

# **The Emergence of New Psychoactive Substance (NPS) Benzodiazepines. A Survey of their Prevalence in Opioid Substitution Patients using LC-MS**

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## **Abstract**

Benzodiazepines have a wide range of clinical uses being among the most commonly prescribed medicines globally. The EU Early Warning System on new psychoactive substances (NPS) has over recent years detected new illicit benzodiazepines in Europe's drug market<sup>1</sup>. Additional reference standards were obtained and a multi-residue LC-MS method was developed to test for 31 benzodiazepines or metabolites in urine including some new benzodiazepines which have been classified as New Psychoactive Substances (NPS) which comprise a range of substances, including synthetic cannabinoids, opioids, cathinones and benzodiazepines not covered by international drug controls.

200 urine samples from patients attending the HSE National Drug Treatment Centre (NDTC) who are monitored on a regular basis for drug and alcohol use and which tested positive for benzodiazepine class drugs by immunoassay screening were subjected to confirmatory analysis to determine what Benzodiazepine drugs were present and to see if etizolam or other new benzodiazepines are being used in the addiction population currently.

Benzodiazepine prescription and use is common in the addiction population. Of significance we found evidence of consumption of an illicit new psychoactive benzodiazepine, Etizolam.

## **Introduction**

Benzodiazepines are useful in the short-term treatment of anxiety and insomnia, and in managing alcohol withdrawal.<sup>1</sup> According to the EMCDDA report on the misuse of benzodiazepines among high-risk opioid users in Europe<sup>1</sup>, benzodiazepines, especially when injected, can prolong the intensity and duration of opioid effects. The report states that high-risk opioid users typically misuse benzodiazepines to self-medicate to treat symptoms of psychiatric disorders, negative emotional states, opioid withdrawal symptoms, and the side effects of alcohol and cocaine use or increase the effects of opioids. For this reason, patients in opioid substitution treatment (OST) may misuse benzodiazepines in order to increase the effects of their opioid medication - possibly prompted by withdrawal symptoms caused by under-dosing of the substitution treatment. The report further notes that benzodiazepine misuse among high-risk drug users, like other forms of high-risk drug use, cannot be measured by direct methods, such as surveys of the general population and also that frequency of benzodiazepine misuse is reported to increase with the length of OST treatment.

The HRB Bulletin Drug Treatment 2017<sup>2</sup> reports on cases of treated problem drug use (excluding alcohol) between 2010 and 2017. While opiates (mainly heroin) remain the main problem drug reported with 45.0% in 2017, the

majority of cases reported problem polydrug use (61.3%) over the period. Benzodiazepines are the second most common additional drugs reported and the proportion of cases who reported benzodiazepines as a main problem drug increased from 4.1% in 2010 to 9.7% in 2017.

The simultaneous use of opioids with benzodiazepines and other central nervous system depressants, such as alcohol, increases the risk of non-fatal and fatal overdose through respiratory depression and this is reflected in the high frequency with which benzodiazepines are identified post-mortem in drug-related deaths.<sup>1</sup>

The 2019 HRB National Drugs related deaths index<sup>3</sup> indicated that benzodiazepines play a major role in poisoning deaths in Ireland and that polydrug use is a significant risk factor for fatal overdose. The percentage of deaths due to polydrug poisonings rose from 44% (n=118) in 2004 to 62% (n=219) in 2016. Of 354 poisoning deaths in 2016, almost two thirds involved polydrugs with an average of four different drugs involved compared to an average of two in 2004. Benzodiazepines were the most common drug group implicated in polydrug deaths in 2016. In 2016 88% of deaths where methadone was implicated (91) involved other drugs, mainly benzodiazepines and 81% of deaths where heroin was implicated (58) involved other drugs, mainly benzodiazepines.

The number of deaths involving licit drugs continues to increase, being implicated in seven out of ten poisoning deaths in 2016. Methadone was implicated in almost one third of poisonings (103, 29%) while diazepam was implicated in over a quarter (96, 27%) of all poisonings and notably alprazolam-related deaths have increased significantly by 283% from 12 deaths in 2010 to 46 in 2016.

