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Metabolites replace the parent drug in the drug arena. The cases of fonazepam and nifoxipam

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Abstract Fonazepam (desmethylflunitrazepam) and nifoxipam (3-hydroxy-desmethylflunitrazepam) are benzodiazepine derivatives and active metabolites of flunitrazepam. They recently invaded the drug arena as substances of abuse and alerted the forensic community after being seized in powder and tablet forms in Europe between 2014 and 2016. A review of all the existing knowledge of fonazepam and nifoxipam is reported, concerning their chemistry, synthesis, pharmacology and toxicology, prevalence/use, biotransformation and their analysis in biological samples. To our knowledge, fonazepam and nifoxipam-related intoxications, lethal or not, have not been reported in the scientific literature. All the available information was gathered through a detailed search of PubMed and the World Wide Web.

Keywords Fonazepam · Nifoxipam · Designer benzodiazepines · NPS · Active metabolites of flunitrazepam

Introduction

During recent years, a great number of new psychoactive substances (NPSs) or reappeared old ones have been introduced into the market of illicit drugs, and they have been reported to be used by drug addicts. According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 101 NPSs were detected for the first time in Europe in 2014 and 100 NPSs in 2015, and these are the highest yearly numbers recorded ever [1].

These substances usually mimic the pharmacological effects of already known and abused drugs, possessing euphoric, stimulating or hallucinogenic effects, while avoiding detection and classification as illegal. Thus, being legally placed and generally available in Internet shops and online sales, they often become the causes of an increasing number of poisonings and fatal intoxications. They also represent an enormous challenge for clinical and forensic toxicologists, as well as policy makers in many countries [2, 3].

A relatively new phenomenon is the occurrence of designer benzodiazepines, like flubromazolam, meclonazepam, fonazepam, nifoxipam and pyrazolam, as they have been highlighted by the EMCDDA since 2011 [4, 5]. Some of them are common active metabolites of classic benzodiazepines (e.g., fonazepam), while others are prescribed medicinal products in some countries (e.g., phenazepam) [4].

Fonazepam and nifoxipam are the desmethyl- and the 3-hydroxy-desmethyl-derivatives of flunitrazepam (one of the 7-nitrobenzodiazepines), respectively, and they are two of its active metabolites. Flunitrazepam has been used as a hypnotic and pre-anesthetic drug for over 20 years, and it is also one of the most widely abused benzodiazepines [6, 7]. Thus, these two metabolites that are also used as new designer benzodiazepines are supposed to possess similar pharmacological and toxicological properties to the parent drug. Nifoxipam could also be a metabolite of fonazepam.

Fonazepam and nifoxipam have recently invaded the drug arena as NPSs and became known in the universal drug community during the last 2 years. A number of nifoxipam seizures were reported between December 2014 and July 2015 in Europe, while in the case of fonazepam, only two recent seizures of the drug have been reported in January 2016 in Germany and in March 2016 in Sweden. These two designer benzodiazepines are sold as research chemicals that are not intended for human or animal consumption [8–10].

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The aim of this article is to review all the available information on chemistry and synthesis of fonazepam and nifoxipam, their pharmacology and toxicology, their biotransformation and their use as drugs of abuse, as well as the published methods for their determination in biological samples. All the reviewed information was gathered through a detailed search of PubMed and the World Wide Web using the keywords 'fonazepam', 'nifoxipam, 'desmethylflunitrazepam', '3-hydroxy-desmethylflunitrazepam', 'pharmacology', 'determination', 'analysis', 'toxicology', 'intoxications', 'fatalities', 'identification', 'biological fluids' and 'legal status'. Nevertheless, no data on their toxicology or extended information on their prevalence were found. Reported seizures around the world and their legal status are also presented.

