# Diazepam Quantification in Street Tablets Using Benchtop $^1$H qNMR Spectroscopy: Method Validation and Real-World Application

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**ABSTRACT**

**Nonmedical use of benzodiazepines and their analogues is increasing, with supplies often sourced from inner circles or open internet sources and crypto markets where products are frequently mislabeled. Therefore, fast, reliable, and simple analytical methods are crucial for accurate identification and quantification. This study aimed to develop and validate a quantitative Nuclear Magnetic Resonance (qNMR) method using a benchtop NMR ($^1$H, 60 MHz) instrument and 3-(trimethylsilyl)propionic-2,2,3,3-$d_4$ acid sodium salt as internal calibrant for the analysis of diazepam. The method proved to be specific and selective, with minor interferences caused by a few cutting agents and structurally related compounds. The precision and accuracy results were within the requirements of the United Nations Office on Drugs and Crime guidelines, yielding an LOD of 0.14 mg/mL, an LOQ of 0.46 mg/mL, and a recovery rate of 95%. The testing batch analysis of 100 street tablets resulted diazepam contents ranging from 0.52 mg to 26.20 mg per tablet. Although the average diazepam content in tablets collected between 2002 and 2023 varied, the limited number of samples per year prevented a reliable assessment of temporal trends. However, the variation highlights the need for continuous and comprehensive monitoring to inform and educate individuals who access these substances and to improve harm reduction services. This study confirmed the high efficacy and suitability of benchtop qNMR for analyzing diazepam content in street samples. Future work should expand the sample size and adapt the method to include other benzodiazepine analogues.**

**Keywords: Non-prescribed diazepam tablets, benchtop qNMR, illicit drug analysis**



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## INTRODUCTION

Since the discovery of chlordiazepoxide, the first benzodiazepine drug, in the 1950s,[1] the use and misuse of these anxiolytic compounds have evolved into a global health crisis.[2,3] A pre-pandemic U.S. study found that 30.6 million adults (12.6%) reported annual benzodiazepine use, with 10.4% use as prescribed, while 2.2% engaged in misuse. Misuse was highest among individuals aged 18-25 years, highlighting significant healthcare concerns.[4] Although the European Union Drug Agency cannot shed light on the scale of new and non-controlled benzodiazepines prevalence, their use alongside alcohol and opioid drugs is highlighted as a health risk.[5]

Legitimately, benzodiazepines are most commonly used for their sedative effect in treating conditions such as panic disorder, anxiety, insomnia, epilepsy, catatonia, and alcohol and benzodi-