Not covered by international drug controls, NPS comprise a range of substances, including synthetic cannabinoids, opioids, cathinones and benzodiazepines. Over the last decade, the EU Early Warning System on NPS has detected an increasing number of new benzodiazepines that have appeared on Europe's drug market with more than half having been detected since 2015.<sup>1</sup> The EMCDDA is currently monitoring 23 new benzodiazepines (3 detected for the first time in Europe in 2017).<sup>4</sup> Some are sold under their own names (e.g. diclazepam, etizolam, flubromazolam, flunitrazolam, fonazepam). In other cases, producers use these substances to manufacture fake versions of commonly prescribed benzodiazepine medicines (e.g. diazepam, alprazolam), which are then sold on the illicit market. The first new benzodiazepines reported were phenazepam in 2007 and etizolam in 2011.<sup>1</sup>

Four drugs — etizolam, diclazepam, flubromazolam and phenazepam — account for over 80% of all tablets containing new benzodiazepines that have been seized in Europe since 2005.<sup>1</sup> In 2016, over half a million tablets containing new benzodiazepines, or similar substances, were seized, some two-thirds up on the number seized in 2015.<sup>4</sup>

In 2016 the Forensic Science Ireland laboratory detected the following illicit new benzodiazepine drugs in seizures: phenazepam, nitrazolam, etizolam and chlorodiazepam.<sup>6</sup> In 2017 Gardaí seized 115,567 benzodiazepine tablets/capsules valued at €1,374,908.<sup>5</sup>

As part of Global Operation PANGAEA Targeting Falsified Medicines in October 2018 the Health Products Regulatory Authority (HPRA), in partnership with Revenue's Customs Service, An Garda Síochána and regulatory and law enforcement agencies worldwide, revealed that from January to October 2018 they have detained, in Ireland, 90,000 dosage units of illegal prescription medicines, valued at over €375,000. This included 25,241 units of sedatives.<sup>7</sup>

Benzodiazepines are frequently prescribed for opioid substitution patients, however the additional consumption of illicitly obtained prescription drugs and/or fake or counterfeit medicines is a significant problem. In October 2018 74% of patients attending our service tested positive for benzodiazepines.

In order to monitor the profile of benzodiazepines being used in the addiction population currently and to determine if any new benzodiazepines are being used, a multi-residue method was developed using Liquid chromatography mass spectrometry (LC-MS) to unambiguously identify 31 Benzodiazepine drugs.

Building on a previously developed method which included 20 drugs, 11 new drugs including etizolam, estazolam, phenazepam, clonazolam, pyrazolam, deschloroetizolam, flubromazepam, flunitrazolam, diclazepam, demoxepam, lormetazepam were tuned and added to the method. Separation and mass spectral conditions were optimised to allow unambiguous determination of 31 drugs.

Benzodiazepine class drugs undergo extensive metabolism through glucuronidation and/or sulfation, which produces conjugated metabolites. Hydrolysis is used to cleave conjugated metabolites prior to analysis which increases the parent drug concentration. A new product Abalonase<sup>®</sup> was employed to achieve a much shorter hydrolysis time than traditional  $\beta$ -glucuronidase.

The method was successfully developed and used to determine what benzodiazepine drugs were present.

## Methods

Confirmatory analysis was carried out using a newly developed in-house benzodiazepine screen for multiple compounds with 2 transitions for each separated in a 6 min gradient.

This included the following drugs or metabolites: demoxepam, flurazepam, 2-hydroxyethylflurazepam, 7-aminoflunitrazepam, alprazolam, chlordiazepoxide, clobazam, flunitrazepam, lorazepam, midazolam, nitrazepam, nordiazepam, prazepam, triazolam, temazepam, bromazepam, clonazepam, 7-aminoclonazepam, oxazepam, estazolam,  $\alpha$ -hydroxyalprazolam and  $\alpha$ -hydroxytriazolam, etizolam, phenazepam, deschloroetizolam, diclazepam, flubromazepam, pyrazolam, clonazepam, lormetazepam, and flunitrazolam.

