The Emergence of New Psychoactive Substance (NPS) Benzodiazepines: A Review

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Abstract

The market for new psychoactive substances has increased markedly in recent years and there is now a steady stream of compounds appearing every year. Benzodiazepines consist of only a fraction of the total number of these compounds but their use and misuse has rapidly increased. Some of these benzodiazepines have only been patented, some of them have not been previously synthesised and the majority have never undergone clinical trials or tests. Despite their structural and chemical similarity, large differences exist between the benzodiazepines in their pharmacokinetic parameters and metabolic pathways and so they are not easily comparable. As benzodiazepines have been clinically used since the 1960s many analytical methods exist to quantify them in a variety of biological matrices and it is expected that these methods would also be suitable for the detection of benzodiazepines that are new psychoactive substances. Illicitly obtained benzodiazepines have been found to contain a wide range of compounds such as opiates which presents a problem since the use of them in conjunction with each other can lead to respiratory depression and death. The aim of this review is to collate the available information on these benzodiazepines and to provide a starting point for the further investigation of their pharmacokinetics which is clearly required.

Keywords: Benzodiazepine, NPS, drug abuse, legal highs

The use and misuse of benzodiazepines

The use and misuse of new psychoactive substances (or "legal highs") has increased significantly around the world in the past 10 years [1] and has to date showed no signs of slowing. In Europe alone the total number of new compounds reported by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has risen rapidly since 2007 with 101 new psychoactive substances reported to the EMCDDA in 2014 [2] and 98 in 2015 [3]. The majority of these compounds have been synthetic cannabinoids, cathinones and phenylethylamines [2]. One group of these compounds, the benzodiazepines, have received limited attention but their use has increased significantly in the past few years. The abuse potential for benzodiazepines was recognised early in their use and led to 33 benzodiazepines being placed under control by the UN Convention on Psychotropic Substances 1971 [4]. Benzodiazepines are one of the most prescribed groups of drugs around the world with the limited available data suggesting that 5.6% of Americans filled a benzodiazepine prescription in 2013 [5]. In England over 5 million doses of diazepam alone were dispensed in 2014, whilst the total number of prescriptions issued for benzodiazepines stood at more than 10.4 million, indicating their widespread use [6] Benzodiazepines are also linked to a significant number of deaths, both via abuse as a drug in their own right and also as part of a deliberate poly pharmacy regime [7]. They are commonly implicated in cases of opioid overdoses, where benzodiazepines are detected in 50 – 80 % of heroin-related deaths and in 40 – 80 % of methadone-related deaths in various countries around the world [7]. Benzodiazepines also account for around 28 – 45 % of drug induced deaths in Europe [2]. A study of 1500 people in 2014 used an internet-based survey to investigate the reasons for the abuse of benzodiazepines and Z-drugs in the United Kingdom (Z-drugs such as zopiclone and zolpidem are structurally different from benzodiazepines but also act via the γ-aminobuytric acid type-A ($GABA_A$) receptor). The study found that the majority of abuse of Z-drugs and

benzodiazepines occurred because users were trying to alleviate stress, to help with sleep or to get high [8]. Unfortunately the study did not differentiate between benzodiazepines or Z-drugs but as a result of their similar effects it is likely that they are used interchangeably. When used in combination with other drugs (such as opioids/opiates) the aim of benzodiazepine use is typically to enhance and/or prolong the high or to reduce the withdrawal effects of the other drugs [9].

Outside normal prescription methods, benzodiazepines are obtained via various routes such as diversion of prescriptions, the illicit market and internet purchasing which is thought to be a rising trend [10]. In 2016 the Research and Development (RAND) corporation published a report suggesting that the UK had the second largest number of online vendors of illegal drugs on the darknet (with the US first) but that UK vendors averaged the most transactions per month [11].

In recent years an increasing number of new psychoactive substances (NPS) benzodiazepines have appeared for sale in various countries. NPS are defined by the United Nations Office on Drugs and Crime as "*substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat.*"[12]. These new psychoactive substances are often sold online and labelled for use as 'research chemicals' only, even though they are implicitly intended for human consumption. Many of these NPS-benzodiazepines have never undergone the clinical testing that is required of licenced medicines and the increasing availability of them may therefore pose serious health risks to poly drug users and benzodiazepine-dependent patients who can no longer obtain their prescription and may turn to other means of obtaining benzodiazepines. We introduce the general way that benzodiazepines are commonly classified (duration of action, half-life and chemical structure) the mechanism of action of benzodiazepines and review what is known to

date about these NPS-benzodiazepines including user experiences, their pharmacology, pharmacokinetics and analytical detection. This data is summarised in **Error! Reference source not found.** and was obtained from a variety of published journal articles except the user experiences which were obtained from chat and comments on internet forums such as Reddit [13], Bluelight [14], Flashback [15] and UK Chemical Research [16]. Caution should be taken when interpreting these user experiences as any experiences are subjective and users may have ingested other compounds at the same time. Instead they serve as a rough guide as to the likely effects that may be expected.

The rise of the NPS-benzodiazepines

The first illicit benzodiazepines identified in Europe to the EMCDDA were phenazepam (fenazepam) and nimetazepam in 2007 [17]. Phenazepam is a prescription drug in the former Soviet bloc [18] and in the intervening years it was detected in an increasing number of cases around the world [19-25]. This led to it being scheduled in the UK and other countries [26-28]. Recently, phenazepam was placed in schedule VI of the 1971 UN Drug control convention [29]. The benzodiazepine-derivative etizolam was the next compound to be detected to the EMCDDA in 2011[30]. It belongs to a class of compounds known as thienodiazepines and is commonly prescribed in Japan [31]. The naming of benzodiazepines and their derivatives is discussed in the next section of this review – "Classification of benzodiazepines". Its appearance mirrored that of phenazepam; a prescription drug in a country outside the UK which subsequently found its way to the UK market. Pyrazolam was the next benzodiazepine to appear on the market and was notable as this was the first benzodiazepine to appear that was not a prescription drug in any country [32]. Following this, multiple benzodiazepines were detected to the EMCDDA that have not been licenced for clinical use anywhere in the world. These benzodiazepines include flubromazepam and

diclazepam in 2013 [33] along with meclonazepam, nifoxipam and deschloroetizolam (a thienodiazepine) in 2014 [34]. Clonazolam and flubromazolam are also thought to have first appeared in 2014 [10] and were subsequently reported to the EMCDDA. Various other benzodiazepines such as adinazolam, nitrazolam and metizolam (another thienodiazepine) have all been reported to the EMCDDA in the 2015 implementation report [35]. Two other benzodiazepines, 3-hydroxyphenazepam (a metabolite of phenazepam [18]) and flutazolam (a Japanese prescription drug [36]) have been detected separately in tablets seized in Sweden in 2015 by the Medical Products Agency (MPA), with the use and spread of flutazolam being monitored and 3-hydroxyphenazepam being subject to an investigation by the MPA [37]. Flunitrazolam, desmethylflunitrazepam (also known as fonazepam) and cloniprazepam were also detected by the MPA in 2016 [38]. Bromazolam [39], desalkylflurazepam (also known as fludiazepam or norflurazepam) [39] and 4-chlorodiazepam (also known as Ro5-4864) [40] are also thought to have appeared at various points in 2016. The years that these benzodiazepines appeared and their year of patient (if available) has been summarised in Table 2 and the timeline can be viewed in Figure 1. Currently hundreds of benzodiazepines have been patented and described in the scientific literature and these are not expected to be the last benzodiazepines that are detected in the so called "explosion" of new psychoactive substances.

NPS-benzodiazepines and thienodiazepines have been implicated in nine drug-related deaths in England and Wales between 2013 – 2014 as either being the cause of death or having contributed to death [41]. In 2016, the Psychoactive Substances Act was introduced in the UK [42] with the aim of stopping the "cat and mouse" game of a NPS being produced to circumvent legislation, being controlled and then another being produced. This legislation restricts the production, sale and supply of drugs that are "psychoactive". It is expected following the introduction of this Act that a fall in supply and use of NPS-benzodiazepines

may be expected. However, phenazepam and etizolam are now both controlled benzodiazepines in the UK under the Misuse of Drugs Act (1971) [43] but are still regularly identified in post mortem cases and in drug-impaired drivers in the UK [21, 22].

Classification of benzodiazepines

Benzodiazepines have traditionally been classified in one of three ways, either by;

1) Their duration of action. Benzodiazepines that have durations of action under 24 hours are short-acting while those with durations of action above 24 hours are long-acting [44].