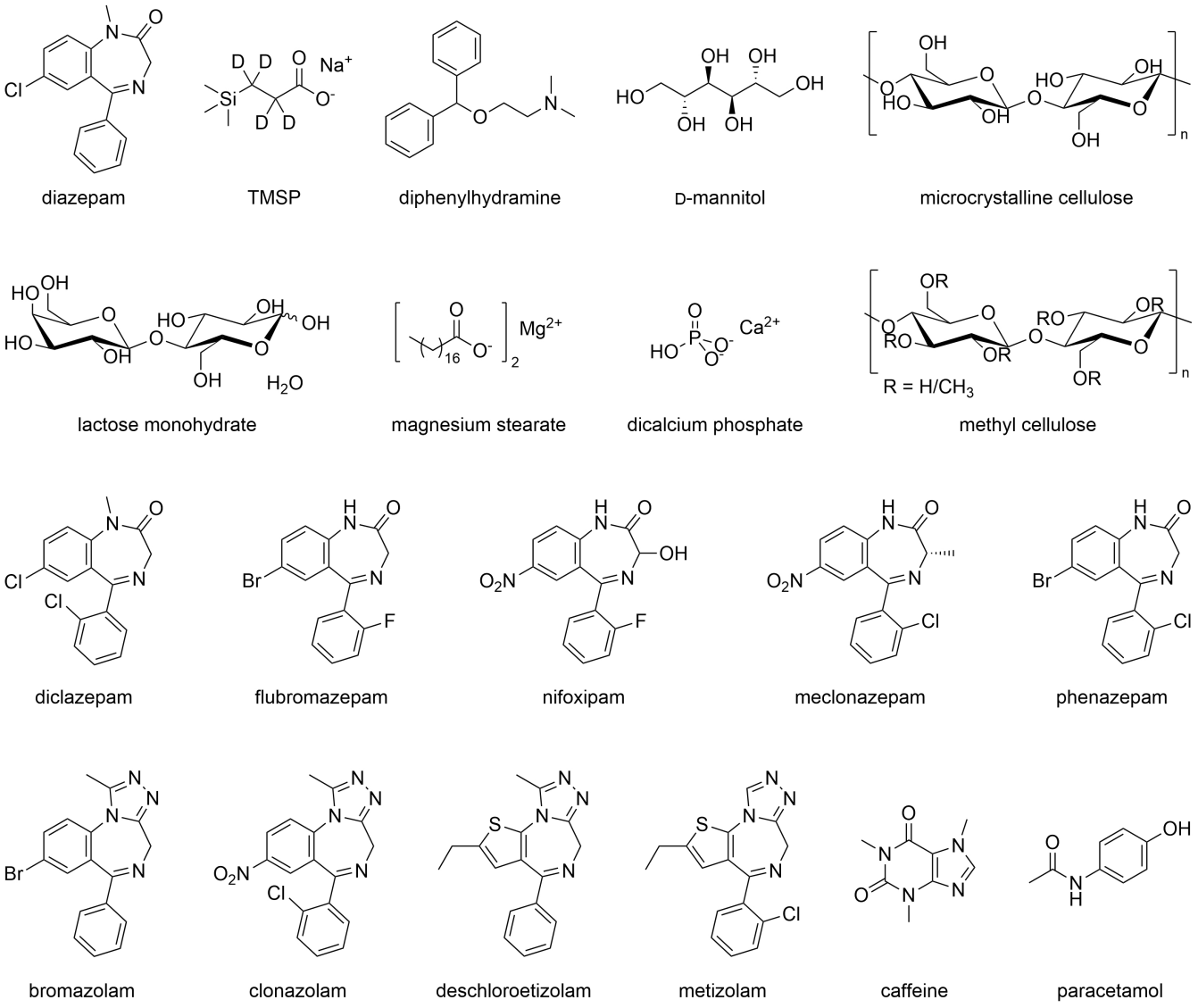


**Fig. 1.** Chemical structures of diazepam and other structurally related compounds alongside the internal calibrant, adulterants and excipients, included in the selectivity and specificity study.

azepine withdrawals.[6] However, their recreational use in party settings is increasingly recognized as a concern.[7, 8] These drugs are frequently co-used with other psychoactive substances, such as alcohol, cocaine, amphetamines, or heroin, either to amplify euphoric sensations or to counteract certain stimulant effects. Prolonged or repeated exposure can result in physical dependence, withdrawal manifestations, and central nervous system dysfunction.[9]

Diazepam, the second benzodiazepine patented by Hoffman-La Roche, was approved for clinical use three years later than chlordiazepoxide, in December 1963, and branded as Valium. It was deemed to have a greater efficacy than chlordiazepoxide, with a shorter duration of action, and a clearer dissociation between its anxiolytic and sedative effects.[1] Although diazepam use in the UK is restricted by medical prescription and categorized as a class C controlled substance,[10] illegal drug distribution channels continue to provide non-medical diazepam, thereby circumventing regulatory controls. Therefore, in addition to street-level face-to-face dealing, technological advancements have moved drug trafficking online, making street diazepam products accessible via open websites and dark web crypto markets.[11, 12] The risks are increased by counterfeit diazepam products containing novel benzodiazepine analogues, which may have a higher abuse potential, increased side effects, and allegedly wider availability through legal loopholes.[13]

Although benzodiazepine analogues are inherently challenging to analyze via mass spectrometry (MS),[14] several analytical methods for their detection, identification, and quantification in biological and bulk samples have been validated.[15-20] NMR spectroscopy minimizes sample preparation workload, is non-destructive and provides relatively fast measurement. As an inherently quantitative analytical technique, qNMR has demonstrated significant potential for the reliable quantification of bulk drugs, despite its lower sensitivity compared to liquid chromatography-mass spectrometry (LC-MS) methods. Crucially, qNMR enables direct quantification without the need for external calibration curves or reference standards of the active compounds.[21, 22] Methods for quantifying diazepam using