Chemistry

Fonazepam (desmethylflunitrazepam) and nifoxipam (3-hydroxy-desmethylflunitrazepam) are two of the metabolites of flunitrazepam that result from the in vivo demethylation of the parent compound followed by 3-hydroxylation of the benzodiazepine ring in the case of nifoxipam [9, 11–13]. Taking into consideration the structure of nifoxipam, we can also assume that it could be the 3-hydroxy metabolite of fonazepam. Fonazepam can also be a photodecomposition product of flunitrazepam [14].

The IUPAC name of fonazepam is 5-(2-fluorophenyl)-7-nitro-1,3-dihydro-1,4-benzodiazepin-2-one, while Ro-05-4435 or Ro 5-4435, N-desmethylflunitrazepam, desmethylflunitrazepam and norflunitrazepam are also used. Its CAS number is 2558-30-7. Fonazepam has the molecular formula $C_{15}H_{10}FN_3O_3$, a molecular weight of 299.26 g/mol, a boiling point of 470.74 °C at 760 mmHg and a melting point of 198.93 °C [9, 15, 16].

The IUPAC name of nifoxipam is 5-(2-fluorophenyl)-3-hydroxy-7-nitro-1,3-dihydro-2H-1,4-benzodiazepin-2-one. It is also named as 5-(2-fluorophenyl)-3-hydroxy-7-nitro-1H-benzo[e][1, 4]diazepin-2(3H)-one, DP370 and 3-hydroxydesmethylflunitrazepam. Its CAS number is 74723-10-7. Nifoxipam has the molecular formula $C_{15}H_{10}FN_3O_4$, a molecular weight of 315.26 g/mol and a log P value of 10.45. It is a crystalline solid that may be more soluble in water than its parent compound, due to the presence of the hydroxyl group in the 3 position of the benzodiazepine ring. In its powder form, it is also stable for 2 years [11, 12, 17, 18].

Synthesis

The synthesis of fonazepam was achieved in 1963 in Hoffmann-La Roche laboratories by Sternbach et al. [19]. It was the product of the direct nitration of the corresponding unsubstituted benzodiazepinone with a mixture of concentrated sulfuric acid and potassium nitrate at 45–50 °C. Fonazepam is formatted in vivo via demethylation of flunitrazepam (the parent drug), during its biotransformation (Fig. 1), and it was identified as its active metabolite in 1976. Nifoxipam is the product of 3-hydroxylation and demethylation of flunitrazepam [13, 20, 21].

Prevalence and use

Fonazepam and nifoxipam are normally used in tablet form and are administered in doses ranging from 0.5 to 3 mg [18, 22, 23]. They are also available in powder form [18]. The most common routes of administration are the oral and sublingual [22–24]. Users of these designer benzodiazepines self-reported the use of other benzodiazepines as well, like alprazolam, clonazepam, etizolam, diazepam, lorazepam, flubromazolam or meclonazepam [22].

