REPORT

Fast chromatographic screening method for 7 drugs of potential threat in drug facilitated crimes

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Abstract: In cases where pharmaceuticals and pharmaceutical candidates are involved in drug facilitated crimes (DFC), like organ theft, robbery, rape and suicides, the analysis of drug powders or solution residues found in crime scenes may give idea on what the victims have ingested. An easy and fast simultaneous determination of 7 drugs; GHB (γ-hydroxybutyrate), GBL (γ-butyrolactone), norketamine, ketamine, fenobarbital, fenitoin and thiopental which have the potential to be used in DFC was performed. The method required no sample preparation and has 12 minutes elution time with a good chromatographic separation. The separation was carried out on a C_{18} monolithic column with UV detection at 215 and 237nm. All r^2 values were ≥0.99 and the linear ranges were between 0.9956-1.0000. The LOD and LOQ values were between 0.56-5.55µgmL^{-1} and 1.69-16.82µgmL^{-1} respectively. The repeatability values were ≤7.35%. This is the first study in the simultaneous screening of the above mentioned drugs using HPLC.

Keywords: Drug facilitated crime and date-rape, GHB, GBL, phenytoin, HPLC determination.

INTRODUCTION

The use of a drug to modify a person's behavior for criminal gain is not a recent phenomenon (Villain *et al*., 2004). However, the recent increase in reports of drug-facilitated crimes (sexual assault, robbery) has caused alarm in the general public. Hypnotic drugs are involved in drug facilitated crimes (DFC) like organ theft, robbery, rape and suicides. Some of these drugs can be difficult to detect (active products at low dosages, chemical instability), possess amnesic properties and can be rapidly cleared from the body (short half-life). For example, to be able to detect GHB (γ-hidroxybutyrate) use, urine sample should be collected in 8-12 hours after the crime (Kintz *et al*., 2003). At this point, fast analysis of the residues in drug vessels, solutions or powders found in the crime scene may provide important evidence on what the victim has drunk. GHB, GBL (γ-butyrolactone), ketamine, phenobarbital and thiopental are used in drug-facilitated crimes (Kintz *et al.*, 2004). There are some preclinical studies on norketamine as a potential chronic neuropathic pain management drug. The preclinical studies suggest that norketamine may have potential as a novel agent for treatment of chronic neuropathic pain, with less side effects than ketamine (Holtman *et al*., 2008; Swartjes *et al*., 2010). Phenytoin is also reported to be used in combination with barbiturates, they interact and can be detected sometimes together in forensic cases (Akvardar *et al*., 2011; Kantarci *et al*., 2013; Du Mont, *et al*., 2010). There are recent reports on phenytoin use in DFC (Kintz *et al*, 2004; Holtman *et al*., 2008; Swartjes *et al*., 2010; Akvardar *et al*., 2011; Kantarci *et al*., 2013).

The victims who are drug-dependent or who have taken these drugs without their will, cannot give explanatory and true information on the drugs they have taken recently or on their effects (Ricaurte and McCann, 2005). And since some are rapidly eliminated from the body fluids, fast, easy and cheap analysis of the drug powder, tablet or solution residues found in crime scenes may give idea on what they have ingested. Various methods are used in DFC as HPLC, capillary electrophoresis, LC-MS/MS, GC, GC-MS etc. in literature (Subhra *et al*., 2014; Kintz, 2014; Adamowicz and Kała 2010; Pandey *et al*., 2012; Uddin *et al*., 2013; Bechtel and Holstege, 2007).

There is a GC-MS study where GHB, ketamine, norketamine, phenobarbital and thiopental were quantified simultaneously (Adamowicz and Kała 2010). However, no study could be found on the simultaneous determination of all the above drugs in literature. In this study, a fast HPLC screening was performed for simultaneous determination of GHB, GBL, norketamine, ketamine, fenobarbital, fenitoin and thiopental which have the potential to be used in drug-facilitated crimes, in powders or solutions. Our unpublished in-house method (Anilanmert *et al*., unpublished results) for a different drug combination was used with slight difference. This is the first study on simultaneous determination of the above mentioned drugs using HPLC.