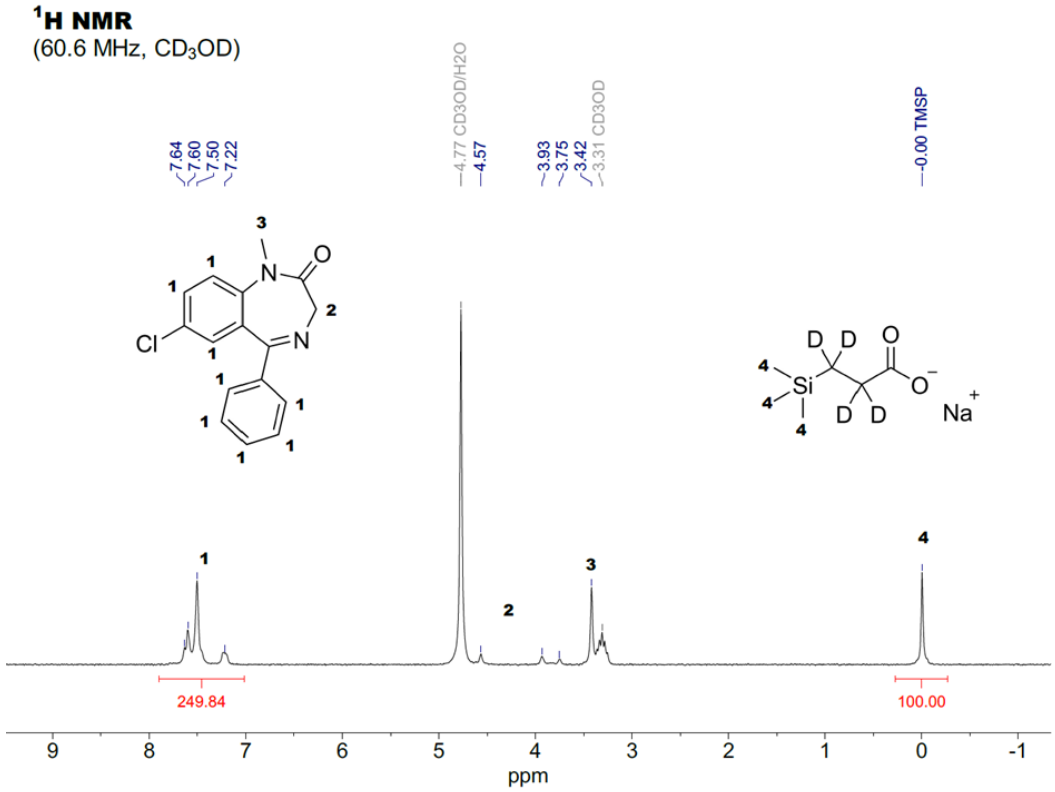


**Fig. 2.** 60 MHz $^1$H NMR spectrum of diazepam and TMSP-$d_4$ as internal calibrant in $CD_3OD$, showing the integrated regions corresponding to the aromatic protons of diazepam (peak pattern **1**) and the resonance of TMSP-$d_4$ (peak **4**).

high-field NMR have been previously described. Junker *et al*. reported a qNMR method ($^1$H, 400 MHz) for acyclovir, captopril and diazepam tablets in DMSO-$d_6$, using caffeine as internal calibrant.[23] Moustafa *et al*. presented a qNMR method ($^1$H, 400 MHz) for diazepam, alprazolam, and chlordiazepoxide in $D_2O$, employing sodium dodecyl sulphate as a surfactant for increased analyte solubility and phloroglucinol as an internal calibrant.[24] Despite the advantages of qNMR methods and the published applications, superconducting high-field NMR instruments require dedicated laboratory space to accommodate the large cryogen-cooled magnet, console and computer, as well as regular refills with liquid nitrogen and helium. As a cost-effective and user-friendly alternative, benchtop NMR systems, also known as low-field Fourier transform NMR, employ permanent magnets and offer low maintenance and high portability, making them suitable for use by non-expert operators.[25] This enables the application of NMR in previously underrepresented sectors, such as forensic science and industrial settings, beyond laboratory research.[26] Benchtop NMR has been effectively applied for both qualitative and quantitative assessments of a wide range of substances, such as pharmaceuticals, excipients, peptides, metabolites, and trace impurities,[22, 27, 28] and for the quantification of 3,4-methylenedioxymethylamphetamine in seized 'ecstasy' tablets.[29, 30] As highlighted by Rock *et al*., data from the National Programme on Substance Use Mortality show clear evidence that deaths involving street benzodiazepine use are increasing. [31] Although most of the benzodiazepine analogues are approved pharmaceutical drugs, they often originate from illicit sources,[31] making the accurate identification and quantification of these substances of paramount importance. Therefore, this study aimed to develop and validate a qNMR method ($^1$H, 60 MHz) to analyze the most common benzodiazepine, diazepam, in tablets seized at music festivals in the UK, using a benchtop NMR instrument and 3-(trimethylsilyl)propionic-2,2,3,3-$d_4$ acid sodium salt (TMSP-$d_4$) as internal calibrant. Accurate measurement of diazepam tablet dose will establish a more reliable basis for medical advice on its misuse, dependence, and withdrawal syndrome, and aid in creating a more effective cessation strategy. Furthermore, it can improve intervention, education, and legislation concerning non-medical benzodiazepine use, thereby helping to reduce morbidity and mortality related to psychoactive substances. Furthermore, reliable and fast on-site analysis will help tailor and enhance the harm reduction advice provided at music festivals.

## METHODS AND MATERIALS

### Chemicals and Samples

Deuterated dimethyl sulfoxide (DMSO-$d_6$), deuterium oxide ($D_2O$), deuterated chloroform ($CDCl_3$), deuterated methanol ($CD_3OD$), ethylene carbonate (TraceCERT®), 1,4-dinitrobenzene, maleic acid (MA, TraceCERT®), and 3-(trimethylsilyl)propionic-2,2,3,3-$d_4$ acid sodium salt (TMSP-$d_4$, 98.0% as per Certificate of Analysis from supplier, purity confirmed as 98.20% via $^1$H benchtop qNMR using maleic acid as internal calibrant, see Table S1 in the Supporting Information) were purchased from Goss Scientific (Cheshire, UK). Diazepam certified reference standard (99.6 ± 4.0%), purity confirmed as 99.06% by $^1$H benchtop qNMR using maleic acid as internal calibrant (see Table S1 in the Supporting Information), was purchased from Chiron (Trondheim,