2) Their elimination half-life (t½). Typically, this is consists of four classifications; ultra-short (t½, <6 h), short (t½, 6 h), intermediate (t½ 6-24 h) and long (t½ > 24 h). The reason for these four classifications is because the duration of action of the benzodiazepines can be extended by active metabolites [45-47].

3) Their chemical structure. The core structure of benzodiazepines is a diazepine ring fused to a benzene ring. A phenyl ring is usually attached to the diazepine ring (Figure 2). Most common benzodiazepines are 1,4-benzodiazepines (Figure 2A) (e.g. diazepam [46]) but 1,5-benzodiazepines (Figure 2B) (e.g. clobazam [48]) also exist. A whole host of derivatives of this basic benzodiazepine structure are possible. Some of them involve the addition of another cyclic system to the molecule, for example a triazole ring (Figure 2C) (e.g. alprazolam [49]), imidazole ring (Figure 2D) (e.g. midazolam [50]) or oxazole ring (Figure 2E) (e.g. cloxazolam [51]). Others involve replacement of the benzene ring with a thiophene or pyridine ring. One such group of benzodiazepine derivatives are the thienodiazepines (e.g. etizolam [52]) (Figure 2F). They differ in structure by the replacement of a benzene ring with a thiophene ring but they have similar anticonvulsant, anxiolytic and sedative properties [52-54]. Thienotriazolodiazepines (Figure 2G) (e.g. brotizolam [54]) have a triazole ring fused to

the diazepine ring, much like the triazolobenzodiazepines. 2,3-benzodiazepines such as tofisopam exist [55] (Figure 2H) but despite them having the benzodiazepine ring structure they exhibit different pharmacological properties compared with the other benzodiazepines; they act via the 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) glutamate receptor but still exhibit anxiolytic activity [56, 57]. To the best of the authors' knowledge there are no reports of abuse of 2,3-benzodiazepines. It may only be possible to classify the NPS-benzodiazepines by structure until more information becomes available. Despite being structurally different, thienodiazepines will be grouped together with benzodiazepines as "NPS-benzodiazepines" in this review.

Mechanism of action of benzodiazepines

The main sites of action of benzodiazepines in the human body are *gamma*-Aminobutyric acid A ($GABA_A$) receptors. $GABA_A$ receptors are ligand-gated ion channels which are endogenously activated by *gamma*-Aminobutyric acid (GABA), the major inhibitor neurotransmitter in the central nervous system (CNS) [58]. Their structure consists of five protein subunits that surround a central pore through which Cl^- ions can permeate [58]. Binding of GABA to the receptor triggers the chloride ion pore to open leading to an inhibition of neural signals. There are seven receptor subunit families (α 1-6, β 1-3 γ 1-3, δ, ε, π, θ) but the most common $GABA_A$ receptor combination is α2β2γ, which comprises around 43 % of all $GABA_A$ receptors in the CNS, with 10 other combinations also identified [59, 60]. These isoforms are preferentially distributed within specific regions of the CNS [61]. As a result, the receptors have different pharmacological properties and this helps to explain the differing pharmacological effects observed with the benzodiazepines. The role of $GABA_A$ receptor subunits and addiction has been reviewed by Tan and colleagues[62], with the α1 subunit containing $GABA_A$ receptors thought to be those that are involved in the

addictive properties of benzodiazepines [62-64]. Benzodiazepines bind between the α1 and γ2 subunits at a site that is distinct from the GABA binding site. They act as positive allosteric modulators, increasing the affinity of GABA to the receptor and potentiating the response of the receptor to GABA [65]. Ethanol also binds to the $GABA_A$ receptor [66] as do another class of drugs, the barbiturates [67]. An exception to the benzodiazepines binding to the $GABA_A$ receptor can be found for 4-chlorodiazepam (Ro5-4864) which recently appeared as an NPS-benzodiazepine [40]. 4-chlorodiazepam binds exclusively to the translocator protein (18 kDa) (TSPO 18 kDa) [68], initially known as the peripheral benzodiazepine receptor [69]. TSPO (18 kDa) is found throughout the body and has a variety of biological functions which have been extensively reviewed [69-71] and it is thought to have considerable potential therapeutic value as a pharmacological target [72, 73]. Certain compounds that bind to TSPO (18 kDa) can exhibit typical benzodiazepine effects such as being anxiolytic without causing some side effects associated with benzodiazepine use such as sedation [74]. However 4-chlorodiazepam has been found to induce anxiety and cause convulsions in rats despite being a sedative [75, 76]. Other benzodiazepines such as diazepam also experience some binding to TSPO (18 kDa) [70, 77] but the majority of their pharmacological effects result from the binding of them to $GABA_A$ receptors [71].

Benzodiazepine pharmacokinetics

The pharmacokinetics of benzodiazepines vary widely. The most common route of administration for prescription benzodiazepines is orally but they are also given intramuscularly, intravenously or rectally [44]. When administered orally there is a wide variation between the time taken to reach t_{max} [44]. For example the NPS-benzodiazepine phenazepam reaches a t_{max} between 2 – 4 hours following a 2 mg dose [78] while flubromazepam is only thought to reach t_{max} after 11.8 hours following a 4 mg dose [79]. The

time of day that benzodiazepines are administered can affect t_{max}; triazolam exhibits a t_{max} of ~13 minutes when taken in the morning compared with ~22 minutes when taken in the evening and the half-life ($t_{½}$) was similarly affected (2.94 hours in the morning versus 3.77 hours in the evening) [80]. It was thought that this is as a result of the longer fasting period prior to the dose [80].

Benzodiazepines can have vastly differing half-lives and this has been well reviewed [44]. An important point of note is that the half-life of active benzodiazepine metabolites can be far greater than that of the parent benzodiazepine. For example, desmethyldiazepam (marketed as nordazepam) is an active metabolite of several benzodiazepines and can have a half-life of 96 ±34 hours following oral administration of prazepam [45] or 120 hours following diazepam [46]. Similarly, desalkylflurazepam is the active metabolite of flurazepam and can have a half-life of 40 – 144 hours following oral administration [47]. Desalkylflurazepam is now known to be sold as a novel psychoactive substance [39]. The main monohydroxylated metabolite of the NPS-benzodiazepine flubromazepam can be detected in urine up to 28 days following ingestion compared to 6 days and 20 hours for the parent compound indicating a higher half-life for the metabolite [79]. Similarly, diclazepam is found only in very low concentrations in serum and urine for just over four days. However its metabolites are detectable for longer time periods; delorazepam is detectable for 6 days in urine and 10 days in serum, lorazepam is detectable for 19 days in both serum and urine and lormetazepam is detectable for 11 days in urine [81].

As well as the variations discussed for maximum plasma concentrations and half-lives, other pharmacokinetic parameters exhibit large differences for the benzodiazepines. For example triazolam has a bioavailability of 44 % [82] versus a bioavailability of 97 % for diazepam [83], diazepam is 97 % bound to plasma proteins [84] while alprazolam is only 70 % bound to plasma proteins [85]. Volumes of distribution also differ; oxazepam and the NPS-

benzodiazepine flubromazepam have relatively low volumes of distribution (0.27 l kg^{-1} [86] and 0.73 l kg^{-1} [79] respectively) versus a high volume of distribution of 4.4 l kg^{-1} for flunitrazepam [87].

The differences briefly mentioned above mean that the pharmacokinetics of benzodiazepines cannot be easily compared and specific knowledge of their individual pharmacokinetic parameters is required to understand how they behave in the body. Typical blood concentrations, half-lives and volumes of distribution (where known) for the NPS-benzodiazepines is provided in Table 1.

The majority of drug metabolism occurs in the liver, primarily by oxidative metabolism mediated by the cytochrome P450 (CYP450) family of enzymes [88]. CYP3A4 is the enzyme most commonly involved in the metabolism of benzodiazepines [89]. However other enzymes are also involved in the metabolism of benzodiazepines such as; CYP3A5, CYP2C19, CYP2B6, CYP2C18 and CYP2C9 [90]. The CYP3A4 enzyme can also conjugate benzodiazepines containing a nitro group with a glutathione group which can result in cytotoxicity in the liver [89]. Polymorphisms in metabolic enzymes can lead to an alteration in the metabolism of specific drugs. There is only limited evidence that polymorphisms of CYP3A4/5 clinically affect benzodiazepine metabolism [91]. However CYP2C19 polymorphisms have been shown to influence the metabolism of benzodiazepines to a significant degree particularly with clobazam [92], etizolam [93] and diazepam [94, 95]. In one study subjects who were CYP2C19 poor metabolisers exhibited an elimination half-life for diazepam which was twice that of normal metabolisers [96]. The effect of polymorphisms could not only lead to greater toxicity but also a longer detection window after administration. The phase II metabolic pathways of benzodiazepines have been less widely studied but are thought to involve uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes particularly UGT2B15 [97], UGT1A9 [98], UGT2B7 [98] and UGT1A4 [99].

