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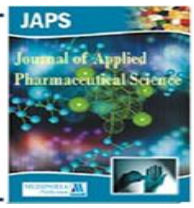

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Recent analytical strategies on "Date-Rape" Drugs and its metabolites

Chanbasha Basheer

ABSTRACT

Recently, much attention has been given to develop simple analytical methods for screening wide range of date-rape drugs. Drug abuse that changes human behavior and guides to crimes has become a serious problem throughout the world. The determination of various classes of abused drugs is an important in many fields of analytical toxicology, such as forensic analysis and antidoping analysis. The detection method must provide more accurate and reliable results from the test specimens. In this regard, attempt is made in this review to summarize the recent applications of these methods in keeping track with complexity of the sample matrices. The following topics are discussed under the headings of different sampling approaches for screening the drugs related to drug facilitated sexual assault and more commonly utilized analytical methods such as liquid chromatography, gas chromatography and capillary electrophoresis separation techniques coupled with mass spectrometry detector. Therefore, relentless efforts should continue to be deployed toward research and development in low cost, fast and efficient ways of detecting illicit drug from all kind of specimens for reliable and more accurate results.

Key words: Club drugs; γ-Hydroxybutyric acid (GHB); Capillary electrophoresis; Gas chromatography; Liquid chromatography; Contactless conductivity detection; Mass spectrometer; Hair and Serum

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INTRODUCTION

Drug facilitated sexual assault (DFSA) also called date-rape is defined as the voluntary or involuntary ingestion of a drug by a victim that results in an act of sexual activity without consent. Drug-facilitated sexual assault, or the use of so-called ''date-rape'' drugs, is a crime in which the executor secretly administers drugs (usually through alcoholic drinks) to the victim prior to a sexual assault. The drugs work in a way that renders the victim physically helpless and unable to remember what has happened. There are different types of DFSA, including situations in which the victim has intentionally taken a recreational drug, combined prescription medicine with illegal drugs, or unknowingly consumed a drug placed in his/her beverage (Bugay et.al., 2010). There are three well-known date-rape drugs: GHB (gamma hydroxybutyrate), Rohypnol (flunitrazepam) and Ketamine (ketamine hydrochloride). Rohypnol is legal in Europe and is prescribed for sleep problems and as an anesthetic, but it is illegal in the United States of America. Ketamine is an anaesthetic for humans and for animals, and GHB is also used to treat sleep problems. Other hypnotic drugs such as Zolpidem and Zopiclone have also been reported in cases of date-rape. Rohypnol (Flunitrazepam), GHB, and Ketamine have many depressant effects that resemble the effects of alcohol. The rapid metabolism and lethargic effects of these compounds make them perfect weapons for an assault. Unfortunately, toxicological screening methods for benzodiazepines target urinary metabolites, such as oxazepam, that have poor cross reactivity with the low-dose benzodiazepines involved in DFSA. GHB methods involve complex derivatization or esterification to the lactone (i.e. lacton g-butyrolactone, GBL), a common GHB substitute

(Salamone, 2001). Thus, more accurate and simple analytical methods are needed which can provide rapid and efficient screening for these compounds.

ANALYTICAL CHALLENGES FOR THE DETECTION OF DATE-RAPE DRUGS

For the ideal analysis of the so called date-rape drugs, the main tasks to be considered are (a) determination of the presence a controlled substance, (b) determination of amount of the substance, and (c) determination of the relationship of drug samples to each other through comparison or ‘‘profiling’’. The challenging part of such analysis is to identify the presence of trace level drug with high sensitivity, selectivity and low detection limits in the human body sample since a very low quantity of drug can cause the effect. Another tricky part of their analysis is owing to the numerous and diverse compounds or therapeutic classes of drugs potentially used. In reality, there are over 50 drugs known or suspected to have been used to commit DFSAs such as Flunitrazepam (Benzodiazepine), GHB and so on. Ketamine (Fig. 1) is an N-methyl-d-aspartate receptor competitor used legally for its dissociative anesthetic properties (Anis et.al., 1983).

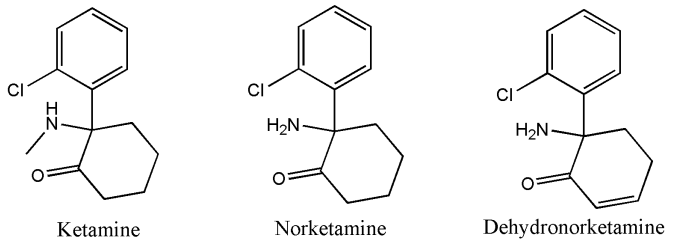

Fig. 1 Structures of ketamines and its metabolites

The lifeless state brought on by administration of ketamine and its metabolites can also produce coma and sedation, which together with possible amnesia and difficulty in fighting off an attacker, has led to its recent implication in drug-facilitated sexual assault.