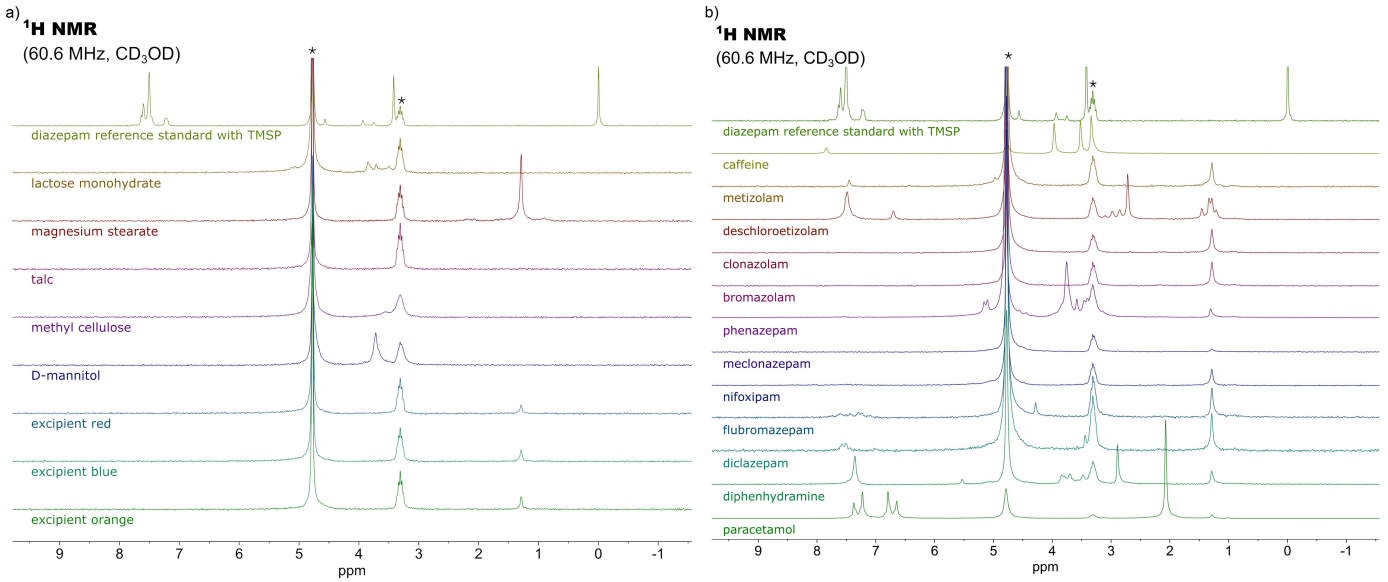


**Fig. 3.** Stacked 60 MHz qualitative $^1$H NMR spectra of compounds in $CD_3OD$ included in the selectivity and specificity testing: (a) excipients and diluents and (b) cutting agents and street samples of structurally related compounds (of unknown concentration). In each stacked plot, the top spectrum shows the diazepam reference material and TMSP-$d_4$ in $CD_3OD$ for comparison. Residual protonated solvent and trace water peaks are marked with an asterisk (*). Line broadening was observed in some spectra, most likely because the obtained suspensions were centrifuged but not filtered.

Norway). Tablet diluents and excipients, such as lactose monohydrate, magnesium stearate, mannitol, methyl cellulose, and talc, were supplied by TICTAC Communications, and caffeine reference material was purchased from Sigma-Aldrich (Dorset, UK). Red, blue, and orange excipient mixtures (Firmapress®: microcrystalline cellulose, magnesium stearate, silicon dioxide, di-calcium phosphate) were purchased from LFA Tablet Presses (Oxfordshire, UK). For specificity and selectivity testing, adulterants (such as paracetamol and diphenhydramine) and benzodiazepine analogue street samples, including diclazepam, flubromazepam, nifoxipam, meclonazepam, phenazepam, bromazolam, clonazolam, deschloroetizolam, and metizolam, were from the collection maintained by TICTAC Communications.

For method validation, diazepam street samples were provided by TICTAC Communications, collected from multiple UK music festivals. The tablets originated either from voluntary submissions to amnesty bins or from items confiscated during entrance inspections. Each sample was homogenized using a clean agate mortar and pestle and subsequently stored in sealed 7 mL glass containers (VWR, Lutterworth, UK) under ambient temperature (i.e., 20–25 °C). A GH-202 analytical balance (repeatability 0.02 mg) from A&D Instruments (Oxfordshire, UK) was used for accurate weight measurements for all experiments.

### Instruments and Methods

One-dimensional $^1$H NMR spectra were acquired at 33 °C using a Nanalysis 60PRO benchtop NMR spectrometer (Nanalysis, Calgary, Canada) operating at 60.60 MHz (1.423 Tesla). Chemical shifts are reported in parts per million (ppm) and coupling constants ($J$) are given as absolute values in hertz (Hz).

Benchtop qNMR measurements employed the following acquisition parameters: a 40-ppm spectral width, 16,384 data points, 32 scans, a spectral center at 10 ppm, a 90° pulse angle, and an automatic receiver gain (fixed for linearity tests). The TMSP-$d_4$ signal displayed the longest relaxation time ($T_1$ = 3.2 s) observed for the benzodiazepine mixtures, requiring a relaxation delay of 20 s, corresponding to > 5 × $T_1$. For qualitative analysis, a relaxation delay of 1 s was used, and each spectrum was acquired with 64 scans. Prior to analysis with MestReNova (v14.2.2), all NMR spectra were manually adjusted for phase and baseline corrections.

The diazepam mass fraction (purity) was determined by comparing the mean integrated area of its characteristic aromatic peak pattern ($\delta$, 7.0 - 8.0 ppm) to the TMSP-$d_4$ resonance ($\delta$, 0.0 ppm), using Equation (1).

$$P_{\text{Diazepam}} = \frac{I_{\text{Diazepam}} \cdot N_{\text{TMSP}} \cdot M_{\text{Diazepam}} \cdot m_{\text{TMSP}} \cdot P_{\text{TMSP}}}{I_{\text{TMSP}} \cdot N_{\text{Diazepam}} \cdot M_{\text{TMSP}} \cdot m_{\text{Diazepam}}} \quad \textbf{(1)}$$

where $P$ = purity, $I$ = integration area, $N$ = number of protons, $M$ = molar mass, $m$ = mass.