Polymorphisms in N-acetyltransferase 2 (NAT2) enzymes can affect the metabolism of benzodiazepines that undergo N-acetylation. This has been observed for a metabolite of clonazepam, 7-aminoclonazepam, where variant NAT2 polymorphisms caused a reduction in the rate of its metabolism [100].

In order to detect benzodiazepine use it is important to be able to detect the parent drug as well as any metabolites. Depending on the type of benzodiazepine "class" and the additional chemical substituent groups on the core structure the benzodiazepines undergo similar phase I metabolism. The common metabolic pathways for 1,4-benzodiazepines and some triazolo/imidazobenzodiazepines are shown in Figures 3 and 4 respectively. Oxidation is the primary phase I metabolic pathway observed for the majority of benzodiazepines. Typically, this involves hydroxylation on the same carbon atom on the diazepine ring, either labelled as position-3 (e.g. phenazepam [101]) or position 4 (e.g. clonazolam [10]). Hydroxylation at the α-position is also thought to occur for some benzodiazepines (e.g. flubromazolam [10]). N-demethylation of the tertiary amine located on the diazepine ring of diclazepam has been described [81] whilst benzodiazepines containing a nitro group (e.g. meclonazepam [10]) undergo reduction. For phase II metabolism, benzodiazepines that contain hydroxyl groups typically undergo phase II glucuronide conjugation (e.g. lorazepam and oxazepam [102]) without any phase I metabolism. Benzodiazepines containing a 3-hydroxy group typically have a shorter duration of action as they are directly metabolised to glucuronidated forms that are inactive [103]. Some benzodiazepines can be detected as benzophenones (they are either directly metabolised to these compounds or experience some form of physical degradation) in urine after administration of the parent drug (e.g. alprazolam [104], nitrazepam [105], and phenazepam [106]). The structures of the NPS-benzodiazepines are provided in Figures 2A-H and Tables 2 – 5 and their metabolic routes are provided in Figures 3, 4 and Table 8.

Once benzodiazepines are metabolised they are mainly eliminated in urine with between <1 % to ~20 % of the parent drug excreted unchanged with glucuronide being the most common metabolite [44]. As benzodiazepines follow common patterns it should be possible to predict the likely metabolites and routes of elimination of the NPS-benzodiazepines.

In order to detect the use of NPS-benzodiazepines, give appropriate clinical treatment people who have been exposed to the NPS-benzodiazepines and to interpret their blood/plasma concentrations it is important to have pharmacokinetic, analytical and clinical data. With this in mind we have collated the current available data on the NPS-benzodiazepines within this review.

Analytical Detection of NPS-benzodiazepines

It is important in toxicological analysis that analytical methodology is able to detect, identify and quantify drugs in a large number of matrices. As benzodiazepines are routinely used in clinical practice throughout the world, a large number of methods exist for their detection and quantification. The analytical methodologies for the determination of benzodiazepines in biological samples (blood, plasma, vitreous, oral fluid, hair, nails and others) have been recently reviewed [107, 108]. It is also important to understand whether analytical methodologies are likely to detect previously unknown benzodiazepines. The common methodological sequence during systematic toxicological analysis is detection, identification and then finally quantitation [109]. Toxicology laboratories commonly utilise immunoassays for presumptive detection before confirmation with other analytical techniques as a result of the large numbers of samples they may acquire. The advantage of the use of an immunoassay for screening is the lack of absolute selectivity of immunoassay antibodies that target the general structure of drug (such as benzodiazepines) rather that the specific drug (such as diazepam or phenazepam) [110]. Two recent publications investigating the cross reactivity of

standard commercial immunoassay drug screening to new NPS benzodiazepines in both blood and urine have shown that new NPS benzodiazepines would be detected by current immunoassay screens [111, 112]. Potential misidentification could occur however for structural isomers such as diclazepam and 4-chlorodiazepam (Ro5-4864) [113]. It is likely that new, as yet unknown, benzodiazepines would be detected by current commercial benzodiazepine immunoassays. This hypothesis was backed up by data from the Swedish STRADA project (a project that monitors the occurrence and trends of new psychoactive substances) where 390 clinical samples tested positive in a benzodiazepine immunoassay screen and subsequently tested negative in a classical LC-MS/MS benzodiazepine screen. Later, 40% of these samples were confirmed as containing NPS-benzodiazepines [114]. Following the presumptive detection of benzodiazepines confirmation and quantitation are needed. Typically HPLC [115, 116] with or without a mass spectrometer is used but GC-MS [117, 118] and capillary electrophoresis [119] have been utilised. As the NPS-benzodiazepines are extremely similar in structure to clinically used benzodiazepines it is expected that they would be able to be detected using similar methods. LC-TOF-MS currently gives the best methodology for the detection of any emerging NPS benzodiazepines as it is possible to search for compounds based on the molecular formula alone [120] although care needs to be taken with any isomers that may lead to misidentification. Sample preparation is an important step in the detection and quantitation of NPS benzodiazepines. The two common techniques used are that of liquid-liquid extraction (LLE) and solid phase extraction (SPE) [109]. SPE gives advantages amongst others of higher selectivity and increased extraction efficiency and recovery over LLE [121]. This could however be a disadvantage when trying to identify new compounds that have not previously been detected and may not elute from a specific SPE column.

One SPE technique that is becoming increasingly popular is the quick, easy, cheap, effective, rugged, safe (QuEChERS) dispersive SPE (dSPE) technique [122]. The use of a primary and secondary amine phase (PSA) allows easier removal of complex matrix components such as blood [123]. The QuEChERS technique has been shown to increase the recoveries of benzodiazepines extracted from various biological matrices such as blood and urine [124] and also from milk-based alcoholic drinks (where benzodiazepines are often added illicitly) which provides a complex matrix for extraction as a result of the high number of proteins and fatty acids [125].

The methods that are currently available for the detection and quantitation of NPS benzodiazepines in body fluids are listed in Table 7. LC-MS has been used to detect both flubromazepam and its metabolites in urine and serum [79] and also pyrazolam [126]. Pyrazolam does not appear to produce metabolites according to one study [126] but is detectable in serum for up to 50 hours but it is excreted in urine for up to 6 days following ingestion of 1 mg which provides a fairly large window of detection for analysis [126]. Diclazepam is found only in very low concentrations for just over four days following ingestion of 1 mg [81]. However its metabolites are discernible for longer time periods with delorazepam detectable for 6 days in urine and 10 days in serum, lorazepam 19 days in both serum and urine and lormetazepam 11 days in urine [81]. Flubromazepam and its metabolites also exhibit a low level of detection in urine using immunoassays [79] [127] [112]. However, by the use of LC-MS, the monohydroxylated metabolite was detectable for 28 days following ingestion in the urine samples, compared with 23 days in the plasma samples providing an extremely long window of detection for the drug [79]. Other NPS-benzodiazepines would be expected to be similarly detectable.

The metabolic pathways for benzodiazepines are fairly similar (see Figures 3 and 4) and this allows metabolites to be predicted and actively searched for when analysing samples using

techniques such as LC-MS [128, 129]. As a result of the aforementioned similar metabolic pathways, care must be taken when interpreting the apparent presence of a metabolite. For example, diclazepam is metabolised to lorazepam, lormetazepam and delorazepam which are all prescription drugs [81]. Likewise 3-hydroxyphenazepam has been sold on its own as an NPS-benzodiazepine but is a metabolite from both phenazepam [18] and a Russian prescription benzodiazepine cinazepam [130]. Desalkylflurazepam is a metabolite of several drugs including flurazepam [131], midazolam [132] and the Japanese prescription drugs flutoprazepam [133] and fludiazepam [134].