An Agilent 1260 HPLC system equipped with a Sciex 4500 QTrap was used for analysis. The method uses Electrospray positive ionisation with multi-reaction monitoring (MRM) - 2 MRM transitions for each compound tested coupled with Enhanced Product Ion monitoring (EPI) giving full scan data. An Xterra RP18 (100 x 4.6mm 3.5 $\mu$ m) with Guard Column: Xterra RP18 (10 x 4.6mm) was utilised. Column temperature was 50°C. Flow rate was 1ml/min.

Mobile Phase A: 100:100:800 Buffer: acetonitrile: water; Mobile Phase B: 100:900, buffer: acetonitrile (Buffer: 0.5% formic acid, 25mM ammonium formate). Ammonium formate, formic acid and HPLC grade solvents were purchased from Fisher Scientific Ireland. Standards were purchased from LGC. Abalonase<sup>®</sup> was purchased from United Chemical Technologies.

Urine samples obtained from participants in opioid substitution treatment at the NDTC are screened on a regular basis for drug and alcohol use by immunoassay. Samples selected at random which tested positive for Benzodiazepine class drugs by immunoassay (CEDIA<sup>®</sup>) screening were subjected to confirmatory analysis for benzodiazepines and results recorded. Samples were subjected to hydrolysis with Abalonase<sup>®</sup>. This was followed by centrifugation, dilution and analysis by LC-MS. Quantification of levels of benzodiazepines was not performed, samples were deemed positive if concentrations were above 10ng/ml and met MRM/EPI matching criteria.

## Results

A total of 200 samples were analysed by LC-MS. 69.5% were male and 30.5% female. The age range of patients was 20.5 to 61.5 years with the mean age being 39.1 years.

There are many common metabolites among the benzodiazepines, details of these and their presence in urine are listed in Table 1.

There were no positives for chlordiazepoxide, clobazam, midazolam, nitrazepam, prazepam, triazolam, triazolam, estazolam, phenazepam, flunitrazepam, deschloroetizolam, flubromazepam, pyrazolam, clonazepam or flunitrazolam. However, many of the parent drugs are not detected to any appreciable extent in urine e.g. chlordiazepoxide metabolises very quickly to nordiazepam.

Positives were as follows: (86.5%) oxazepam, (84.5%) temazepam, (79.5%) nordiazepam, (12.%) 2-hydroxyethylflurazepam, (66.5%) alprazolam, (64.4%)  $\alpha$ -hydroxyalprazolam, (8%) lorazepam, (7%) lormetazepam, (4%) flurazepam, (3.5%) etizolam, (0.5%) bromazepam, and (0.5%) 7-aminoclonazepam (Figure 1). Lorazepam could be present from intake of Lorazepam and/or Lormetazepam or Diclazepam (Table 1). One patient was positive for six benzodiazepines or metabolites: nordiazepam, temazepam, oxazepam, alprazolam, and  $\alpha$ -hydroxyalprazolam and etizolam, probably indicating diazepam, alprazolam and etizolam use.

Samples were also subjected to a routine suite of immunoassay drug tests in the laboratory. All 200 samples were also tested for opiate, cocaine, alcohol and EDDP (methadone metabolite). 186 samples were tested for cannabis and 189 for amphetamine. Positive results were as follows: opiate 41%, cannabis 55.4%, cocaine 33%, amphetamine 2.1%, alcohol 1%, EDDP 92.5% and buprenorphine 6.5%.