Benzodiazepines are consist of a 1, 4-diazepine ring with a benzene ring combined to carbons 6 and 7 and typically a phenyl group attached to carbon 5 (Fig. 2). These drugs are used in the treatment of insomnia and as preanesthetic medication. However clinical observations have shown that are frequently misused by polydrug users and is often involved in date-rape cases (Negrusz et.al., 1999). The lower dose compounds, such as alprazolam, lorazepam, midazolam and triazolam, have a shorter duration of action along with a faster clearance rate (Salamone, 2001). These characteristics make analysis very challenging. GHB is made up of a hydroxyl group, three straight chain carbons and a carboxyl group (Fig. 3). γ-Butyrolactone (GBL), and 1,4-butanediol were introduced as ‘‘legal’’ substitutes and can all be transformed into GHB in vivo. GHB can bind to receptors for GABA (γ-aminobutyric acid) and has been used as a date-rape drug (Wells, 2001). The development of rapid and efficient methods for the determination of GHB is therefore important for clinical and forensic toxicology. However, despite the simple structure of the molecule its analysis is challenging. The GHB molecule is very

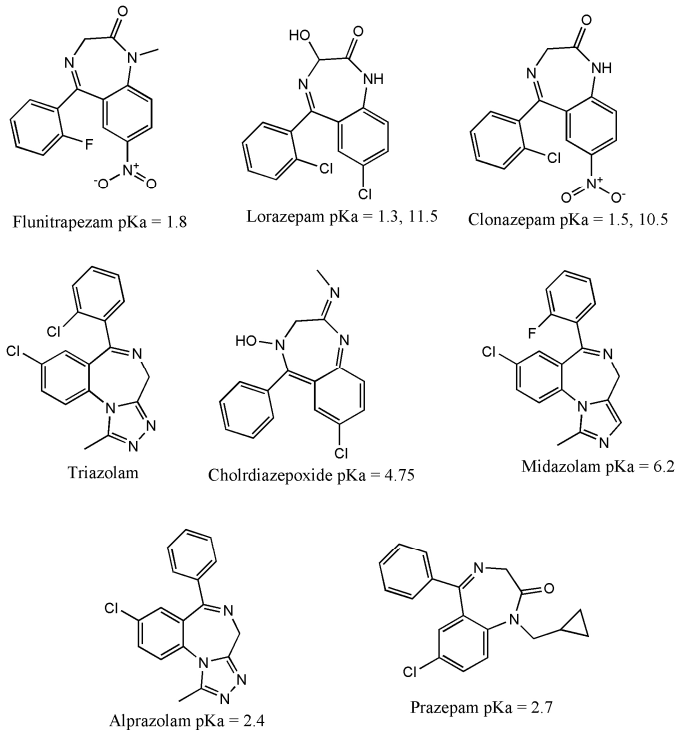

Fig. 2. The structures of benzodiazepines

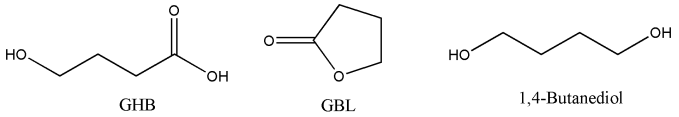

Fig. 3. γ-Hydroxybutyric acid and its analogs γ-butyrolactone and 1,4-butanediol.

small and polar and the chromatographic analysis proves challenging in most of the cases. A highly sensitive and selective detection method is required for biological and biomedical investigations of this compound. Moreover the intrinsic UV absorbance of the carboxylic group, even at low wavelengths (e.g. 210 nm), is not sufficient to provide adequate detection sensitivity (Zacharis et.al., 2008).

Many of these drugs are well-known recreational drugs of abuse, prescription medications, or pharmaceuticals. Most are fast-acting, strong central nervous system depressants that mimic ethanol intoxication. Because the central nervous system depressant effects of these drugs tend to imitate one another, it is highly unlikely that one can determine the drug used in a DFSA case simply by symptoms alone. Moreover Drug-facilitated sexual assault victims often do not report the incident until several hours or, in fact, several days after the event, because of amnesia and doubt about what may have happened, and possibly other psychological reasons, so it is very important to know how long after a single dose intake the presence of the drug could be detected and determined in biological fluids. Hence the method should be capable of recognize these drug from a biological sample even after long hours from the drug administration.