Biotransformation

It is known that flunitrazepam undergoes biotransformation via N-demethylation, 3-hydroxylation and glucuronidation, and/or reduction of the nitro group to an amine with subsequent acetylation (Fig. 1) [13, 20, 21]. Its formation is mediated by CYP2C19, CYP3A4 and CYP1A2 [25–29]. Over a 7-day period, an average of 84 % of an oral dose is eliminated in urine. At least 11 metabolites of flunitrazepam have been identified in urine. 7-aminoflunitrazepam (10 % of a dose), 3-hydroxyflunitrazepam (3.5 %), 7-acetamidonorflunitrazepam (2.6 %) and 3-hydroxy-7-acetamidoflunitrazepam (2.0 %) are the major ones, while less than 0.2 % of the initial dose is excreted unchanged [13, 20]. We can assume that fonazepam and nifoxipam undergo similar biotransformation pathways (Fig. 1). There is no available information on the biotransformation of fonazepam. On the other hand, Meyer et al. [30] proved the metabolic pathway of nifoxipam based on its identified urinary metabolites. Thus, nifoxipam was mainly reduced to the respective 7-amino benzodiazepine and then acetylated (Fig. 1). The acetylated metabolite was further conjugated with glucuronic acid. In that study, other metabolic steps, such as hydroxylation or sulfate conjugation were not observed, probably because the metabolic patterns and the metabolites detected depend on the ingested dose and the timespan between intake and sampling which were not known in detail. The suggested metabolic pathway of nifoxipam is presented in Fig. 1 [30].
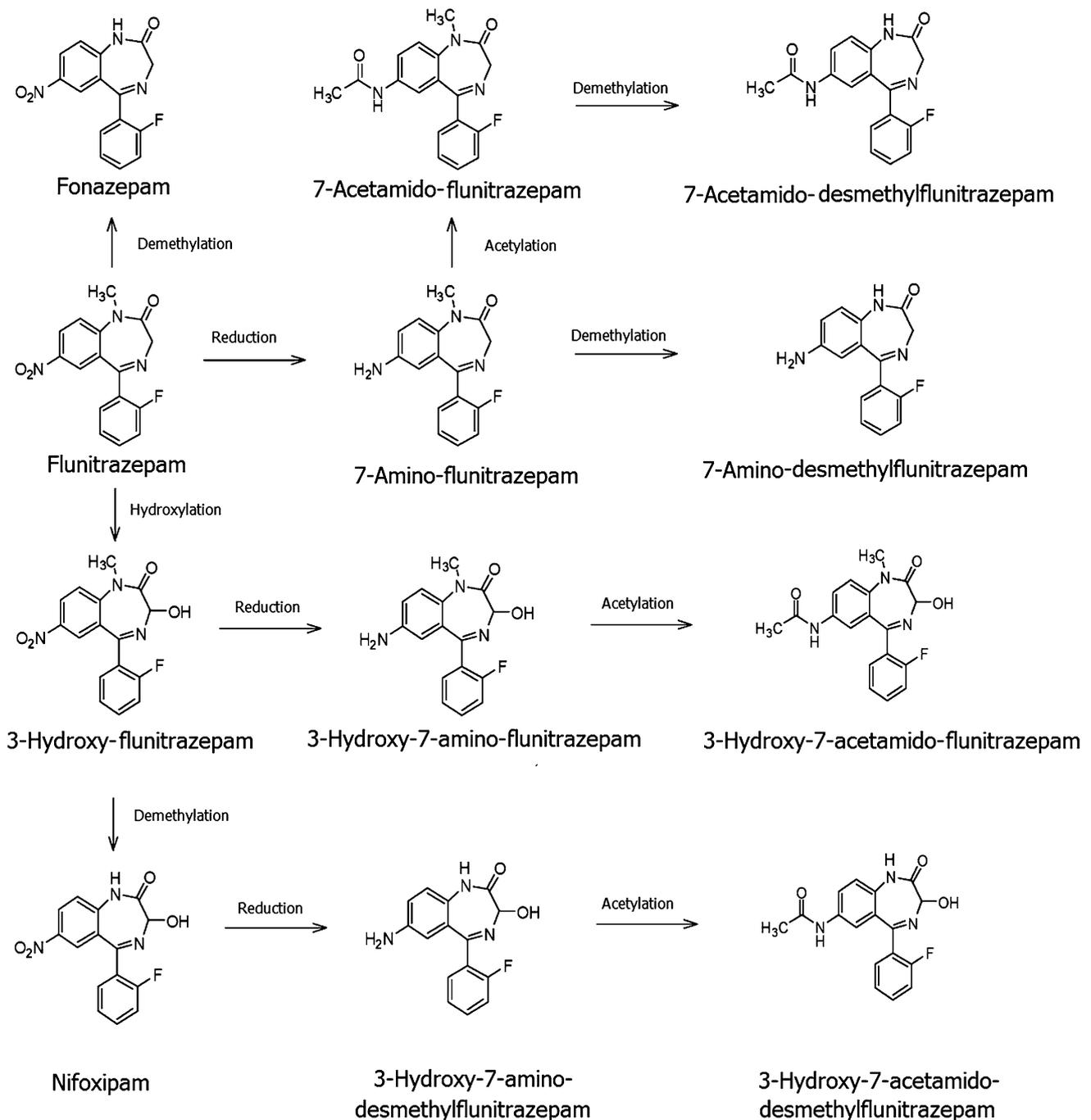
Fig. 1 Phase I biotransformation pathways of flunitrazepam, fonazepam and nifoxipam in humans, as suggested by Wendt [20] and Meyer et al. [30]