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MATERIALS AND METHODS

Reagents and solutions

GBL was provided from the pharmacology department of Marmara University Faculty of Medicine. GHB was synthesized from GBL in laboratory. Ketamine HCl and phenytoin sodium was provided by Pfizer (Turkey), phenobarbital by Bayer (Turkey) and thiopental by Ibrahim Ethem Ulagay (Turkey). Norketamine was purchased from Tocris. Chromatographic grade methanol and acetonitrile was purchased from Merck (Darmstadt, Germany). Deionized water was purified with Millipore Elix 5 water purification system (Watford, UK). Onyx 100x4, 6mm C_{18} monolithic column (Phenomenex) was used.

Instrumentation and chromatographic conditions

An Agilent (U.S.A.) 1200 HPLC system with a G1310A isopump Gradient Pump Agilent 1260 G1329B autosampler and an Agilent 1200 G1315D DAD Detector (190-400 nm) was used. DAD detector was set at 215 nm for GHB, GBL, ketamine, norketamine, phenobarbital, phenytoin and 237 nm for thiopental. 50.0 mM phosphate buffer (pH=2.70), methanol and acetonitrile were used as the mobile phase. Chromatographic separation was achieved at room temperature. Elution was performed at ambient temperature. Acetonitrile (A), methanol (B), 50 mM pH=2.7 phosphate buffer (C) was used as the mobile phase. The gradient elution started with a 4 minutes ramp from 3.0:7.0:90.0 (A: B: C) (1.30 $mLmin^{-1}$) to a composition of 4.0:6.0:90.0 (1.50 $mLmin^{-1}$). A second ramp to 9.0:11.0:80.0 (1.80 $mLmin^{-1}$) was applied for 1.50 minutes. Composition was switched to 35.0:5.0:60.0 (3.80 $mLmin^{-1}$) in 3.5 minutes. Another ramp to 40.0:5.0:55.0 A: B: C (4.00mL/min) was applied to for 1.0 minute and kept constant for 1.0 minute. Finally, the composition was returned to the first composition in 1 minute. Elution time was 12 minutes and the conditioning time was 1.0 minute for 7 analytes.

Preparation of standard solutions and analysis

A stock mix solution including 1.50 $mgmL^{-1}$ GHB, 1.00 $mgmL^{-1}$ GBL, 1.00 $mgmL^{-1}$ norketamine, 1.00 $mgmL^{-1}$ ketamine, 0.50 $mgmL^{-1}$ phenobarbital and 0.50 $mgmL^{-1}$ phenytoin and 1.00 $mgmL^{-1}$ thiopental was prepared. These mix solutions were diluted in the appropriate volumes regarding the signal intensities in HPLC-DAD instrument for constructing the calibration graph and 25.0 µL portions were directly injected to HPLC. Methanol was used as the blank. Linearity, precision, specificity/selectivity, limit of detection (LOD), limit of quantification (LOQ) were investigated during the validation of the method. Precisions were calculated as RSD% ($n \geq 3$). LOD value was calculated using the formula: LOD= 3.3xs/slope (Latha and Ramachandran, 2013; ICH Guideline, 2005; Gonzales and Herrador, 2007). LOQ values were calculated according to the formula: LOQ: 10xs/slope (Gonzales and Herrador, 2007). External standard method was used.

RESULTS

HPLC method is very fast for 7 analytes (12 minutes) since high flow rates as 3.8, 4.0 $mLmin^{-1}$ were possible because of the low back pressures which can obtained with monolithic columns. All analytes, especially the ones with different polarities were separated with a good resolution. Retention times and wavelengths, which each analyte was observed were given in table 1. When the analyte chromatograms were compared with blank methanol chromatograms (fig. 1), no interaction was observed with the blank chromatogram. No interaction was observed also among the analytes. These showed that the method worked specific and selective for all these drugs. The equations of the calibration curves, which were linear over a wide range with regression coefficients >0.99 were given in table 2. Repeatabilities were determined at each concentration level and given as the mean repeatabilities in table 2, which were≤ 7.35%. The LOD and LOQ values were given in table 1.