**Table 1**

Benzodiazepine	Metabolites
Flurazepam	2-hydroxyethylflurazepam
Flunitrazepam	7-aminoflunitrazepam
Alprazolam	α-hydroxyalprazolam
Chlordiazepoxide	demoxepam, nordiazepam, oxazepam,
Clobazam	desmethyloclobazam
Lorazepam	lorazepam (no metabolites known)
Midazolam	α-hydroxymidazolam
Prazepam	oxazepam
Triazolam	α-hydroxytriazolam
Temazepam	oxazepam
Diazepam	oxazepam, temazepam, nordiazepam
Bromazepam	3-hydroxybromazepam
Clonazepam	7-aminoclonazepam
Oxazepam	oxazepam (no metabolites known)
Nitrazepam	7-acetamidonitrazepam, 7-aminonitrazepam
Estazolam	3-hydroxyestazolam
Etizolam	α-hydroxyetizolam
Phenazepam	3-hydroxyphenazepam
Deschloroetizolam	9-OH-methyl-, 2-OH-ethyl-, 6-OH-deschloroetizolam
Diclazepam	delorazepam, lorazepam, and lormetazepam
Flubromazepam	hydroxy-flubromazepam
Pyrazolam	pyrazolam (no metabolites known)
Clonazolam	7-aminoclonazolam
Lormetazepam	lorazepam
Flunitrazolam	7-acetamidoflunitrazolam, 7-aminoflunitrazolam

**Figure 1**

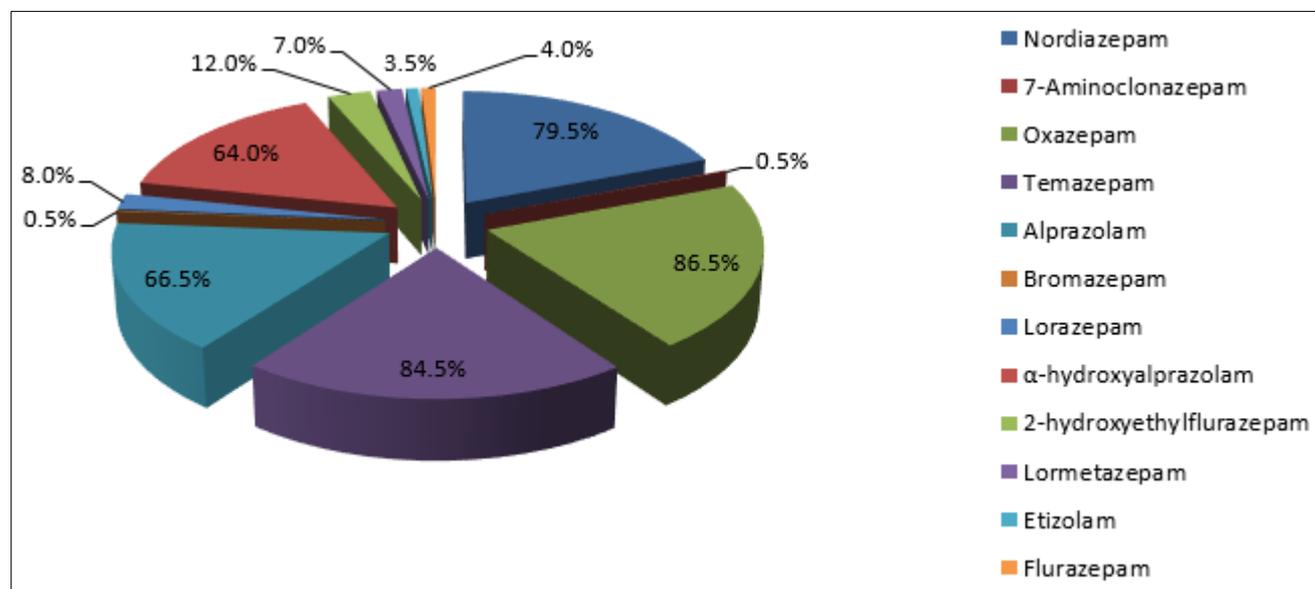
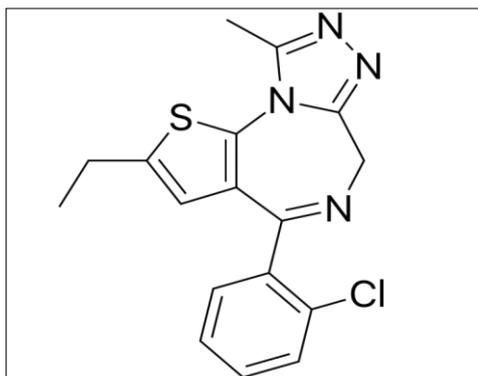


Figure 2



## Discussion

Because there are many common metabolites among the benzodiazepines tested it is difficult to determine the parent drug taken, only benzodiazepines with specific metabolites or parent compounds can be unambiguously identified e.g. flurazepam, flunitrazepam, clonazepam, clobazam, estazolam, triazolam, clonazolam, pyrazolam, phenazepam, midazolam, bromazepam, nitrazepam, etizolam and alprazolam.