BIOLOGICAL SPECIMENS FOR DATE-RAPE DRUG ANALYSIS

Urine is the most useful specimen in the majority of date drug investigations. Because drugs and metabolites are concentrated in urine, they tend to be more readily detectable in this specimen. Urine sample should be collected as soon as possible after the crime, not to exceed 96 hours after the suspected drug exposure (LeBeau et.al., 1997). If possible, 100 mL of urine should be collected to ensure that enough of the specimen is available for the laboratory to perform a sensitive, thorough analysis while also retaining a suitable portion for retesting (LeBeau et.al., 1997). These specimens should be refrigerated or frozen until analysis. Recent reports suggest that the addition of sodium fluoride to these samples may also be beneficial, particularly when there will be an extensive delay between collection and analysis (Kerrigan et. al., 2002; LeBeau et.al, 2007). In blood specimens, the detection limits most drugs used to facilitate crimes fall below the typical laboratory detection limits within 24 hours after ingestion, therefore restrict the usefulness of this specimen. In cases in which blood can be collected a short time after drug ingestion, the combination of blood and urine specimens may provide a clearer picture as to the window of exposure to the drug. Blood specimens should be placed into collection tubes containing sodium fluoride. At least 7 to 10 mL of blood should be collected when it can be provided within 24 hours of the suspected drug exposure (LeBeau et.al., 1997). It is important to note that blood samples should be accompanied by a urine specimen.

Often, a victim of a DFSA does not present to medical or law enforcement people until weeks or even months after the alleged crime. At this point, it is no longer possible to find evidence of a drug in blood or urine specimens. In these cases, samples of hair have shown great promise, particularly when newer analytical instrumentation with superior sensitivity is used (Negrusz, et.al., 2002; Frison et.al., 2003 and Goulle et.al., 2003). Hair samples pose more complication in interpretation compared to blood and urine specimen due to the lake of information about its association of DFAS drugs with it. It requires skilled person to interpret the results of the segmental analysis. It has also been reported that the human biological fluids cerebrospinal fluid (CSF) and saliva were employed as specimens for date-rape drug analysis (Zacharis et.al., 2008).

SAMPLE PREPRATION TECHNIQUES FOR VARIOUS BIOLOGICAL SPECIMENS

For date-rape drug analysis, specimens specifically for drug analysis will need to be collected from complainants, in turn requiring changes in sexual assault evidence collection kits and protocols. Similarly the sample preparation methods also are validated in order to preconcentrate the DFSA drugs in the sample. It needs special sample treatment procedure since the steps involved in the sample collection is different for date-rape drug analysis.

A standard procedure has been reported in many literatures for hair specimen. Based on the collected head hair specimens from sexual assault complainants, we could use possible microscopical hair comparisons. A common method for the hair sample treatment protocol similar to that of the one reported by Negrusz et al., 2000. They showed a remarkable difference in the resolution between the untreated and treated samples. The sample undergoes solid phase extraction (SPE) (Negrusz et al., 2000 and Lee et.al., 2011) before analysis.

The hair samples were cut approximately 1.5 cm from the root end, pulverized and 50 mg aliquots were analyzed and internal standard and methanol and it was sonicated for one hour. The tubes were then centrifuged and the supernatant was stored at 4°C. The hair samples were again centrifuged the supernatants were pooled, then, glacial acetic acid and deionized water were added. The SPE columns were conditioned with methanol, deionized water, and glacial acetic acid. The bed of the column was washed with deionized water hydrochloric acid and methanol. The collection tubes were placed in the rack and the drugs were eluted using mixture of methylene chloride: isopropanol: ammonium hydroxide. The eluent was evaporated to dryness at room temperature using a stream of nitrogen. The dry residue was reconstituted in ethyl acetate and derivatized using heptafluorobutyric anhydride (HFBA).

For urine samples, the similar procedure was followed for solid phase extraction, before that the sample undergoes enzymatic hydrolyses with β-glucuronidase and sodium acetate buffer (Negrusz et.al., 2000). The sample was sonicated and incubated for 2 hours at 37˚C. Following incubation, the sample undergoes SPE.

Blood samples were extracted directly as follows: after priming the SPE column with of methanol and phosphate buffer, samples were allowed to pass through the column under positive air pressure. The cartridge was rinsed successively with of methanol–water, orthophosphoric acid, methanol–water and dichloromethane. The analytes were eluted with 2 ml of the elution solvent and the eluant was dried under a stream of nitrogen. The residue was dissolved in 30 ml LC mobile phase, transferred to autosampler vials fitted with 200-ml inserts and 5 ml of the solution were injected.