NPS Benzodiazepine Stability

With any detection, identification and quantification of a drug it is important to have information on the stability of the drug and any possible changes in the drug concentration that may happen during transportation and/or storage [135]. There have been numerous studies on the stability of benzodiazepines in matrices such as blood and urine at temperatures from 20 °C to -80 °C [136-139]. Nitrobenzodiazepines (such as flunitrazepam, clonazepam and nitrazepam) and chlordiazepoxide have been found to be the most unstable especially in bacterially-contaminated specimens [136, 140]. Two studies have been carried out investigating the stability of NPS benzodiazepines (pyrazolam, diclazepam, flubromazepam, meclonazepam, phenazepam, etizolam, nifoxipam, deschloroetizolam, clonazolam, flubromazolam and flutazolam) but only in urine for 1 month and 7 months [128, 141]. These studies showed that flubromazepam, clonazolam, nifoxipam and meclonazepam (the latter three are nitrobenzodiazepines) were unstable in urine (at ambient temperature and at -4 °C). Meclonazepam was only detected at 8 % of its original concentration after 4 weeks at -4 °C and -20 °C after 4 weeks). Meclonazepam has also been shown to be unstable in plasma in glass, but not in polypropylene tubes at -20 °C [142]. These studies indicate that

any future nitrobenzodiazepines are likely to be unstable and suggest that all NPS benzodiazepines should be investigated for stability and that they should all be collected in tubes containing fluoride oxalate (1 %) and then stored at the lowest temperature possible (ideally -20 °C or lower) before analysis.

Prediction of the pharmacological, toxicological and pharmacokinetic properties of benzodiazepines.

The lack of both *in vivo* and *in vitro* pharmacological testing of the new psychoactive substances that are emerging can be overcome to an extent with the use of quantitative structure activity relationship (QSAR) modelling. This technique creates a model that relates biological activity to structural descriptors of the compound and is based on a learning set with known biological activity. Systematic *in vivo* and *in vitro* work has also been carried out in order to investigate the structural characteristics that relate to pharmacological activity. From these studies estimations of activity of novel 1,4–benzodiazepines (Figure 2A) can be estimated for half-life (t½), volume of distribution (Vd), bioavailability (F) [143] as well as the potential toxicity of benzodiazepines [144], showing that hydrazone fragments, primary amines and saturated heterocyclic ring systems lead to increases in toxicity [144]. The biological activity of benzodiazepines was initially studied by Hester who determined the effects of substituents on the biological activity. This determined that triazolobenzodiazepines (Figure 2C) were more potent than the corresponding 1,4-benzodiazepine [145, 146]. As for the 1,4–benzodiazepines, the R1, R3, R7 and R2' positions (Figure 2A) are important for biological activity [147, 148]. The removal of the phenyl group removes the GABA potentiation by the compound but it can still bind to the GABA site [149]. QSAR studies identified the relative importance of each site to activity and also which functional groups could be added at various positions for optimal biological activity.

The R7 position was the most important position for increasing receptor affinity (30 % in the QSAR model) with the 10 optimal functional groups being CH_2CF_3 > I > Br > CF_3 > Cl > $C(CH_3)_3$ > NO_2 > F > N_3 > $CH{=}CH_2$ [150]. At the R1 position (37 % in the QSAR model) the most optimal groups were OH > F > NH_2 > H > NHOH > C_2H_5 > Cl > CF_3 > Br > CH_3 [156] and the *tert*-butyl substitution led to inactivity [157]. At the R2' position (15 % in the QSAR model) the order of the most optimal groups was NO_2 > F > CN > Cl > CF_3 [150, 151]. The influence that substitution at the R3 position has on biological activity is unclear and difficult to predict as a result of the formation of enantiomeric forms [152, 153] but it is thought to have limited influence on the biological activity [154]. In the literature there are measured binding affinities for desmethylflunitrazepam (fonazepam) and meclonazepam (log IC_{50}) of 0.176 and 0.079 respectively with predicted values of 0.565 and 0.357 respectively [152, 153]. These results show that although QSAR can be useful for prediction it is not a replacement for traditional *in vivo* and *in vitro* testing.

The composition of illicitly-sold NPS-benzodiazepines

A major issue with the purchase of drugs online is that there is no guarantee of the quality of composition. Alprazolam is one of the most widely-prescribed benzodiazepines in the world therefore it is not surprising that it is often illicitly sold. However, the wide variety of drugs that are sold and stated to contain alprazolam is both remarkable and concerning. Mimic alprazolam tablets have been found to contain melatonin [155] or the opioid fentanyl [156]. EcstasyData.org is an independent testing laboratory, created primarily to reduce the potential harm of illicit ecstasy by providing data on the composition of ecstasy tablets [157]. However, a variety of other drugs are often sent in and tested. This independent testing laboratory utilises GC-MS, thin layer chromatography (TLC) and colour tests for analysing and identifying the materials that are supplied to them [157]. Other drugs that have been

found in alprazolam tablets include other clinically-used benzodiazepines, synthetic cannabinoids, synthetic opiates, Z-drugs, piperazines, barbiturates and clinically-used anaesthetics and antihistamines. Clonazepam tablets have been identified as containing the NPS-benzodiazepine clonazolam [157]. Etizolam tablets have been found to contain alprazolam, flubromazepam (an NPS-benzodiazepine) and also diphenylprolinol, a compound used as a designer drug [157]. Diclazepam tablets have been identified as containing nimetazepam [157], a widely-prescribed and abused drug in southeast Asia [158] and an NPS-benzodiazepine in Europe itself [17] and some illicit tablets of nimetazepam (also known as Ermin 5) have been found to contain phenazepam [159]. In addition, in the 2016 EMCDDA drug report it was noted that alprazolam tablets had been identified as containing flubromazolam and diazepam tablets had been identified to contain phenazepam [3]. This is a huge problem for drug users as they may be inadvertently taking a drug potentially many times more harmful than expected as a result of the lack of information regarding drug-drug interactions. As mentioned previously, it is well known that the concurrent use of opioids, opiates and benzodiazepines can increase the risk of death [7, 160]. There have been sporadic reports of the use of benzodiazepines as either diluents or adulterants in heroin however this does not appear to be as common [161]. The majority of the data from EcstasyData.org is from the United States but samples are sent in from across the world with many appearing to have been purchased online in China [157]. With the increase of NPS-benzodiazepines in recent years, this may become even more problematic.

Summary

The use and abuse of benzodiazepines is already common throughout the world. In recent years there has been a large increase in new psychoactive substances. Benzodiazepines are only a small subsection of the total number of new psychoactive substances but that number

is steadily increasing. NPS-benzodiazepines are appearing in a variety of countries across the world. NPS-thienodiazepines are appearing at a much slower rate, perhaps as a result of a lower usage clinically and the already widespread availability of benzodiazepines. NPS-benzodiazepines have been implicated in deaths in England and Wales and the increasing availability of all novel psychoactive substances led to the introduction of the Psychoactive Substances Act within the UK in 2016. It remains to be seen whether this will affect the supply and use of NPS-benzodiazepines because phenazepam and etizolam were placed under control in the UK under the Misuse of Drugs Act 1971 but are still regularly identified in post-mortem cases and in drug-impaired drivers within the UK. The same may be expected for the NPS-benzodiazepines. The pharmacokinetics and metabolic pathways of NPS-benzodiazepines are not currently well understood and there can be huge variation in pharmacokinetic parameters between individual compounds. Further investigation is clearly needed to establish the exact pharmacology of these new psychoactive substances.