High-field $^1$H NMR measurements were conducted at 28 °C using a Bruker Avance III HD NanoBay 400 instrument equipped with a 5 mm $^1$H/$^{13}$C/$^{15}$N/$^{31}$P QNP probe featuring a z-gradient system, located at King's College London. Practical work and benchtop NMR measurements were carried out at TICTAC Communications.

### Method Validation

The validation experiments adhered to the United Nations Office on Drugs and Crime (UNODC) guidelines for quantitative spectroscopic techniques,[32] testing the specificity and selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity and working range, as well as assessments of accuracy, precision, and recovery. Additionally, an external quality control assessment was performed by a different laboratory to ensure the consistency and reliability of the results. Identical samples were analyzed on a high-field NMR instrument at a different laboratory, using their in-house qNMR method ($^1$H, 400 MHz). The

results were compared with those acquired in the present study to highlight the robustness and ruggedness of the technique.

Specificity and selectivity experiments evaluated potential interference in the benzodiazepine analyte region of the $^1$H NMR spectrum (aromatic peaks near 7.5 ppm) and the TMSP-$d_4$ internal calibrant peak. For comparison, street tablet samples of structurally related compounds, including diclazepam, flubromazepam, nifoxipam, meclonazepam, phenazepam, bromazolam, clonazolam, deschloroetizolam, and metizolam, were analyzed. The investigation further included comparative analysis of adulterants (caffeine, paracetamol, and diphenhydramine), common tablet excipients and diluents (lactose monohydrate, magnesium stearate, mannitol, methylcellulose, talc, and mixtures of blue/red/orange excipients), seized samples of verified composition, and legitimate pharmaceutical diazepam products. Chemical structures of the compounds are provided in **Fig. 1**.

LOD is the lowest concentration of an analyte that can be reliably distinguished from the background noise, with a signal-to-noise ratio (SNR) $\geq 3$. LOQ refers to the lowest analyte concentration that can be quantitatively measured with confidence, with an (SNR) $\geq 10$. For the determination of LOD and LOQ, a diazepam certified reference standard solution (1.14 mg/mL in $CD_3OD$), containing 100 µL of TMSP-$d_4$ stock solution (12.50 mg/mL) was prepared and analyzed in triplicate. The recorded SNR was extrapolated to calculate the LOD and LOQ.

Precision testing evaluates the degree of agreement among repeated measurements of the same quantity under identical conditions. Precision was evaluated at three concentration levels: low (2.50 mg/mL), medium (5.00 mg/mL), and high (10.00 mg/mL). Each level was analyzed in triplicate during both morning and afternoon over five consecutive days. According to acceptance criteria, the relative standard deviation (RSD) should not exceed 20% at low concentrations and 15% at higher concentrations. Accuracy reflects the extent to which measured results deviate from the accepted reference value, encompassing both systematic methodological and laboratory-related errors. In alignment with the UNODC guideline, deviations should not exceed ±20% at low concentration levels and ±15% at higher concentrations.

The recovery rate measures the difference between the amount of added and extracted analyte, with an accepted RSD below 15%. A tablet mixture was prepared using 5 mg of the diazepam certified reference standard and 165 mg of blue excipient mix, and the amount of diazepam was obtained both through calculation and analytical experiments. The blue excipient mix was part of the TICTAC Communications collection and chosen to mimic the appearance of legitimate diazepam tablets.

As an external quality control, ten tablets were quantified by a different laboratory (King's College London) using a high-field NMR instrument. The results were compared to those acquired on the benchtop NMR unit.

### Testing Batch Analysis

For the analysis of the street samples, each tablet was first weighed intact and then pulverized using an agate pestle and mortar. An aliquot of 30 mg of the resulting fine powder was accurately measured using a five-digit balance in a glass weighing boat (VWR, Lutterworth, UK). The weighed portion was transferred to a 1.5 mL Eppendorf tube (Appleton Woods, Birmingham, UK) containing 1 mL of $CD_3OD$. The suspension was mixed with vortex for 10 min, and subsequently centrifuged at 8000 rpm for 1 min. From the supernatant, an aliquot of 600 µL of the diazepam extract was placed to a 5 mm NMR tube (GPE Scientific, Bedfordshire, UK) followed by the addition of 100 µL of freshly prepared TMSP-$d_4$ stock solution (12.30 mg/mL in $CD_3OD$). The sample was mixed and then placed in the sample warmer (Nanalysis, Calgary, Canada) to reach the magnet temperature. This test sample was transferred to the instrument and allowed to equilibrate for an additional 5 min to prevent any potential thermal disturbance before analysis. Each test sample was measured in triplicate, with individual measurements lasting 15 min. Quantitative evaluation, including determination of LOD and LOQ, linearity, and quantification assay, was conducted using $^1$H benchtop qNMR, following the acquisition parameters specified in the section **Instruments and methods**. For qualitative analysis (i.e., selectivity and specificity study), 64 scans and a relaxation delay of 1 s were used to expedite measurements while ensuring that compounds with low solubility could still be detected.