Recent analytical strategies for date-rape drugs

Although DFSAs have likely occurred for centuries, reports of these crimes have significantly increased since the mid-1990. DFSAs have increased over the past several years and sexual violence represents a serious public health problem (Deveaux et.al., 2008). When a case of DFSA is suspected, the quality and promptness of biological specimen collection, the use of specific and sensitive analytical techniques, and the collaboration between the clinician and the toxicologist, are essential keys for successful toxicological investigations. Drug-facilitated sexual assault cases require toxicological analysis in addition to the usual forensic biological examinations. Accordingly, specimens specifically for drug analysis will need to be collected from complainants, so it requires changes in sexual assault evidence collection kits and

protocols. A number of methods have been developed to determine these drugs, most focus on the application of gas chromatography-mass spectrometry (GC/MS), liquid chromatography-mass spectrometry (LC/MS) or capillary electrophoresis/MS (CE/MS) for their determination in serum or biologically related samples.

Methods based on Liquid Chromatography-Mass Spectrometry (LC-MS)

The LC–MS and LC–MS/MS are currently the techniques of choice for the screening of abused drugs in different matrixes. Recently, introduction of tandem mass spectrometry coupled to liquid chromatography (LC–MS/MS) has largely improved the performance of the technique by enhancing sensitivity and analyte identification, therefore, LC–MS/MS has led to increase usage in high-throughput analysis. An analytical strategy developed by Chen et al., 2009 for the high throughput screening of abused drugs in human urine is performed by liquid chromatography–heated electrospray ionization/ tandem mass spectrometry. This technique demonstrated for the analysis of various abused drugs including amphetamine and its analogues; ketamine and its metabolite; cocaine and morphine and some new abused drugs. The real suspected urine samples were stored at −30˚C in a refrigerator before analyzed. In this study a novel heated electrospray ionization (HESI) source coupled to selected reaction monitoring (SRM) scan mode was used. To evaluate the ion transition of all abused drugs in the SRM mode, the individual drug standard solution was infused into HESI source by using a syringe pump and ionized in positive ionization mode. All abused drugs in this study were ionized by using positive HESI ionization mode and the protonated molecule, [M+H]+ was the base peak for all abused drugs in the full scan spectrum. The mass ion chromatograms of suspected urine sample were shown in Fig. 4.

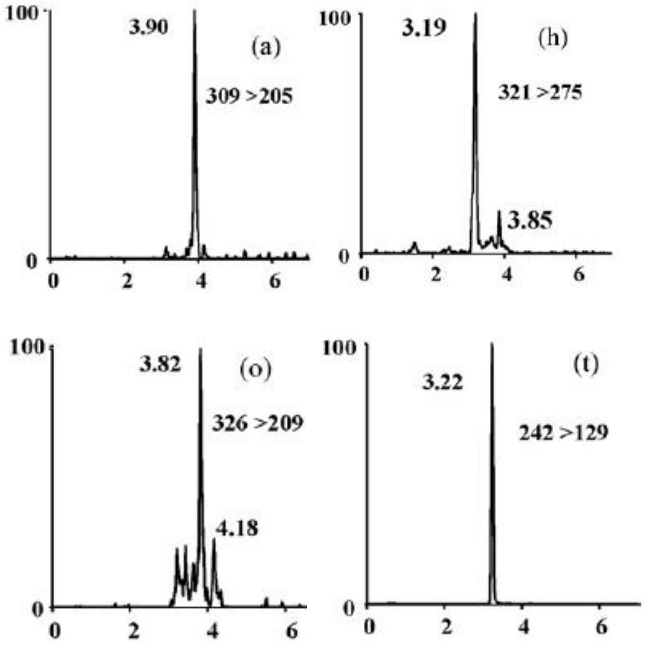

Fig. 4. Mass ion chromatogram of the sample produced by LC–HESI-MS/MS. (a) Alprazolam; (h) lorazepam; (o) midazolam; (t) ketamine.