Table 1: Pharmacological Details of NPS Benzodiazepines

Drug	Formula	mW (g mol^{-1})	Typical Recreational dose (mg)	"Therapeutic"/DUID range in blood (mg l^{-1})	T1/2 (h)	Vd (l kg^{-1})	User reports of effects	Refs
3-hydroxyphenazepam	$C_{15}H_{10}BrClN_2O_2$	365.6	0.5 – 2	-	-	-	Anxiolytic, slight muscle relaxant, strongly sedating	[14, 162]
4-chlorodiazepam	$C_{16}H_{12}Cl_2N_2O$	319.2	-	-	-	-	No reports	-
Adinazolam	$C_{19}H_{18}ClN_5$	351.8	20	0.1 – 0.46	1 – 3	2.2	No reports	[163-167]
Bromazolam	$C_{17}H_{13}BrN_4$	353.2	1	-	-	-	No reports	-
Clonazolam	$C_{17}H_{12}ClN_5O_2$	353.1	0.5 - 1	0.0019 – 0.011	-	-	Slight euphoria, strongly sedating	[168, 169]
Cloniprazepam	$C_{19}H_{16}ClN_3O_3$	369.8	2.5	-	-	-	Slight anxiolytic, higher doses (>5-10 mg) required for muscle relaxation, sedation in most users	[170]
Desalkylflurazepam	$C_{15}H_{10}ClFN_2O$	288.7	5	-	-	-	Strongly sedating and long lasting effects	[16]
Deschloroetizolam	$C_{17}H_{16}N_4S$	308.4	4 - 6	-	-	-	Effects lasting 12 – 24 hours, anxiolytic, sedative effect, slight euphoria	[14]
Desmethylflunitrazepam (fonazepam)	$C_{15}H_{10}FN_3O_3$	299.3	0.6	-	-	-	Anxiolytic, muscle relaxant, sedation	[171, 172]
Diclazepam	$C_{16}H_{12}Cl_2N_2O$	319.2	1 - 2	0.0021 – 0.057	42	-	Effects lasting 5 – 12 hours, anxiolytic, useful for 'tapering' dependence of other benzodiazepines, low cognitive impairment, low recreational value.	[81, 169] [162]
Etizolam	$C_{17}H_{15}ClN_4S$	342.1	0.25 – 3	0.019 - 0.17	3.4 – 7.1	0.91	Anxiolytic, euphoric, muscle relaxant, used as a sleep-aid	[169]
Flubromazepam	$C_{15}H_{10}BrFN_2O$	333.1	4	0.0047 – 1.2	106.4	-	Effects lasting 18 – 24 hours, anxiolytic, mild euphoria, blackouts, sedating and muscle relaxant effects,	[169] [88, 177]

							short-term memory loss	
Flubromazolam	$C_{17}H_{12}BrFN_4$	371.2	0.15 – 0.25	0.0048 -0.10	-	-	Effects lasting 12 – 18 hours, anxiolytic, high tolerance to lower doses quickly observed, blackouts and memory loss, strongly sedating, higher doses of 2.5 – 4 mg have effects reported to last up to 3 days and strong memory loss and cognitive impairment. Ingestion of 3 mg of flubromazolam 19 hours prior to hospitalisation has been reported in a patient. Severe respiratory failure, hypotension, central nervous system depression and brain damage were observed.	[79, 169, 173]
Flunitrazolam	$C_{17}H_{12}FN_5O_2$	337.3	0.1	-	-	-	Strong sedative, slight amnesia reported, anxiolytic	[168]
Flutazolam	$C_{19}H_{18}ClFN_2O_3$	376.8	4 - 12	0.014	~3.3	690 L	Strong anxiolytic, hypnotic, short acting (3 – 4 hours)	[174, 175]
Meclonazepam	$C_{16}H_{12}ClN_3O_3$	329.7	2 - 3	0.01 – 0.1	80	100 L	Low sedation, anxiolytic, muscle relaxant	[10, 175, 176]
Metizolam (desmethyletizolam)	$C_{16}H_{13}ClN_4S$	328.8	2	0.000011	-	-	Anxiolytic and muscle relaxant, effects not as strong as etizolam	[168, 177]
Nifoxipam	$C_{15}H_{10}FN_3O_4$	315.3	0.5 - 2	-	-	-	Effects lasting 12 – 18 hours, anxiolytic , moderately sedating, mild euphoric, High doses can cause users to feel sleep-deprived, muscle relaxant	[175, 178]
Nimetazepam	$C_{16}H_{13}N_3O_3$	295.3	5	0.0000134	12 - 21	-	No reports	[179-181]

Nitrazolam	$C_{17}H_{13}N_5O_2$	319.3	0.5 – 2	-	-	-	Anxiolytic, hypnotic, strongly sedating	[182]
Phenazepam	$C_{15}H_{10}BrClN_2O$	349.6	0.5 – 1	0.030 – 0.070	6 – 80	4.7 – 6.0	Anxiolytic, extremely sedating, short-term memory loss often leads to users redosing, blackouts at higher doses, psychotic episodes, insomnia	[101, 118, 168, 183]
Pyrazolam	$C_{16}H_{12}BrN_5$	354.2	1	0.074	17	-	Effects lasting 6 – 7 hours, anxiolytic, low sedation, low hypnotic effect, low recreational value	[126] [168, 169]

Figure 1: Timeline of the reporting of NPS-benzodiazepines to the EMCDDA

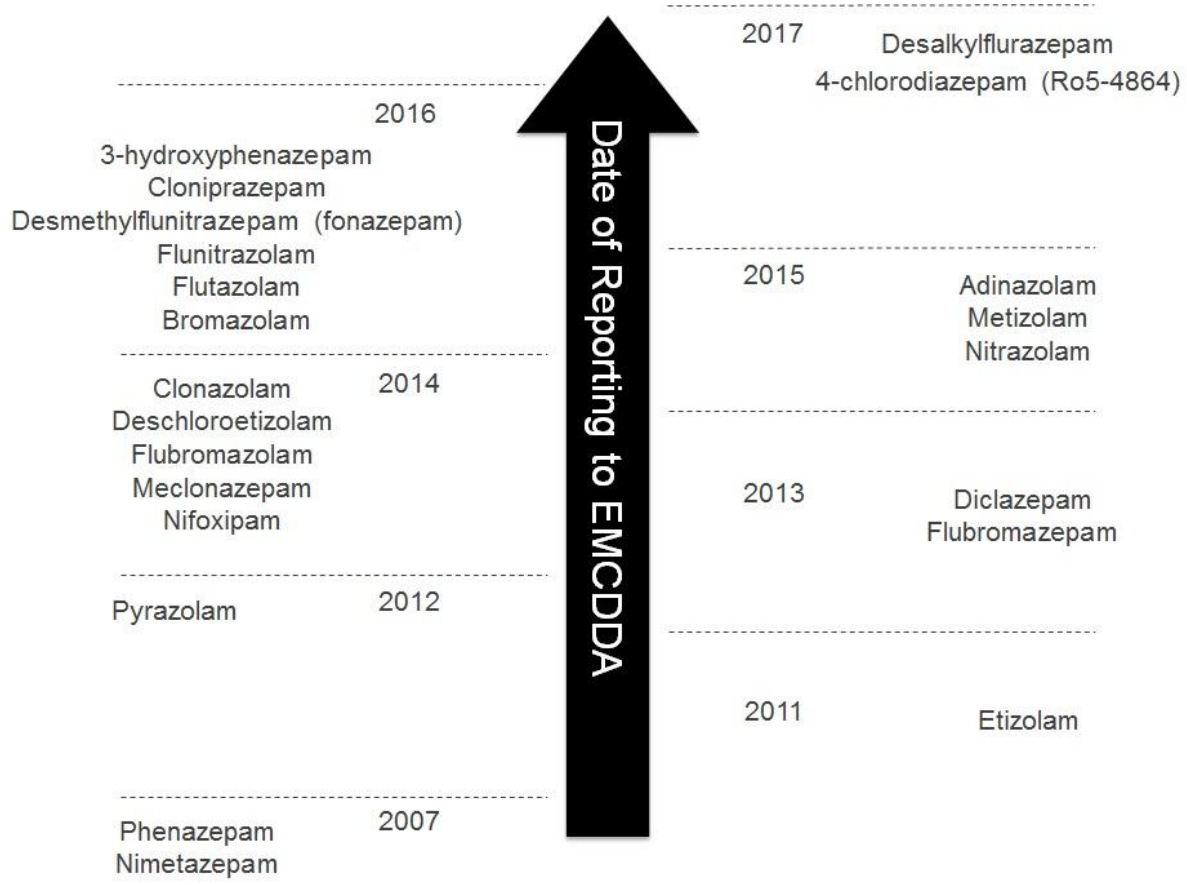
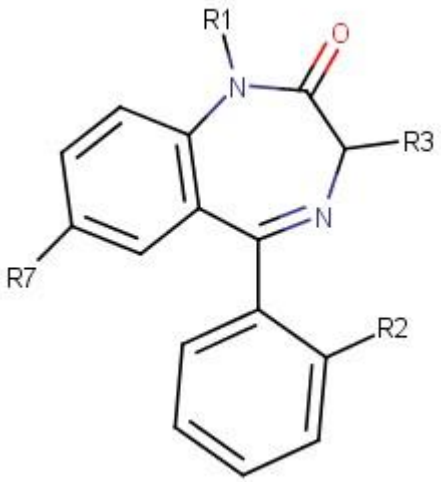


