutipanlai S and Phonchai A (2022). A stir bar sorptive extraction device coupled with a gas chromatography flame ionization detector for the determination of abused prescription drugs in lean cocktail samples. *Anal. Methods.,* **14**(26): 2557-2568.

Øiestad EL, Karinen R, Christophersen AS, Vindenes V and Bachs L (2014). Analyses of beverage remains in drug rape cases revealing drug residues - the possibility of contamination from drug concentrated oral fluid or oral cavity contained tablets. *J. Forensic Sci.,* **59**(1): 208-210.

Olsen V, Gustavsen I, Bramness JG, Hasvold I, Karinen R, Christophersen AS and Mørland J (2005). The concentrations, appearance and taste of nine sedating drugs dissolved in four different beverages. *Forensic Sci. Int.,* **151**(2-3): 171-175.

Ribeiro MMAC, Barreto DN, Flávio da S. Petruci J and Richter EM (2022). Simultaneous determination of scopolamine and butylscopolamine in pharmaceutical and beverage samples by capillary zone electrophoresis. *Microchem. J.,* 172, Part A: 106985

Stelmaszczyk P, Gacek E and Wietecha-Posłuszny R (2022). Green aspects in the procedure of detection ketamine, flunitrazepam and diazepam in drinks based on dried sample spot analysis. *Green Anal. Chem.*, **3**: 100029.

Tan D, Han C, Yu Z, Sun X, Chen J, Dhanjai, Shao X and Wang D (2022). Dummy molecularly imprinted polymers for class-selective extraction of amphetamine-type stimulants from alcoholic and nonalcoholic beverages. *J. Chrom. A* **1663**: 462759.

Teoh WK, Mohamed Sadiq NS, Saisahas K, Phonchai A, Kunalan V, Md Muslim NZ, Limbut W, Abdullah AFL and Chang KH (2023). Detection and discrimination of sedative-hypnotics in spiked beverage dry residues using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy combined with chemometrics. *J. Forensic Sci.*, **68**(1): 75-85

The White House Council on Women and Girls (2015). Rape and sexual assault: A renewed call to action. Federal Action on Rape, Sexual Assault and Domestic Violence, **201401**: 1-34

Vincenti F, Montesano C, Babino P, Carboni S, Napoletano S, De Sangro G, Di Rosa F, Gregori A, Curini R and Sergi M (2021). Finding evidence at a crime scene: Sensitive determination of benzodiazepine residues in drink and food paraphernalia by HPLC-HRMS/MS. *Forensic Chem.*, **23**: 100327.

Ye J, Wang S, Zhang Y, Li B, Lu M, Qi X, Wei H, Li Y and Zou M (2021). Surface-enhanced shifted excitation Raman difference spectroscopy for trace detection of fentanyl in beverages. *Appl. Opt. ,* **60**(8): 2354-2361.

Yen Y Te, Lin YS, Chen TH, Chyueh SC and Chang HT (2020). A carbon-dot sensing probe for screening of date rape drugs: Nitro-containing benzodiazepines. *Sens. Actuators B Chem.,* **305**: 127441.