Parkin et.al., 2008 reported that an efficient quantidatve assay using Ultra-performance liquid chromatography–mass spectrometry (UPLC–MS/MS) following extraction of urine sample using mixed-mode (cation and C_8) solid-phase cartridges for the analysis of ketamine and it metabolites. Separation was done by UPLC system with C_{18} column and detection was performed on a triplequadrupole mass spectrometer with electrospray ionization working in positive ionization mode. They were chosen three ion transitions for confirmatory purposes. As ketamine and norketamine (including their stable isotopes) are available as reference standards, the assay was additionally validated for quantification purposes to study elimination of the drug and primary metabolite following a small oral dose of ketamine. They evaluated selectivity, specificity, linearity, limit of detection, limit of quantitation, precision, recovery and dilution integrity for ketamine and its primary metabolites norketamine and dehydronorketamine (Fig. 5). All other assay analysis including inter-day, intra-day analysis were employed and for linearity of the assay six different concentration ketamine and norketamine and deutarated internal standard were added. With careful analysis and proper validated methodology, the authors achieved very low limits detection 0.03 ng/mL and 0.5 ng/mL for ketamine and norketamine respectively.

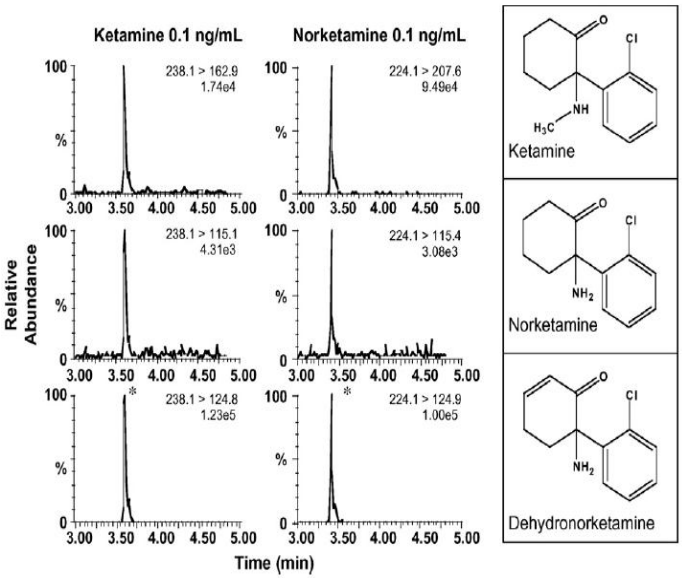

Fig 5. Selected ion chromatograms from an extracted urine calibrant of the quantifying and two qualifying ions of ketamine, norketamine and dehydronorketamine at 0.1 ng/mL.

Another interesting study, reported by Honeychurch et.al., 2008, demonstrated the determination of the potential date-rape drugs flunitrazepam and nitrazepam by liquid chromatography with dual-electrode-detection using a carbon fibre veil electrode (LC-DED). The LC-DED assay for the simultaneous determination of nitrazepam and flunitrazepam using the reductive–reductive mode of detection conditions which were optimized for their determination in a beverage sample. They utilized a LC-DED system that employed 3 mm diameter glassy carbon electrodes as the working electrodes in both the generator and detector cells.

They improved the efficiency of the detection system by substituting a carbon fibre veil electrode (CFVE) for the glassy carbon electrode in the generator cell which has shown superior electrochemical property. This material is known to have a very large surface area coupled to a low resistivity. The much larger surface area of the CFVE would allow for a higher percentage of the target analytes being converted in the generator cell and hence increase the sensitivity of the method.

Cyclic voltammetric investigations were performed to determine the behavior of nitrazepam and flunitrazepam at these CFVEs. The chromatographic and electrochemical conditions were then optimized, and the LC-DED assay was applied to the determination of nitrazepam and flunitrazepam in a spiked soft drink (Fig. 6).

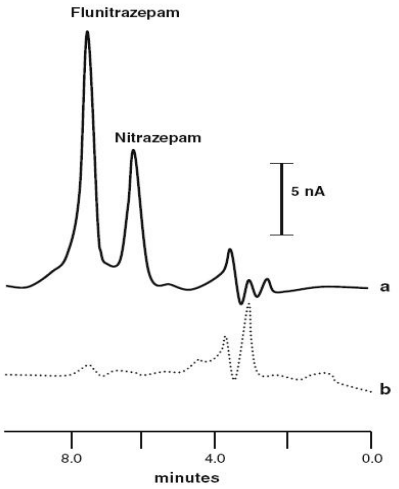

Fig. 6. Representative chromatograms of a beverage sample (Pepsi Max) obtained by LC-DED for (a) fortified at 1.0 μg ml− 1 of nitrazepam and 1.47 μg ml−1 flunitrazepam and (b) unadulterated

The cyclic voltammetric investigations show that both nitrazepam and flunitrazepam exhibited similar electrochemical redox reactions at the CFVE to that observed with other more traditional materials such as Hg . As the result of these findings they proved that it was possible to use the CFVE for the LC-DED determination of these compounds. Further Hydrodynamic Voltammetry investigations were made to identify the optimal applied potential for the detector cell.