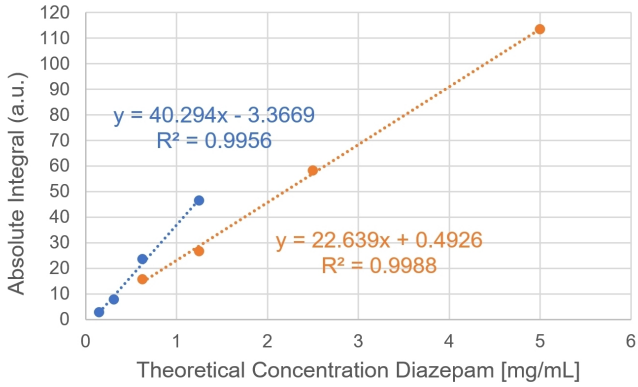


**Fig. 4.** Linearity and working range testing for diazepam. Six concentrations were selected and analyzed in two series, each employing a different fixed receiver gain. The concentrations ranged from 0.6 to 5 mg/mL, $R^2$ = 0.9988, and from 0.15 to 1.25 mg/mL, $R^2$ = 0.9956, respectively.

## RESULTS AND DISCUSSION

Several solvents were tested before $CD_3OD$ was selected for the internal calibrant stock solution and the sample preparation. DMSO-$d_6$ was excluded to minimize the risk of overlap between the labile $^1$H peaks and analyte peaks. Benzodiazepines have poor solubility in $D_2O$; therefore, this was excluded from consideration. $CDCl_3$ proved to be unsuitable due to its poor solubility of the internal calibrant. The internal calibrant acts as a reference material in qNMR and should be an inert compound that does not overlap with any other peaks from the matrix.[21] In this study, TMSP-$d_4$ was selected as the internal calibrant. The peaks of ethylene carbonate and 1,4-dinitrobenzene in the spectrum overlapped with either the analyte or matrix peaks. Conversely, the $^{13}$C satellite peaks in the aromatic region of the MA spectrum overlapped with the analyte peaks, potentially leading to significant errors, particularly when the MA concentration was several times higher than that of the analyte. To accurately determine the purity of the non-certified but highly pure TMSP-$d_4$, a quantification against MA of known purity was carried out (see **Table S1** in the Supporting Information). This resulted in a purity of 98.20% for the internal calibrant, TMSP-$d_4$, which was used for all calculations.

In this study, a stock solution of the internal calibrant, TMSP-

a)

| | Blue mix |
|---|---|
| Mass of diazepam certified reference standard, m(diazepam) | 5.10 mg |
| Mass of cutting agent mix, m(cutting agent mix) | 165.12 mg |
| Mass fraction of diazepam theoretical | 2.97% |
| Mass fraction of diazepam measured | 2.81% |
| Recovery | 95% |

b)

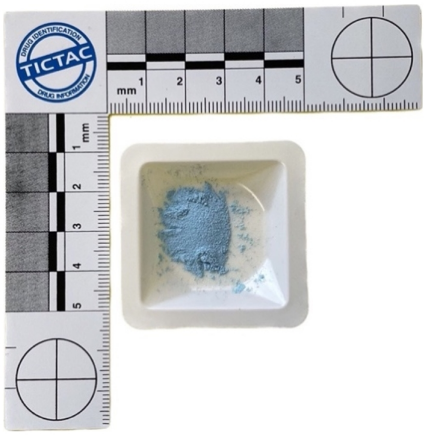


**Fig. 5.** Recovery assessment using blue excipient mixture to evaluate the extraction efficiency (recovery, %), showing (a) the experimental recovery results and (b) the photograph of the blue excipient mixture combined with a diazepam tablet.

$d_4$ in $CD_3OD$ was used for practical reasons related to sample throughput. It should be noted that the additional volume transfer step introduced by using an internal calibrant stock solution, rather than the direct addition of TMSP-$d_4$, might prompt errors if pipettes are not properly calibrated or if the solvent evaporates over time. To avoid potential issues, TMSP-$d_4$ could alternatively be added as a neat compound. However, throughout this study, the potential risk of random or systematic error was accepted to enable the development of a practical method.

**Fig. 2** presents the $^1$H NMR spectrum of diazepam recorded in $CD_3OD$ using TMSP-$d_4$ as the internal calibrant. For diazepam, the aromatic peaks of the eight hydrogen atoms were chosen as the integration region for quantification. Despite covering a relatively wide range, between 7.0 and 8.0 ppm, these peaks were well separated from the residual solvent peaks, and the integration area was interference-free, as compared to the peak integral of TMSP-$d_4$ at 0.0 ppm, which was used to calculate the amount of diazepam.