Pharmacology

Little is known about the pharmacology of fonazepam and nifoxipam. As it has already been mentioned, they are two active metabolites of flunitrazepam along with 7-aminoflunitrazepam (Fig. 1) and, consequently, a similar pharmacological behavior is expected [13, 20].

Flunitrazepam is a low-dose sedative with therapeutic doses ranging from 0.5 to 2 mg and it is used in short-term treatment of moderate insomnia and as a premedication for minor surgical procedures [31, 32]. Fonazepam and nifoxipam, as benzodiazepine derivatives, probably possess similar pharmacological activity by binding the benzodiazepine receptors but they are not used therapeutically.

More specifically, they bind with a subset of $GABA_A$ receptors, increasing the affinity of the neurotransmitter GABA for these receptors within the central nervous system, exactly as flunitrazepam does [31, 33, 34] and diminishing the transmission of several important signal substances such as dopamine, noradrenaline, serotonin and acetylcholine [32]. Research using neural artificial networks has predicted the binding affinity of fonazepam to benzodiazepine/$GABA_A$ receptors [35]. The drug was reported to have a binding activity (log IC_{50}) of 0.176 and a predicted value of 0.565 [35, 36]. Structure-activity relationship studies showed that position 7 of the benzodiazepine ring is the most important location on the molecule for increasing the receptor affinity, accounting for 30 % of the total connection weight. Increased lipophilicity and electronic charge by the presence of the nitro group have been directly related to increases in receptor affinity for both fonazepam and its parent drug, flunitrazepam. The fact that the latter possesses a higher binding activity as well as a predicted value (0.580 and 0.778, respectively) than its metabolite could be explained by the presence of the methyl group on the nitrogen of the benzodiazepine ring [35].

According to information gathered from users, the common oral dosage of nifoxipam is between 0.5 and 1 mg, reaching 2 mg in some cases. Its onset time of action after oral intake is 45–120 min and the duration of action is approximately 10–75 h [17, 37]. Users report that the intake of a 2 mg tablet of nifoxipam causes *"only a small relaxing feeling and body sedation"*, while a total dosage of 8 mg can cause benzodiazepine-like sedation and anti-anxiety effects along with slight euphoria and *"a little heavy"* feeling accompanied with nightmares [22]. Other nifoxipam users have experienced anxiety relief, greater hypnotic action than diazepam, as well as euphoria within 10–15 min after intake of 1 mg along with a drink [23]. In any case, euphoria and amnesia are expected effects after their use [17]. Anticonvulsant properties of fonazepam and nifoxipam are also expected, in part or entirely, due to binding to voltage-dependent sodium channels rather than benzodiazepine receptors [38].

Toxicology

To our knowledge, toxicology data on fonazepam and nifoxipam has not been published. It is assumed that as fonazepam and nifoxipam are active metabolites of flunitrazepam, they could result in respective toxicity and adverse effects, and that their abuse could have similar addictive potential as the parent drug.

Sedation is one of the most common physical effects of flunitrazepam and its active metabolites. These metabolites are moderately sedating and can potentially lead to a lethargic state. In some cases, users experience sleep deprivation that increases proportional to dosage and this sense becomes eventually enough to force a person into complete unconsciousness [17].