The parent compounds only of the following drugs were looked for: midazolam, nitrazepam, clobazam, bromazepam, pyrazolam, clobazam, phenazepam and estazolam. However these parent compounds are only present in trace amounts in urine and it is therefore possible that their metabolites were present but not detected.

The pattern of results we observed indicated that the main benzodiazepines being used are probably diazepam, chlordiazepoxide, temazepam and alprazolam. There is some evidence of flurazepam use but of particular interest is the detection of the new benzodiazepine, etizolam, in 3.5% of samples.

This study was confined to samples screening positive by immunoassay for benzodiazepines. Information on the selectivity of CEDIA<sup>®</sup> benzodiazepine immunoassay for newer benzodiazepines is limited and requires further study.

Of the patients tested 36.5% are being prescribed benzodiazepines by the NDTC, the majority of these are prescribed diazepam. One patient was prescribed bromazepam only and one was prescribed flurazepam in addition to diazepam. Alprazolam is not prescribed to any of the patients and it is notable that 66.5% of patients have tested positive for this drug indicating patients either being prescribed these drugs elsewhere or consumption of illicitly obtained prescription drugs or counterfeit drugs.

The evidence of consumption of the illicit new psychoactive benzodiazepine, etizolam is alarming. Etizolam is not registered as a medicinal product in Ireland. It cannot be determined whether users are consuming etizolam knowingly or consuming illicit counterfeit drugs being sold as diazepam (or some other drug) but with etizolam actually present as a substitute for the stated active substance "as sold".

Etizolam (Figure 2) is a benzodiazepine analogue that provides sedation, euphoria, and anxiety-reduction. It has been used medically in some regions for issues like anxiety, depression, insomnia, and panic disorder. The substance is currently used by many people in non-medical settings, with etizolam being purchased online. It has gathered some popularity on the NPS market in the UK and Europe.<sup>7</sup>

Etizolam is a recognised medicine in Japan, Italy and India the primary application of etizolam being for the treatment of generalised anxiety disorder with depressive symptoms. Additional medical applications include treatments for sleep problems and convulsions, as well as replacement therapies for alcohol addiction. Etizolam has appeared in the form of 'blotters' (similar to LSD paper doses). Its high potency ( $\approx 5x$  diazepam) allows an effective dose of a few milligrams to be present on a paper dose.<sup>8</sup>

In the UK under Statutory Instrument S.I. 2017/ 634, S.I. 2017/ 631 and S.I. 2017/ 632 which come into force on 31 May 2017 sixteen 'designer' benzodiazepines including etizolam which are not registered as medicinal products in the UK were designated as drugs to which section 7(4) of the 1971 Misuse of Drugs Act applies. The circular states that the designer benzodiazepines being controlled under the order are not licensed medicines in the United

Kingdom, but are imported specifically for abuse as NPS. Since the harms and potential harms were deemed to be commensurate with benzodiazepines already controlled as Class C, the Advisory Council on the Misuse of Drugs (ACMD) recommended their control.<sup>9,10</sup> At time of writing etizolam is not controlled in Ireland.

Etizolam was implicated in, or potentially contributed to, the cause of death in 299 drug-related deaths, Scotland 2017.<sup>11</sup> In Ireland etizolam was present in 7 poisoning deaths in 2015 and 6 poisoning deaths in 2016.<sup>3</sup>

In conclusion, there is a serious issue with misuse of alprazolam and its increasing involvement in drug related deaths. Given the evidence presented here of its consumption by the polysubstance drug using population in addiction treatment, it is not surprising that the NPS drug etizolam is now featuring as a drug contributing to drug related deaths in Ireland.

#### **Declaration of Conflict of Interest:**

The author have no conflicts of interest to declare.

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