DISCUSSION

The use of date rape drugs is increasing (Sankhla *et al*, 2017). The incidence of drug facilitated sexual assault is unclear. Many victims fail to report the incident for some reasons. For the victims who report the incident, the elapsed time may be too long for drugs to be reliably detected in body fluids. Because of this, the powders, solutions, beverages and food found in crime scenes gain importance as an evidence to understand if victim has been given a drug and which drug may be used in the crime, in case the drug is eliminated from the body fluids of the victim.

In the literature, even not including all the analytes that this study has targeted, there are determination studies on a wide spectrum of pschoactive drugs including some date rape drugs (Juhascik *et al*., 2004; Adamowicz and Kala, 2010; Verplaetse *et al*., 2013; Chen *et al*., 2016; Kourtesi *et al*., 2015; Anilanmert *et al*., 2016; Montesano *et al*., 2014). These methods did not include all the targeted drugs in this study. Sophisticated techniques like GC-MS, LC-MS/MS, DART-MS may not be available in all hospital laboratories, or may not be available for a limited time period or may be too busy because of a huge routine trace detection of drugs in biological materials in forensic laboratories. In such cases, use of HPLC instruments are very suitable for providing fast results in the screening and determination of date-rape drugs in solutions or powders, found in the crime scenes. Immunoassay techniques may also be used, however there is a risk of false positive because of cross-reaction probabilities. If the solution or powder found positive for

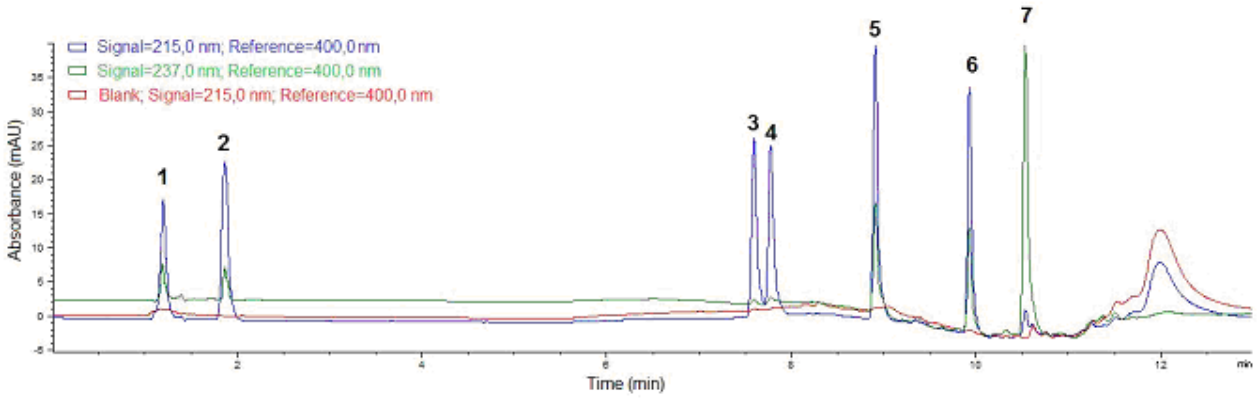

Fig. 1: Chromatograms of mix solution; GHB (1): 75.0µgmL^{-1}, GBL (2): 50.0µgmL^{-1}, norketamine (3): 50.0µgmL^{-1}, ketamine (4): 50.0µgmL^{-1} , fenobarbital (5): 25.0µgmL^{-1} , fenitoin (6): 25.0µgmL^{-1} and thiopental (7): 50.0µgmL^{-1}

Table 1: Wavelengths, retention times, LOD, LOQ values for the analytes.