The predominant symptoms due to flunitrazepam overdose include ataxia, drowsiness, dizziness, hypotension, muscle relaxation, respiratory depression and coma that can be controlled successfully with supportive therapy [17, 18, 39]. Other adverse effects are loss of motor control, lack of coordination, slurred speech, confusion and gastrointestinal disturbances that last 12 h or more [40]. They also cause lack of coordination, which may lead to falls and injuries, and impair psychomotor functions, such as reaction time and driving skill with increased likelihood of road traffic accidents [17].

Chronic use of flunitrazepam can lead to physical dependence and appearance of withdrawal syndrome after discontinuation. Likewise, nifoxipam is extremely physically and psychologically addictive. Within a couple of days of continuous use, tolerance is developed along with sedative/hypnotic effects, while after cessation, it returns to baseline in 1–2 weeks. As with all benzodiazepines, withdrawal or rebound symptoms may occur after ceasing treatment abruptly following a few weeks or longer of steady dosing, while benzodiazepine discontinuation is difficult and potentially life-threatening for individuals that use it regularly without progressive reduction of the dosage. An increased risk of hypertension, seizures and death has been reported [17, 41].

It has to be mentioned here that nifoxipam presents cross-tolerance with all benzodiazepines, reducing in this way their pharmacological effects [17].

The use of flunitrazepam in combination with alcohol or opioids is always a particular concern since both of these central nervous system depressants potentiate each other's toxicity [17, 42–44]. The same is expected for its active metabolites. So, similar dangerous and potentially lethal combinations could include the simultaneous use of fonazepam or nifoxipam with other depressants, dissociatives and stimulants that usually result in fatal levels of respiratory depression, muscle relaxation, sedation, amnesia, and vomiting during unconsciousness [17].

Paradoxical effects, like increased seizures in epileptics, aggression, disinhibition, increased anxiety, irritability, violent behavior and suicidal behavior have been described during the therapeutic use of benzodiazepines although they are rare with an evidence rate below 1 % [45, 46]. These effects occur with greater frequency in recreational users, individuals with mental disorders, children and patients on high-dosage regimens [47, 48]. Similar paradoxical effects could be expected to appear to recreational users of fonazepam or nifoxipam.

Seizures

The first seizures of fonazepam were made in Europe in 2016. In January 2016, fonazepam was detected in a seized sample of 1 g of white/yellow powder that was sent by mail from an online chemical company based in China. The sample was identified as fonazepam by the Medical Center at the University of Freiburg, Institute of Forensic Medicine, Forensic Toxicology Department in Germany [10, 49]. Two months later, in March 2016, fonazepam was detected in 51 tablets (27 white, 15 blue and 9 grey tablets) seized by police in Linköping, Sweden. It was confirmed by gas chromatography–mass spectrometry (GC–MS) using a reference standard [10, 50].

The first seizure of nifoxipam was reported in April 2014 in Visby, also in Sweden. The drug was analytically confirmed, by the Swedish National Laboratory of Forensic Science, as the active ingredient of 20 brown tablets seized by the police using GC–MS, liquid chromatography–mass spectrometry (LC–MS) and nuclear magnetic resonance spectroscopy [12, 51]. In December 2014, four round, light-brown tablets marked as "Nifoxipam 1 MG" were sent from the UK to Finland where they were seized. The identification of its active ingredient, nifoxipam, was performed by using GC–MS and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [12, 52]. A month later (January 2015), the Norwegian federal police seized 101 brown tablets found in a mail package sent from the UK to Lørenskog, Norway. Nifoxipam was identified by GC–MS [12, 53].