Methods based on Gas Chromatography-Mass Spectrometry (GC-MS)

Negrusz A. et al., 2000 developed a highly sensitive NCI–GC–MS (Negative chemical ionization GC–MS) method for quantitation of 7-aminoflunitrazepam and Flunitrazepam in urine. They employed enzymatic hydrolysis with β- glucuronidase followed by solid phase extraction, then detection of flunitrazepam and its metabolites. By using micro-plate enzyme immunoassay, flunitrazepam and its metabolites were detected in the urine of 10 subjects after a single 2-mg oral dose of Rohypnol up to 21 days in one volunteer, 10 days in two cases, 8 days in 4 subjects, and 5 days in three subjects. Detectable levels of flunitrazepam and its metabolites were observed in urine five days after drug intake in all test subjects regardless of urine treatment used. The authors, therefore, recommended commercially available micro-plate enzyme immunoassay in sexual assault investigation when flunitrazepam or other benzodiazepine use is expected.

The application of NCI–GC–MS allowed investigators to detect and quantify 7-aminoflunitrazepam in urine samples (using two ions) after a single 2-mg dose of Rohypnol up to 28 days in three out of ten subjects, 21 days in one subject, and 14 days in the remaining six subjects. Unchanged Flunitrazepam was present in urine samples up to 5 days in only one subject, 3 days in seven subjects, and 24 h in two subjects. The study clearly indicated that the urine specimens could most likely be collected from drug-facilitated rape complainants for at least 14 days after an alleged incident involving a single dose of the drug, and still be employed successfully to detect the presence of the drug metabolite. The limit of quantitation for 7-aminoflunitrazepam (after derivatization with HFBA) was 10 $pgmL^{-1}$ and 100 $pgmL^{-1}$ for flunitrazepam. NCI–GC–MS offers much better sensitivity than the conventional EI–GC–MS and has been successfully applied to the detection of various forensically relevant benzodiazepines.

The same group (Negrusz et.al., 2000) demonstrated deposition of clonazepam and its major metabolite 7-aminoclonazepam in hair by NCI–GC–MS analysis of segmented hair samples collected from people taking therapeutic doses of clonazepam. The study revealed that the 7-aminoclonazepam concentrations in hair are higher than concentrations of the parent drug, and it remains in hair for much longer periods of time. The data further suggest that 7-aminoclonazepam can be detected in hair after a single dose of clonazepam, as would be expected in drug-facilitated sexual assault scenarios.

Methods based on Capillary Electrophoresis (CE)

During the past decade, capillary electrophoresis (CE) emerged as a promising, effective and economic approach for separation of a large variety of substances, including those encountered in clinical toxicology. Reliable and automated CE instruments became commercially available and promoted the exploration of an increasing number of CE methods for illicit and licit drugs in body fluids. The widespread applicability of CE, its enormous separation power and high-sensitivity detection schemes make this technology an attractive and promising tool for the detection of date-rape drugs. CE is inherently simple and well suited for the determination of small ionic species. Baldacci et. al., 2003 developed an assay for urinary GHB that is based upon liquid–liquid extraction and capillary zone electrophoresis (CZE) with indirect UV absorption detection. The assay was successfully applied to urines collected after administration of 25 mg sodium GHB/kg body mass. Negative electrospray ionization ion-trap tandem mass spectrometry was used to confirm the presence of GHB in the urinary extract. All urines were filtered prior to analysis using disposable syringe filters For extraction, urine samples (0.1 ml, plain or diluted urine) were placed in 5 ml glass tubes and spiked with 25 ml of an aqueous solution containing 1

mg/ml sodium OSA (internal standard). Then, 500 ml of a saturated aqueous solution of NaH_2 $PO_4.H_2$ O and 4 ml of ethyl acetate were added. After shaking with a horizontal shaker (5 min) and centrifugation at about 1500 *g* (3000 rpm) for 3 min, the organic phase (upper phase) was transferred into a new glass vial and evaporated in a water bath under a gentle stream of air at about 40˚C. The residue was redissolved in 100 ml of water (for CE analysis) or in 100 ml of a mixture of methanol–water (80:20, v/v) containing about 0.1% of concentrated ammonia solution (for ESI–MS analysis).