Table 2: Benzodiazepine patent years and EMCDDA report years

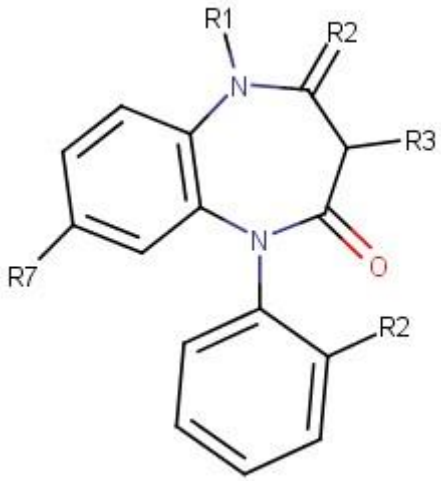
Compound	Year patented	Year reported to the EMCDDA
3-hydroxyphenazepam	Not reported	2016 [46] [37]
4-chlorodiazepam (Ro5-4864)	1964 [191]	2016 [40]
Adinazolam	1976[192]	2015 [35]
Bromazolam	1976 [193]	2016 [39]
Clonazolam	1971 [194]	2014 [35]
Cloniprazepam	Not reported	2015 [184]
Desalkylflurazepam	Not reported	2016 [39]
Deschloroetizolam	1998 [196]	2014 [34]
Desmethylflunitrazepam (fonazepam)	1963 [197]	2016 [38]
Diclazepam	1964 [198]	2013 [33]
Etizolam	1978 [199]	2011 [30]
Flubromazepam	1962 [200]	2013 [33]
Flubromazolam	1978 [201]	2014 [34]
Flunitrazolam	Not reported	2016 [38]
Flutazolam	1970 [202]	2015 [37]
Meclonazepam	1975 [203]	2014 [34]
Metizolam	1988 [204]	2015 [35]
Nifoxipam	1985 [205]	2014 [34]
Nimetazepam	1963 [206]	2007 [17]
Nitrazolam	1971 [194]	2015 [35]
Phenazepam	1974 [207]	2007 [17]
Pyrazolam	1979 [208]	2012 [32]

Figure 2A-D: Structure of benzodiazepines and derivatives

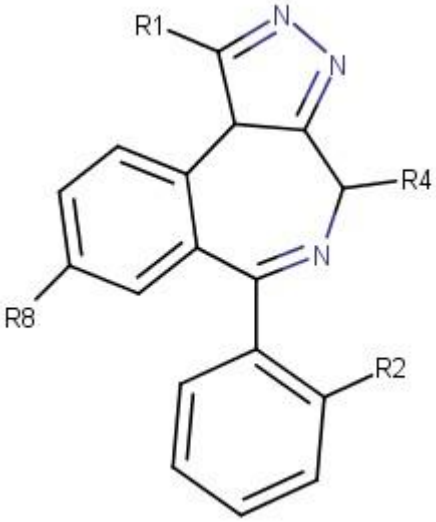
A) 1, 4 - Benzodiazepine



B) 1,5 – Benzodiazepine



C) Triazolobenzodiazepine



D) Imidazobenzodiazepine

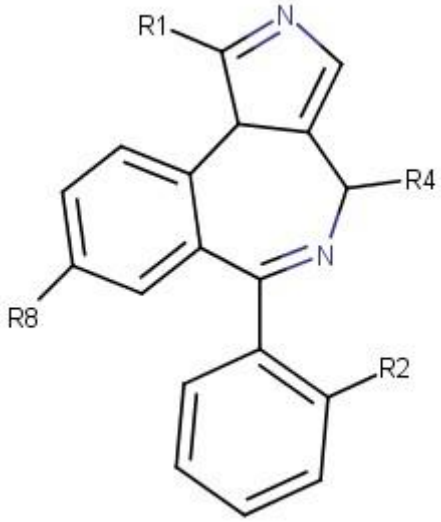
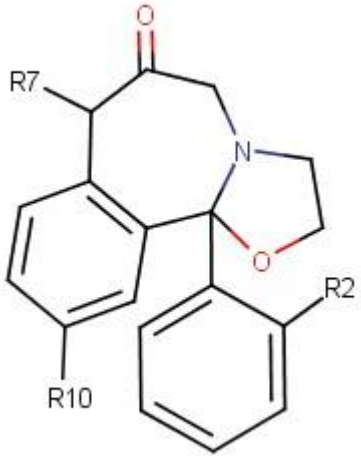
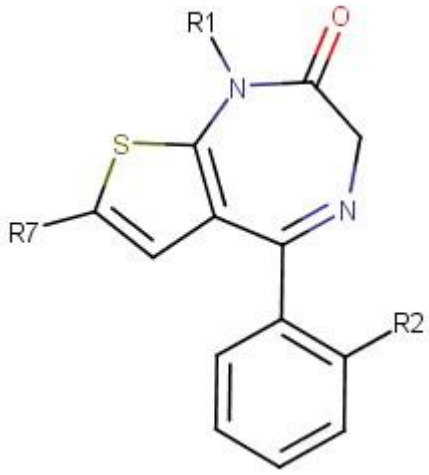


Figure 2E-H: Structure of benzodiazepines and derivatives

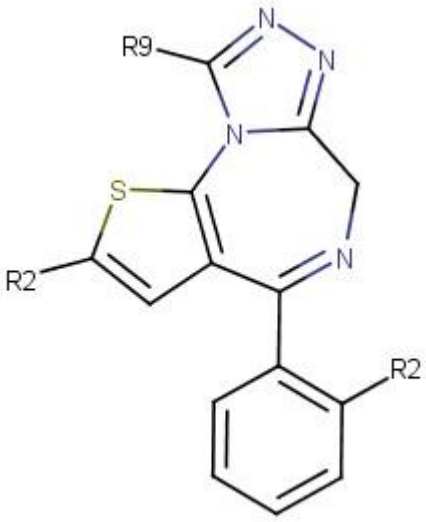
A) Oxazolobenzodiazepine



B) Thienodiazepine



C) Thienotriazolodiazepine



D) 2,3 – Benzodiazepine

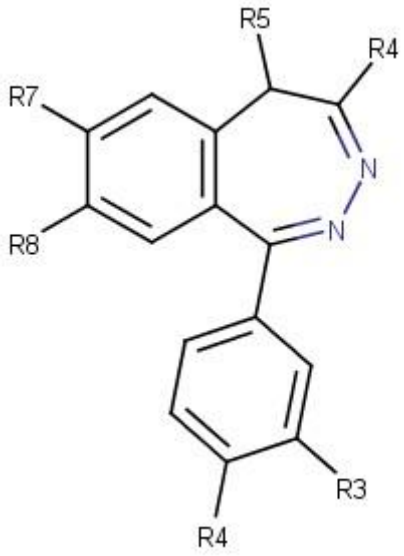


Table 3: 1,4-benzodiazepine based NPS structures

	From Figure 2A				
Compound	**R_1**	**R_2,**	**R_3**	**R_7**	
3-hydroxyphenazepam	H	Cl	OH	Br	
4-chlorodiazepam (Ro5-4864)	CH_3	H	H	Cl	Note: 4-chlorophenyl ring instead of phenyl ring at position 6
Cloniprazepam	Methylcyclopropane	Cl	H	NO_2	
Desalkylflurazepam	H	F	H	Cl	
Desmethylflunitrazepam (fonazepam)	H	F	H	NO_2	
Diclazepam	CH_3	Cl	H	NO_2	
Flubromazepam	H	F	H	Br	
Meclonazepam	H	Cl	CH_3	NO_2	
Nifoxipam	H	F	OH	NO_2	
Nimetazepam	H	H	OH	NO_2	
Phenazepam	H	Cl	H	Br	

Table 4: Triazolobenzodiazepine based NPS structures

	From Figure 2C			
Compound	R_1	$R_{2'}$	R_8	
Adinazolam	$CH_2N(CH_3)_2$	H	Cl	
Bromazolam	CH_3	H	Br	
Clonazolam	CH_3	Cl	NO_2	
Flubromazolam	CH_3	F	Br	
Flunitrazolam	CH_3	F	NO_2	
Nitrazolam	CH_3	H	NO_2	
Pyrazolam	CH_3	None	Br	Note: pyridine ring instead of phenyl ring at position 6