In April 2015, the Slovenian police seized a light-brown tablet of 0.21 g and the active ingredient was confirmed to be nifoxipam by high-performance liquid chromatography–time-of-flight-mass spectrometry (HPLC–TOF-MS). In May 2015, the Danish federal police seized 25 light-beige tablets of 0.1 g that were sent from the UK to Denmark. The tablets were seized at the Copenhagen International Post Office and were marked by the street and chemical name of their ingredient. The presence of nifoxipam was analytically confirmed by GC–MS and LC–QTOF-MS using a database [12, 54, 55]. Two more nifoxipam-related seizures were reported in France in April and July 2015. In the first case, 50 beige tablets sent from the UK were seized at the airport [12, 56], while the second seizure concerned a sample of 105 mg beige powder bought as flubromazepam (Lexomil®) [12, 57].

It seems that the problem of trafficking of these designer benzodiazepines concerns, for the time being, only Europe, while there is almost no information available in the USA, Australia or Japan despite the extensive use of other benzodiazepine derivatives for therapeutic or recreational purposes at these places [4, 58, 59].

Determination of fonazepam in biological specimens

Fonazepam and nifoxipam showed significant cross-reactivity, showing a high degree of detectability for the new designer benzodiazepines, by the most common commercially available immunochemical assays for screening purposes in urine (CEDIA, EMIT II Plus, HEIA, KIMS II) [60, 61]. For the confirmation of the results, several analytical methods, using known chromatographic techniques, have been developed for the determination of fonazepam as a metabolite of flunitrazepam in biological specimens. On the other hand, only three articles have been published that describe analytical methods for the detection and quantification of nifoxipam in biological specimens [30, 60, 61], and in one of these studies nifoxipam was determined as the parent drug along with its metabolites [30]. A summary of all the developed chromatographic methods for the determination of fonazepam and/or nifoxipam is shown in Table 1.

Liquid-liquid extraction and solid-phase extraction techniques have been used for the isolation of fonazepam and nifoxipam from biological specimens. Different chromatographic techniques have been used for the development of sensitive methodologies, such as GC combined with an electron capture detector [62–65] or MS [66–69], and LC combined with MS [70, 71], MS/MS [30, 61, 72–76], ultraviolet detection [77, 78], diode array detector [79] or photodiode array detection [71].

El Mahjoub and Staub [78] used an on-line column switching HPLC method and evaluated two different extraction columns for the determination of fonazepam as a metabolite of flunitrazepam among other metabolites. The procedure was based on direct injection of benzodiazepines on the extraction column followed by the transfer of the compounds to the analytical column.

Another sensitive analytical technique, capillary electrophoresis combined with micellar electrokinetic chromatography, has been described by Huang et al. [80] for the determination of fonazepam along with the other active metabolite, 7-aminoflunitrazepam, and their parent drug, flunitrazepam, in urine after isolation via SPE.

A nano-LC–high-resolution-MS/MS chromatographic method was described for the identification of nifoxipam and its phase I and II metabolites together with other new designer benzodiazepines and their metabolites in urine. Urine samples needed no specific preparation and they were injected directly in the LC system for analysis [30].

No methods for the detection and determination of fonazepam and nifoxipam in seized materials, like tablets or powder, have been described in the literature.

Table 1 Summary of analytical methods developed and used for the determination of fonazepam and nifoxipam in biological specimens