### Validation Results

Throughout the selectivity and specificity testing, the tablet excipients and diluents (microcrystalline cellulose, di-calcium phosphate, silicon dioxide, magnesium stearate, mannitol, methyl cellulose, talc, and lactose monohydrate) showed poor solubility in $CD_3OD$, leading to no interferences either with the TMSP-$d_4$ or with the analyte peaks (**Fig. 3**). Most spectra did not display any peak patterns in the corresponding $^1$H NMR spectra (**Fig. 3a**), except for the residual methanol solvent, TMSP-$d_4$ ($\delta$ at 0.0 ppm), and a grease impurity ($\delta$ at 1.3 ppm). However, lactose and mannitol showed peaks around 3.5 ppm. Some cutting agents and some of the diazepam structurally related compounds showed a varying degree of overlapping within the analyte region (**Fig. 3b**). Some compounds exhibited low peak intensity in the aromatic region, which can be attributed to low concentration, low solubility in $CD_3OD$, or the presence of impurities. Aromatic peaks of other benzodiazepines (such as diclazepam and phenazepam) would overlap in the aromatic region, and thus an identification step should be carried out prior to quantification. The spectrum of diazepam shows a multiplet between 7.4 and 7.7 ppm for the aromatic hydrogens. The methylene group displays an AB pattern around 3.8 and 4.6 ppm, partially overlapping with the water peak. The methyl peak is observed at 3.4 ppm, overlapping with the methyl peak of the methanol. The spectrum clearly distinguishes the peak patterns of the three cutting agents (paracetamol, caffeine, and diphenhydramine) from those of diazepam. No peak overlap was observed between TMSP-$d_4$ and any of the excipients, cutting agents, or structurally related compounds. Although there is potential overlap with other aromatic compounds, the aromatic peaks of diazepam exhibit a characteristic pattern and were chosen due to the absence of interference from water, solvent, or the internal calibrant peaks in $CD_3OD$.

Sensitivity assessment determined the LOD for diazepam to be 0.14 mg/mL and an LOQ to be 0.46 mg/mL. As previously mentioned, the threshold for SNR was set as 3 (LOD) and 10 (LOQ) or higher. However, a higher SNR can be achieved by increasing the number of scans or the sample concentration, thereby enabling lower LOD and LOQ values expressed in units of mg/mL.[29]

Although qNMR methods are inherently linear, making confirmation of their linearity unnecessary, to fully comply with the UNODC guidelines, the linearity for diazepam at lower concentrations was confirmed. As shown in **Fig. 4**, linearity assessment yielded an $R^2 > 0.99$. The working range spanned concentrations between 0.15 and 5.00 mg/mL, providing reliable and consistent results that proved the method suitable for quantifying diazepam in street tablets, exceeding 30 mg of diazepam per tablet (three times the highest available pharmaceutical strength).

Three concentration levels (2.5 mg/mL, 5 mg/mL, and 10 mg/mL) were used to assess the accuracy and precision (see **Tables S2 – S4** in the Supporting Information). The RSD for the precision experiments was expressed as a percentage for the three concentrations: 1.45 %RSD, 0.82 %RSD, and 1.22 %RSD, respectively. Accuracy testing revealed deviations of 3.25%, 4.28%, and 3.89% from the expected value. Accuracy was assessed using the purity of the diazepam certified reference material as the expected value. All precision results fell within 15% of the UNODC guideline acceptance criteria.

To test the recovery rate, 5.10 mg of diazepam reference standard (99.06%) was thoroughly mixed with 165.12 mg of the blue,

**Table 1.** Comparison of external quality control results and percentage relative standard deviations (%RSD) for ten randomly selected diazepam samples, analyzed using both 60 MHz and 400 MHz $^1$H qNMR methods

| Tablet identification number | Diazepam mass fraction in % | | Deviation 60 MHz $^1$H qNMR from 400 MHz $^1$H qNMR in % |
|---|---|---|---|
| | 60 MHz $^1$H qNMR result | 400 MHz $^1$H qNMR result (external quality control) | |
| 38512 | 1.16 | 1.29 | 10.08[a] |
| 32938 | 5.24 | 5.22 | 0.38 |
| 32937 | 6.78 | 6.86 | 1.17 |
| 32935 | 2.55 | 2.51 | 1.59 |
| 31894 | 7.21 | 7.30 | 1.23 |
| 31784 | 5.08 | 5.10 | 0.39 |
| 31640 | 4.64 | 4.72 | 1.69 |
| 30728 | 5.72 | 5.69 | 0.53 |
| 30664 | 4.42 | 4.34 | 1.84 |
| 30204 | 2.49 | 2.48 | 0.40 |

[a] the diazepam content in this sample was calculated to be below the LOQ of the method.

ready-made excipient mix. Selectivity and specificity testing (see **Fig. 3a**) confirmed that the spectrum of the blue mix showed no interference with either the target diazepam or the TMSP-$d_4$ peaks. Analyte recovery from spiked tablet mixtures was reproducible and consistently met UNODC acceptance criteria. The blue spiked powder was found to contain 2.81% diazepam, whereas the theoretical content of diazepam was calculated as 2.97%. Therefore, the recovery rate was 95%, proving that the method can be used reliably (**Fig. 5**).

Ten identical NMR samples of diazepam tablets, seized at festivals between 2009 and 2022, served as external quality controls and were analyzed using $^1$H qNMR (both 60 and 400 MHz) in a different laboratory, showing deviations within acceptable limits (see **Table 1**). Of note, these ten tablets are different than the ones included in the testing batch results. The highest deviation (10.08%, sample 38512) was observed at the lowest diazepam concentration (1.29% mass fraction diazepam, see **Table 1**). This could be attributed to the fact that a concentration of 1.29% diazepam in the 30 mg ground tablet material corresponds to 0.33 mg/mL in the resulting NMR sample, which falls below the LOQ of the method (0.46 mg/mL). Excluding this highest deviation value, the range was between 1.84% and 0.38%. For lower concentrations, increasing the number of scans would be advisable; however, in this validation, all experiment parameters were kept the same to ensure consistency.