Table 5: Thienotriazolodiazepine based NPS structures

	From Figure 2G		
Compound	R_2	$R_{2'}$	R_9
Deschloroetizolam	CH_2CH_3	H	CH_3
Etizolam	CH_2CH_3	Cl	CH_3
Metizolam	CH_2CH_3	Cl	H

Table 6: Oxazolobenzodiazepine based NPS structures

	From Figure 2E		
Compound	R_2'	R_7	R_{10}
Flutazolam	F	CH_2CH_2OH	Cl

Figure 3: General metabolic pathways for 1,4-benzodiazepines

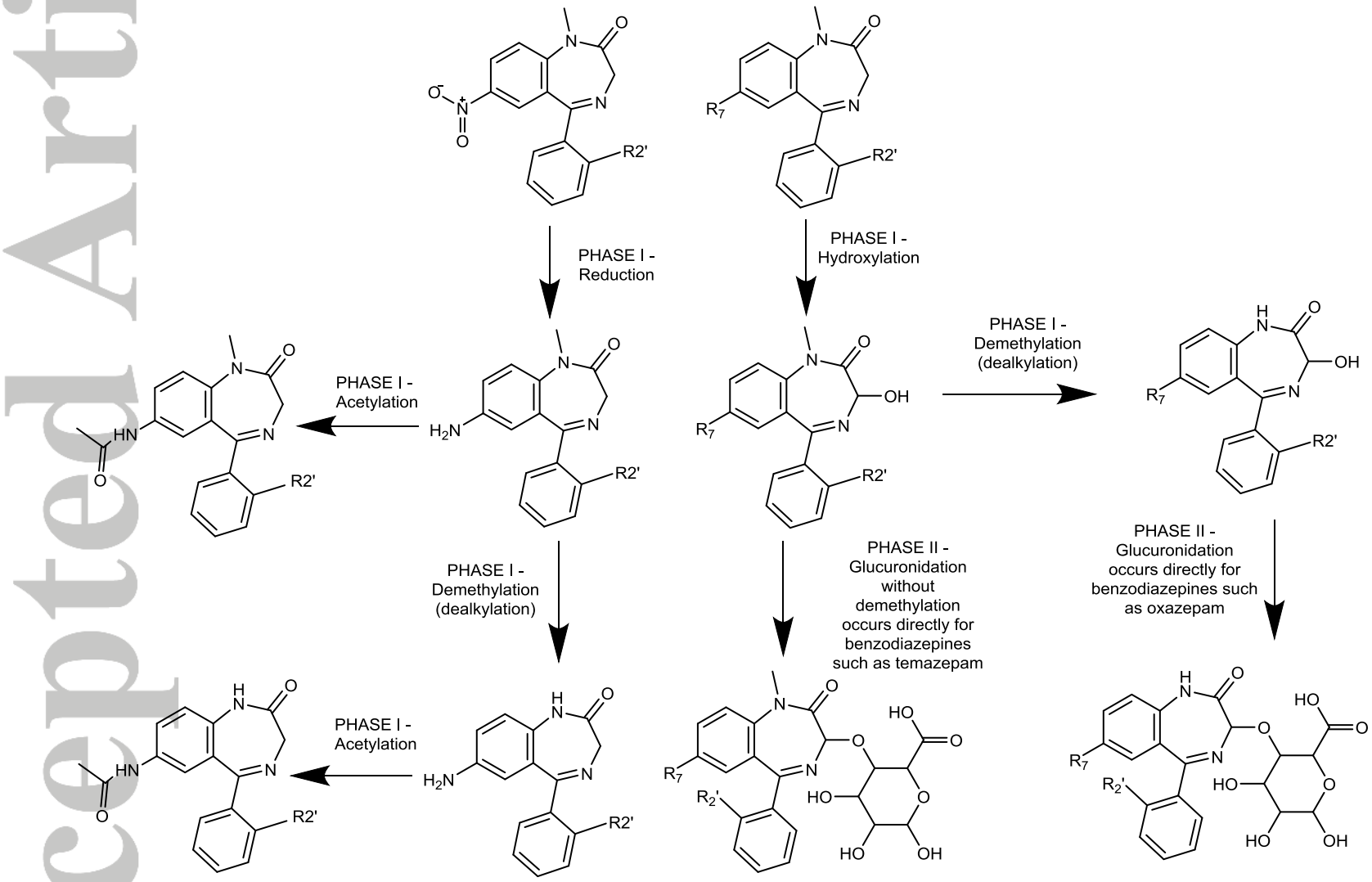


Figure 4: General metabolic pathways for triazolobenzodiazepines (also applies to imidazobenzodiazepines)

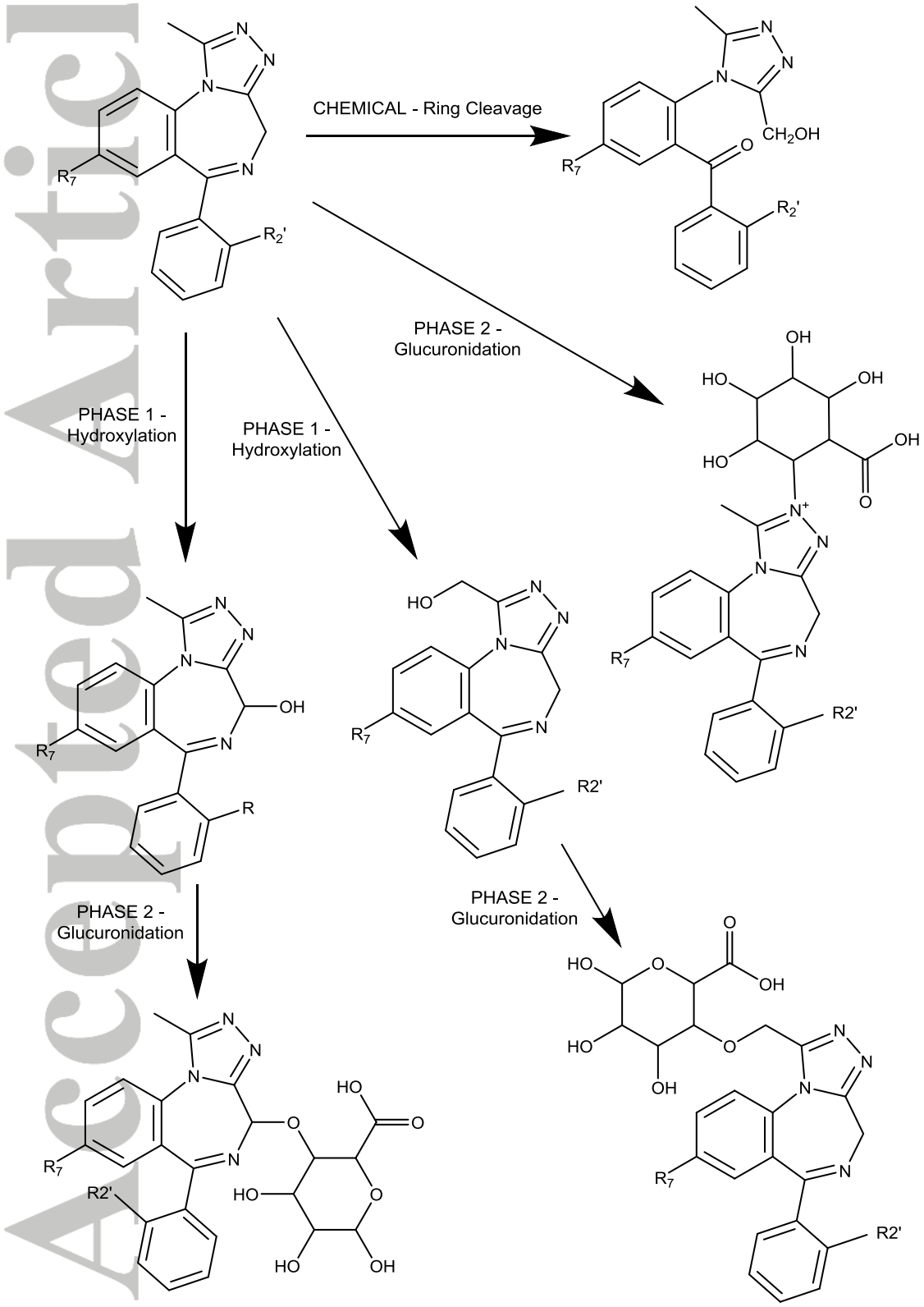


Table 7: Analytical methods for the analysis of NPS-benzodiazepines in biological matrices