Reference	Biological fluid	Extraction method	Analytical technique	LOD/LOQ (ng/mL)	Recovery (%)	Concentration range (ng/mL)	Application
Fonazepam							
de Silva et al. [62]	Blood	LLE [alkalinization, benzene/dichloromethane (90:10, v/v)]	GC–ECD	n.r.	n.r.	n.r.	Application to clinical biological specimens
Faber et al. [63]	Serum	LLE (benzene)	GC–ECD	n.r.	98.6	10–300	n.r.
Drouet-Coassolo et al. [64]	Plasma	LLE (alkalinization, butyl acetate)	GC–ECD	0.5/1.5	n.r.	1–50	Pharmacokinetic study
Berthault et al. [77]	Serum	LLE [alkalinization, diethyl ether/chloroform (80:20, v/v)]	HPLC–UV (242 nm)	2.5/10	57	10–1000	n.r.
He and Parissis [79]	Plasma / Urine	SPE (chloroform) (hydrolysis with β-glucuronidase in urine samples)	HPLC–DAD (gradient)	4.53/15.1 / 4.33/14.4	59.3 / 99.27	25–500 / 20–400	Application to forensic toxicology and pharmacokinetic studies in humans
Salamone et al. [60]	Urine	Hydrolysis with β-glucuronidase, LLE [alkalinization, dichloromethane/isopropanol (90:10, v/v)]	GC–MS	n.r.	n.r.	50–300	Pharmacokinetic study
Coller et al. [65]	Hepatic microsomal incubations	LLE [alkalinization, hexane/diethyl ether (50:50, v/v)]	RP HPLC–UV (210 nm)	n.r.	n.r.	0.2–10 μM	Study of oxidative metabolism of flunitrazepam
Bogusz et al. [70]	Serum, blood, urine	SPE (methanol/0.5 M acetic acid)	LC–APCI–MS	1.0/n.r.	99	1–500	Application to real samples from subjects suspected of DUI of drugs
Nguyen and Nau [66]	Urine	SPE [dichloromethane/isopropanol/NH₃ (80:20:2, v/v/v)]	GC–MS	n.r.	n.r.	n.r.	n.r.
Borrey et al. [67]	Urine	SPE [hydrolysis with β-glucuronidase, elution with methanol, derivatization with pyridine/acetic anhydride (1:1, v/v)]	GC–MS	LOD = 18 (scan mode) LOD = 1.2 (SIM mode) LOQ < 50 (scan mode)	93.4–99.2	20–1000 (scan mode) 2–100 (SIM mode)	n.r.
El Mahjoub and Staub [78]	Urine	Online extraction with column switching. First column: elution with 20 mM phosphate buffer (pH 7.2)/ACN (94:6, v/v). Second column: elution with 30 mM phosphate buffer (pH 7.5)/ACN (94:6, v/v)	HPLC–UV (245 nm)	n.r.	n.r.	n.r.	n.r.

Table 1 continued

Reference	Biological fluid	Extraction method	Analytical technique	LOD/LOQ (ng/mL)	Recovery (%)	Concentration range (ng/mL)	Application
Kollroser and Schober [72]	Plasma	SPE [dichloromethane/isopropanol/NH$_3$ (78:20:2, v/v/v)]	LC–APCI–MS/MS	2.0/5.0	93.7–103	5–100	Application to forensic and clinical toxicology
Jourdil et al. [73]	Plasma Urine	Automated SPE [dichloromethane/isopropanol/NH$_3$ (70:30:2, v/v/v)]	LC–ESI–MS/MS	0.04/0.5	81	n.r.	n.r.
Pirnay et al. [68] Pirnay et al. [69]	Rat plasma	LLE (dichloromethane/1,2-dichloroethane/heptane/isopropanol), derivatization with BSTFA with 1 % TMCS	GC–MS	n.r/0.125	81 (plasma)	0.125–12.5	Pharmacokinetic and toxicokinetic studies in rats.
Dussy et al. [71]	Whole blood Plasma	LLE (1-chlorobutane) or SPE [chloroform/isopropanol/NH$_3$ (69:29:2, v/v/v)]	HPLC–PDA-MS and PDA	n.r./3.0	n.r.	3–100	Application to forensic toxicology
Huang et al. [80]	Urine	SPE [dichloromethane/isopropanol/NH$_3$ (78:20:2, v/v/v)]	CE–MEKC	12/n.r.	n.r.	n.r.	n.r.
Forsman et al. [74]	Urine	Hydrolysis with β-glucuronidase, SPE [dichloromethane/isopropanol/NH$_3$ (80:20:2, v/v/v)]	LC–MS/MS	n.r.	n.r.	n.r.	n.r.
Verplaetse et al. [75]	Whole blood and urine	SPE [acetone/chloroform (1:1, v/v) and 2 % ammoniated ethyl acetate]. Hydrolysis with β-glucuronidase in urine samples	LC–ESI–MS/MS	0.050/2.0	n.r.	2–500	Application to forensic toxicology
Jeong et al. [76]	Urine	Centrifugation of urine samples, addition of internal standard, direct injection of 5 μL	LC–MS/MS	3.0/10.0	n.r.	10–100	n.r.
Nifoxipam							
Salamone et al. [60]	Urine	Hydrolysis with β-glucuronidase, LLE [alkalinization, dichloromethane/isopropanol (90:10, v/v), derivatization with MTBSTFA, 1 % TBDMS]	GC–MS	n.r.	n.r.	50–300	Pharmacokinetic study
Meyer et al. [30]	Urine	Dilution in internal standard, centrifugation	Nano-LC–HR-MS/MS	n.r.	n.r.	n.r.	Identification of nifoxipam metabolites and application in drug testing analysis
Bergstrand et al. [61]	Urine	n.r.	LC–MS/MS	n.r.	n.r.	100–1000	Application to clinical and forensic toxicology