The method has been validated and all other parameters such as running buffer, internal standard, pH were optimized with suitable techniques. Experiments performed using a running buffer comprising 4 m*M* nicotinic acid and histidine (pH 6.2) indicated that butyric acid could be a suitable internal standard. Certain drugs utilized in sexual assault, such as GBL and benzodiazepines, are neutral at physiological pHs. Since the mobility of GHB is strongly pH dependent buffers with pH values ranging from 4.2 to 6.7 (0.5 pH interval) were employed and both peak position and magnitude were determined to change as function of pH. Electropherograms obtained for various concentrations of (blank, plain, diluted urine) urine samples for best separation.

ESI ion-trap MS was also applied for the confirmation of urinary GHB. Samples were introduced via the syringe of the infusion pump into the liquid chromatography ESI interface of the instrument. Application of an aqueous standard solution containing sodium GHB revealed a parent ion with *m*/*z* of 103.4. Isolation of this ion and fragmentation led to the MS–MS mass spectrum with an *m*/*z* 85.1 ion. Direct urine injection is not possible as the high salt content would extremely pollute the source and the heated capillary. Introduction of the extracts, however, was found to lead to interesting data. With infusion of an extract prepared from the 12-fold diluted blank urine GHB could not be detected. However, with an extract prepared from a 12-fold diluted blank urine fortified with sodium GHB, the presence of GHB could clearly be recognized in the mass spectrum (Fig. 7) and confirmed by MS–MS. The same was found to be true for a urinary drug level of 5 mg/ ml. Signal magnitudes, however, for that sample were quite low, indicating that 5 mg/ml is close to the detection limit. Not surprisingly, GHB could be unambiguously detected in the extract of the 60 min volunteer urine (Fig. 7). These data confirm the validity of the CZE assay with indirect detection. Furthermore, analysis of an extract prepared from the undiluted 30 min urine also revealed the presence of GHB, suggesting a GHB content .5 mg/ ml. ESI–MS appears to provide the better selectivity and thus better sensitivity than CZE with indirect detection.

Another method for the determination of γ-hydroxybutyric acid (GHB) in urine and serum samples with capillary electrophoresis using capacitively coupled contactless conductivity detection (CE-C^4D) was developed by Gong et.al., 2008. Contactless conductivity detection is a relatively new alternative detection method for capillary electrophoresis, which due to its mode of operation has been termed C^4D (for "capacitively coupled contactless conductivity detection").

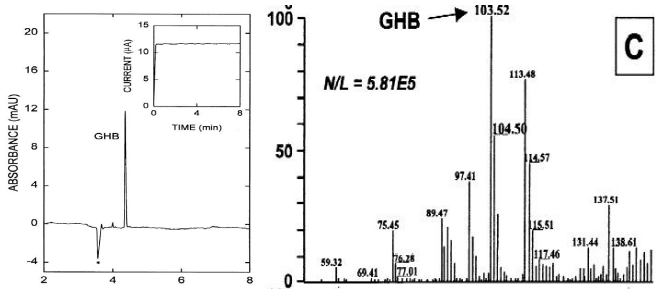

Fig. 7 Electropherogram of a sample containing 100 mg/ml sodium GHB and Mass spectra of extracts prepared from 12-fold diluted blank urine that was fortified with 20 mg/ml sodium GHB

Any charged species can be detected, and the method is thus a versatile and inexpensive alternative, in particular for non-UV active species. In this study, all samples were kept in a freezer at −18˚C until the experiments and were diluted 1:4 with the buffer solution prior to analyses. Separations were carried out in fused silica capillaries (25 μm) in order to minimize Joule heating, which has a more significant effect on baseline stability for conductivity detection then for other modes of detection. Capillaries were rinsed with NaOH followed by deionized water, and running buffer, before commencing experiments. Standard solutions and samples were dissolved in the running buffer to ensure injection under non-stacking conditions and were introduced electrokinetically by applying 5 kV for 7 s. The measurements had been carried out using a method based on ion-chromatography. The background electrolyte composition was optimized and the identification was done by internal standardization and quantification. γ-Hydroxybutyric acid (AHB) was employed as a suitable internal standard because it has a similar structure and CE properties of GHB; the trace amounts of AHB present in biological fluids could be ignored (Fig. 8).