Analyte	Matrix	Analytical Method	Internal Standard	Extraction	Limit of detection (ng ml^{-1})	Linear range (ng ml^{-1})	Limit of quantitation (ng ml^{-1})	Ref.
Adinazolam	Plasma	HPLC-UV	Alprazolam	LLE (ethyl acetate)	~5	10 – 800	10	[185]
3-hydroxyphenazpem Phenazepam	Blood Urine Vitreous Muscle Brain Liver	LC-MS/MS	Diazepam-d5	LLE (hexane:ethylacetate 7:3)	0.3 7	16 - 100 0.7 - 200	0.7 16	[162]
Clonazolam Meclonazepam Nifoxipam	Urine	LC-MS	Methamphetamine-d5 Pethidine-d4	LLE	Not provided	Not provided	Not provided	[186]
Clonazolam, Diclazepam Etizolam Flubormazepam Flubromazolam Pyrazolam	Blood	UPLC-MS/MS	Diazepam-d5	LLE (ethyl acetate:heptane 4:1)	Same as LOQ	Not provided	1.4 1.6 1.4 3.3 0.37 3.5	[169]
Pyrazolam Dicalzepam Flubormazepam Meclonazepam Phenazepam Etizolam Nifoxipam Deschloroetizolam Clonazolam Flubromazolam Flutazolam	Urine	LC-MS/MS	Temazepam-d5 Estazolam-d5	B-glucuronidation followed by dilute and shoot	4 2 2.5 1 5 2 10 2 5 2 5	10 – 1000 2 – 200 2.5 – 250 1 – 100 5 – 500 5 – 500 10 – 1000 5 – 500 5 – 500 5 – 500 5 – 500	10 2 2.5 1 5 5 10 5 5 5 5	[128]
Desmethylflunitrazepam (fonazepam)	Numerous methods reviewed by Katselou [178]							
Diclazepam	Plasma/urine	LC-MS	Diazepam-d5 Lorazepam-d4 Nordazepam-d5 Temazepam-d5	B-glucuronidation then LLE using 1-chlorobutane and borate buffer (pH 9)	0.25	0.25 – 100	Not provided	[81]

Etizolam	Plasma/Urine	HPLC	Alprazolam	LLE	1	1-100	Not provided	[187]
Etizolam	Plasma	HPLC	N/A	N/A	0.3	Not provided	0.6 ng/ml	[93]
Etizolam	Plasma/Urine	GC-MS/MS	Fludiazepam	SPE	Not provided	5-50	Not provided	[188]
Flubromazepam	Plasma/Urine	LC-MS/MS	Nordazepam	LLE (1-chlorobutane)	1	1-100	Not provided	[79]
Meclonazepam	Plasma	GC	None	LLE (butyl acetate)	0.1	0.6 – 20	0.6	[142]
Meclonazepam	Urine	LC-MS-QTOF	None	B-glucuronidation	Not provided	Not provided	Not provided	[186]
Metizolam	Urine	LC-MS/MS	Methyl-clonazepam & -OH-ethylthoephyllin	LLE (dichloromethane/n-heptane/isopropanol (25/65/20)	0.025	0.05 – 50	0.05	[177]
Nifoxipam	Numerous methods reviewed by Katselou [178]							
Nimetazepam	Urine	LC-MS/MS	Nitrazepam-d5	SPE	0.05	0.05 – 100	0.1	[189]
Phenazepam	Plasma	GC	7-bromo-5-(2-bromophenyl)-1,3-dihydro-1,4-benzodiazepin-2-one	LLE (diethyl ether)	1-2	1-150	Not provided	[190]
Phenazepam	Plasma	GC-MS	Prazepam	SPE	Not provided	Not provided	1	[118]
Phenazepam	Plasma/Plasma	LC-MS/MS	Diazepam	SPE	1.44	5 - 1000	3.06	[24]
Phenazepam	Plasma	Dual-column GC	Norclobazam	LLE (ethyl acetate)	5 ng g^{-1}	10 – 500 ng g^{-1}	10 ng g^{-1}	[191]
Phenazepam	Plasma	LC-MS/MS	Diazepam	LLE (acetone)	12	100 – 1600	28	[25]
Pyrazolam	Plasma/Urine	LC-MS/MS	Alprazolam	LLE (1-chlorobutane)	1	1-100	Not provided	[126]

Table 8: Metabolic pathways and metabolites of NPS -benzodiazepines

Compound	Major Phase I metabolites (both *in vivo* and *in vitro*)	Reference(s)
3-hydroxyphenazepam	None Known	[184]
4-chlorodiazepam (Ro5-4864)	There appears to be a lack of information on the metabolic routes of this benzodiazepine but they are possibly similar to those observed for diclazepam such as N-demethylation and 3-hydroxylation	No reference
Adinazolam	N-desmethyladinazolam N,N-didesmethyladinazolam α-hydroxyadinazolam, Estazolam	[184] [192]
Bromazolam	No experimental studies to date but possible metabolites are hydroxylation at the α or 4 positions as is the case with other triazolobenzodiazepines	No reference
Clonazolam	7-aminoclonazolam, 7-acetaminoclonazolam Hydroxyclonazolam	[10, 193]
Cloniprazepam	Monohydroxylated cloniprazepam Clonazepam (dealkylation) Reduction of the 7-nitro to a 7-amino group 7-aminoclonazepam (dealkylation and reduction) Hydroxylation and dealkylation Hydroxylation and dealkylation Oxidation of the 3-hydroxy group to a 3-keto group	[184]
Desalkylflurazepam	It is unclear as to whether this would go further phase I metabolism or instead proceed directly to phase II metabolism as is the case when it is a metabolite from flurazepam	[47]
Deschloroetizolam	Monohydroxylation (probable 9-methyl) Monohydroxylation (probable 2-ethyl) Monohydroxylation (probable position 6) Dihydroxylation (positions undetermined)	[10, 176]
Desmethylflunitrazepam (fonazepam)	3-hydroxynorflunitrazepam Monohydroxylation (position undetermined) 7-aminonorflunitrazepam	[184]
Diclazepam	Delorazepam, Lorazepam Lormetazepam	[81, 176]
Etizolam	Hydroxylation on the α-carbon of the 9-methyl group (also known as α-hydroxyetizolam) Hydroxylation on the α-carbon of the 2-ethyl group (also known as 8-hydroxyetizolam)	[176, 187]
Flubromazepam	Monohydroxylation (possibly 3-hydroxy, undetermined) Debromination and monohydroxylation (possibly 3-hydroxy, undetermined) Monohydroxylation (either on the phenyl ring or the benzene ring, undetermined)	[79, 176]
Flubromazolam	α-hydroxyflubromazepam 4-hydroxyflubromazepam Dihydroxylation (α-hydroxy and 4-hydroxy)	[10, 176]
Flunitrazolam	No experimental studies to date but possible metabolites are reduction of the 8-nitro group to a 8-amino group and hydroxylation at position 4 of the diazepine ring.	No reference
Flutazolam	Oxazole ring-opening and elimination The above metabolite is thought to be the main metabolite present in plasma but other metabolic pathways do exist: N1-dealkylation (loss of CH_2CH_2OH), 3-hydroxylation Hydroxylation on either the fluorophenyl or chlorophenyl ring Both N1-dealkylation and 3-hydroxylation	[36]
Meclonazepam	7-aminomeclonazepam, 7-acetaminomeclonazepam	[176, 193]
Metizolam	2 mono-hydroxylated compounds Di-hydroxylated compound Hydroxylation (likely to be 2-ethyl or 6- position)	[177, 184]
Nifoxipam	7-aminonifoxipam, 7-acetaminonifoxipam	[176, 193]
Nimetazepam	Nitrazepam, 7-aminonimetazepam	[189]
Nitrazolam	8-aminonitrazolam	[184]

	Mono hydroxylated metabolite (likely either 4- or α- position)	
Phenazepam	3-hydroxyphenazepam Hydroxylation and methoxy addition (positions undetermined)	[18, 101]
Pyrazolam	No detectable metabolites in serum or urine	[126]

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The Emergence of New Psychoactive Substance (NPS) Benzodiazepines: A Review

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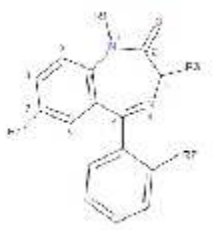

This paper critically reviews both the stage of knowledge of the emerging NPS-benzodiazepines and also the analytical methods that can be used to detect them.