ACN acetonitrile, *APCI* atmospheric pressure chemical ionization, *BSTFA* N,O-bis-(trimethylsilyl)trifluoroacetamide, *CE* capillary electrophoresis, *DUI* driving under the influence, *GC–ECD* gas chromatography–electron capture detector, *GC–MS* gas chromatography–mass spectrometry, *HPLC–DAD* high-performance liquid chromatography–diode array detection, *HR* high-resolution, *LC–ESI–MS/MS* liquid chromatography–electrospray ionization–tandem mass spectrometry, *LLE* liquid–liquid extraction, *LOD* limit of detection, *LOQ* limit of quantification, *MEKC* micellar electrokinetic chromatography, *MTBSTFA* (N-tert-butyldimethylsilyl)-N-methyltrifluoroacetamide, *n.r.* not reported, *PDA* photodiode array, *RP* reverse-phase, *SIM* selected ion monitoring, *SPE* solid-phase extraction, *TBDMS* tertiary butyldimethylsilyl trifluoroacetamide, *TMCS* trimethylchlorosilane, *UV* ultraviolet detection

Legal status

Fonazepam and nifoxipam are newly introduced compounds in some European countries. Fonazepam is a controlled substance on Schedule IV of the Controlled Substances Act in the USA as a derivative of flunitrazepam [81]. Nifoxipam is illegal in the UK under the Psychoactive Substance Act, which came into effect on 26 May, 2016 [11]. It has also been controlled in Denmark since 18 February 2016, after amendment of the Executive Order on Euphoriant Substances. Thus, it may only be used for medical or scientific purposes [12].

It is not known to be specifically illegal within other countries around the world. However, people that possess fonazepam and nifoxipam or intend to sell or consume them may still be prosecuted, under certain circumstances, by "analogue" laws [82].

Conclusions

Fonazepam and nifoxipam, two designer benzodiazepines, appeared in Europe after 2014 as NPSs replacing the known controlled benzodiazepines in the drug arena. Although both drugs are active metabolites of flunitrazepam, extended information on their pharmacology and toxicology is not available. Due to their chemical structure, it is assumed that they possess similar pharmacological action and toxicological behavior with the parent drug, flunitrazepam, binding to the same $GABA_A$ receptors. These new designer benzodiazepines are available online labeled as a "research chemical" and "not intended for human or animal consumption". Since they have recently appeared in the drug arena to cover the legal loophole, the international drug enforcement agencies are expected to take actions and measures worldwide against their use or abuse. Increased public vigilance by hospitals, law enforcement and medical examiners is also needed. The final aim is to prevent the expansion of fonazepam and nifoxipam abuse, and the possible intoxications and deaths related to these new designer benzodiazepines.

Compliance with ethical standards

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