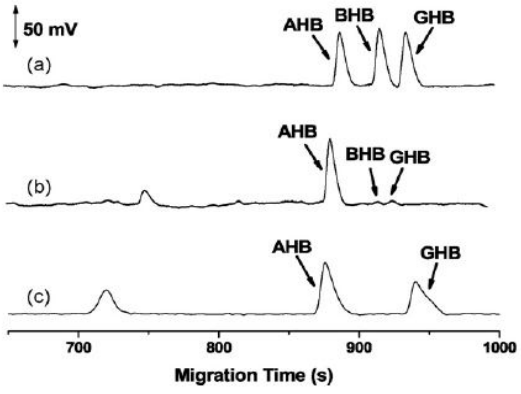

Fig. 8 Electropherograms of (a) a standard mixture of AHB, BHB, and GHB (50 μg/ml), (b) a negative urine sample) of fourfold dilution in the presence of internal standard (AHB) and (c) a positive sample of fourfold dilution in the presence of internal standard (AHB)

While CZE is limited in its ability to detect neutral compounds, micellar electrokinetic chromatography (MECC) proposed for the analysis of a group of date-rape drugs by Bishop et.al., 2004, permits the analysis of uncharged molecules by providing a secondary separation through the addition of a surfactant that forms into micelles. In this paper, an anionic surfactant, sodium

dodecyl sulfate (SDS), was used in the micellar mode to separate a variety of DFSA and club drugs (Fig. 9).

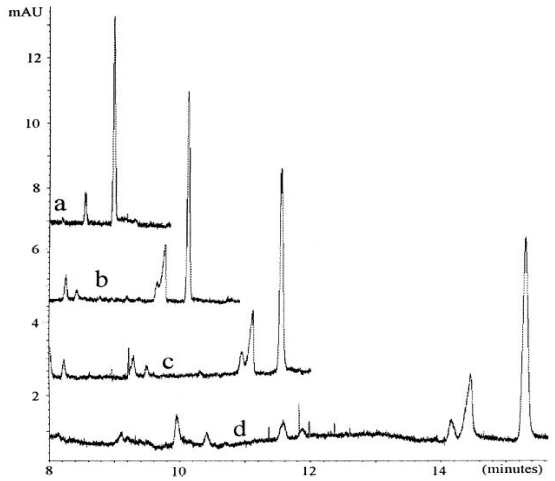

Fig. 9. Extraction of GHB from an interfering peak in beer utilizing changes in both SDS and acetonitrile concentrations: (a) blank beer sample diluted 1:10 with a run buffer of 20 mM SDS with 7% acetonitrile; (b) beer spiked with GHB using a run buffer of 30 mM SDS with 10% acetonitrile; (c) beer spiked with GHB using a run buffer of 30 mM SDS with 15% acetonitrile; (d) beer spiked with GHB using a run buffer of 30 mM SDS with 20% acetonitrile

The buffer system also contains an organic modifier, which has been proven useful to alter the selectivity by affecting the partition coefficients and capacity factors of the analytes. A variety of beverages frequently consumed at parties and bars were chosen for analysis by this method. In CZE, positive, neutral, and negative compounds are separated into zones within the capillary by exploiting their mobility differences. In the case of micellar electrokinetic chromatography, micelles provide further separation of these distinct zones. The sodium dodecyl sulfate surfactants begin forming aggregates at the critical micelle concentration which tends to vary with buffer additives. A better separation was observed when suitable buffer concentration employed.

Compared to customary GC–MS, electrokinetic capillary instrumentation is less expensive and somewhat simpler to operate. However, in addition to the structural information provided by MS, the GC–MS detection sensitivity is higher than that of MECC (Micellar electrokinetic capillary electrophoresis) and CZE with on-column UV absorption detection. Nevertheless, for most methods reported in this review shows better sensitivity than those of commercial immunoassays which are typically employed for rapid urine screening.

CONCLUSION

The analysis of drugs involved in drug facilitated sexual assaults cases requires different sampling techniques, sample treatment procedure compared to usual toxicological investigations. The three classes of drugs that have recently received the most attention are benzodiazepines, γ-hydroxybutyric acid (GHB) analogs and the ketamines owing to their suspected widespread use as date-rape drugs. For the analysis of date-rape drugs, considerable progress has been made over the past 20 years in terms of sensitivity, fast and throughput screening of the drugs and its metabolites. Especially, LC-MS with various detection techniques plays important role in the quantification of club drugs. GC-MS based methods also equally employed for the detection and quantification of date-rape drugs. However, GC methods require extensive derivatization for better sensitivity. CE is an aqueous chromatography technique that has been proven to be useful in both toxicology and in the analysis of seized drugs (Bishop et.al., 2004). The advantages of using CE over traditional GC are that it does not require extensive extraction or derivatization steps.

For future progress, there will have to be significant changes in the initial response to complainants, in evidence collection kits, in the kind of forensic analysis done, and follow-up procedures. Improvements in drug detection methodology and knowledge of drug elimination will help in designing those changes in order that they prove most useful in assisting victims, and identifying and convicting perpetrators of drug-facilitated sexual assault.

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