Analyte	λ (nm)	Retention time (min)	LOD (µgmL^{-1})	LOQ (µgmL^{-1})
GHB	215	1.215 (±0.028)	5.50	16.67
GBL	215	1.889 (±0.026)	5.55	16.82
norketamine	215	7.494 (±0.012)	3.07	9.31
ketamine	215	7.687 (±0.033)	1.48	4.47
phenobarbital	215	8.856 (±0.018)	2.71	8.23
phenytoin	215	9.819 (±0.244)	3.63	11.00
thiopental	237	10.570 (±0.010)	0.56	1.69

Table 2: Linear ranges, regression values and the equations of the calibration graphs for each analyte

Drug	Linear range (µgmL^{-1})	Equation of the calibration graph	r^2	Repeatability[a] (RSD%)
GHB	15.0-1500.0	y=0.743(±0.010)x-6.912(±8.530)	0.9996	4.96
GBL	10.0-1000.0	y=0.838(±0.006)x+3.863(±3.554)	0.9999	7.35
norketamine	50.0-1000.0	y=1.093 (±0.034)x+13.16(±21.96)	0.9990	1.61
ketamine	50.0-1000.0	y=1.109 (±0.00)x+60.60(±8.19)	0.9996	6.37
phenobarbital	2.5-500.0	y=1.263 (±0.042)x+21.26(±9.56)	0.9957	6.13
phenytoin	5.0-500.0	y=1.257(±0.084)x+30.03(±27.06)	0.9956	6.61
thiopental	5.0-500.0	y=1.421(±0.00)x+0.176(±0.542)	1.0000	4.98

[a] mean of minimum 3 analysis, minimum 5 concentration level

date rape drugs in HPLC analysis, the result can be confirmed using and hyphenated MS technique, especially LC-MS/MS.

With techniques like HPLC, the studies are mostly in the tendency of determining a limited number of drugs or drugs from the same pharmaceutical families (since the similar polarities and chemical similarities provide easiness in the detection procedure, because of the nature of the instrument). Forexample in an HPLC-UV study, a method for 6 benzodiazepines was developed for the forensic screening of adulterated non-alcoholic drinks. 30 minutes isocratic elution was performed on a C_{18} column (250mm × 4.6 mm, 5µm) at 45ºC temperature, with a 15mM phosphate buffer: methanol (50:50 v/v) mobile phase, at a flow rate 1.4 mLmin^{-1}. Calibration curves were in the range of 0.5 - 10 µgmL^{-1} (Soltaninejad *et al*., 2016).

A fast micellar liquid chromatographic procedure with UV detection was developed for the simultaneous determination of three commonly used stupefacients, from 3 different pharmaceutical groups (lidocaine, ketamine and diazepam), using a C_{18} reversed-phase column. A micellar mobile phase 0.15 M sodium dodecyl sulfate and 6% (v/v) pentanol, pH 7, and UV detection at 230 nm were used to determine the three stupefacients in food samples (Subhra *et al*., 2014). LOD and LOQ values were in the range of 0.004–0.03 µgmL^{-1} and 0.004-0.03 µgmL^{-1}, in order.

In this study, analytes from three different pharmaceutical family with different chemical structures, which have not been determined simultaneously with HPLC before, were determined with good repeatability and an adequate linear range for the determination in powders, solutions. No

derivatization is necessary. The validation parameters were also fit for the purpose. Use of monolithic column provided a chance of fast analysis with 12 minutes elution time, especially for the molecules which are far from each other from the point of structure and polarity. The linear ranges and LOD and LOQ values were enough for screening the drugs in concentrations found in residues, powders or solutions in crime scenes. This method can be modified and improved with addition of more analytes in other studies.

CONCLUSION

Simultaneous pharmaceutical analysis of the drugs found in crime scenes will be important from the point of finding out some clues on which type of drug may be used DFC and determining the reasons of deaths. The method is suitable for routine screening of these drugs in powders or solutions found in the crime scene.

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