### Testing Batch Results

Although benzodiazepine drugs have been the first-choice treatment of anxiety and insomnia for years, their abuse potential, withdrawal syndrome and rebound effects have raised concerns and limited their clinical use.[33, 34] Nevertheless, benzodiazepines have been reported to be well tolerated by patients, and the large gap between their therapeutic and toxic doses (therapeutic index) is reflected in prescribing patterns.[35] Other studies have emphasized that the adverse effects associated with benzodiazepine use are not only dose-dependent, but they are also influenced by duration of the treatment, the type of benzodiazepine used (i.e., anxiolytic or hypnotic), and the individual-related factors.[35-37]

As a long-acting benzodiazepine, diazepam is typically prescribed in 2, 5 or 10 mg dosage forms, with the lowest (2 mg) being close to placebo and the highest (10 mg) one showing significant effects.[38] The pharmaceutical diazepam products added to the TICTAC Communications collection between 1988 and 2023 were blue, white and yellow round tablets. The most common markings were D 2, D 5 and D 10, reflecting the dose of the tablet. Several were marked C DC on one side, and the majority were half-scored (see **Fig. S1** in the Supporting Information).

A total of 100 diazepam tablets seized or collected at various music festivals were included in the testing batch to highlight the practicability of the method for high-throughput analysis. These ranged from 1998 to 2023, spanning 25 years. Although the origin could not be definitively traced, blister pack information indicated that the product was manufactured in the UK, India, and France. Since many of them might have been purchased online rather than from a pharmacy, the diazepam content remains unknown and cannot be standardized or certified by users. Therefore, this study aimed to shed light on the actual amount of diazepam in street or other non-pharmaceutical samples.

The lowest diazepam dose was 0.52 mg (0.30%) in tablet no. 21955, while the highest reached 26.25 mg (15.72%) in tablet no. 19-1650. Out of the 100 samples analyzed, four of them (no. 33705, 19-1633, 22-046, 22-277) failed to display the diazepam peaks of interest. A possible explanation is that their diazepam level was below the limit of detection (0.14 mg/mL), hence not covered by the working range of the developed method. This would support the allegation that they were illicitly produced, as the lowest pharmaceutical commercially available diazepam

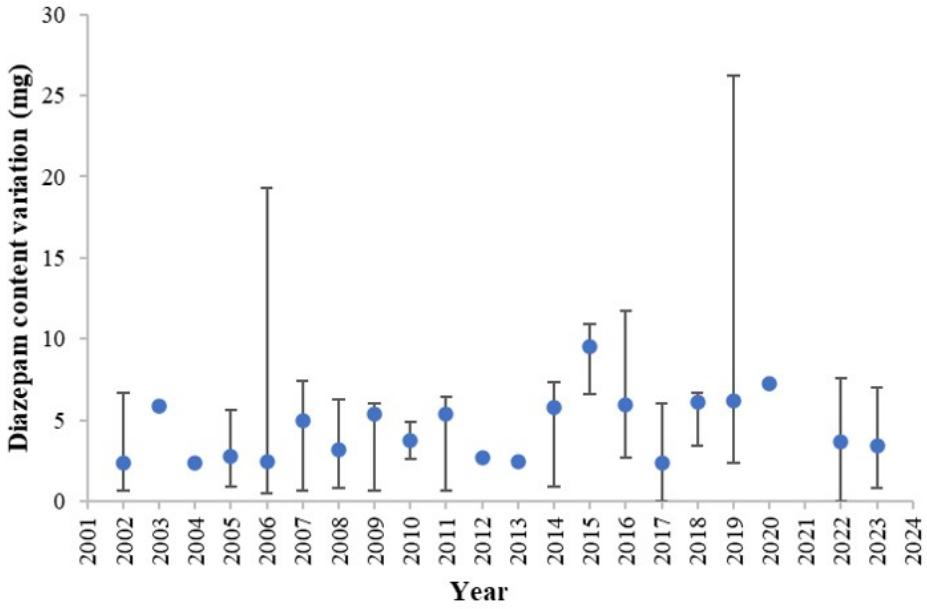


**Fig. 6.** The trend in the content of diazepam tablets sampled from 2002 to 2023 (n = 97). The median diazepam content per year is represented by a blue dot, with the minimum and maximum values indicated by the error whiskers.

tablet contains 2 mg of the active ingredient. The lower diazepam content in tablets further corroborated the crypto market research, which found that a diluted product maximizes the profits.[11,12]

As shown in **Fig. 6**, the diazepam content fluctuated from 2002 to 2023. The content increase observed between 2017 and 2019 may be associated with the surge in popularity of the so-called 'Sleeping beauty diet'.[39] This fad, which became widely discussed around 2017, promoted the use of benzodiazepines to induce prolonged sleep and thereby avoid food intake. Over time, continuous use could have resulted in drug tolerance and dependence, prompting individuals to consume progressively higher doses to achieve the same sedative effect. Therefore, the higher doses in street tablets might be a consequence of the demand. The lowest diazepam content, 0.52 mg (0.30%), was recorded in 2006, while the highest, 26.25 mg (15.72%), was recorded in 2019. In the same year, a few more high-strength tablets were recorded, ranging between 13 and 18 mg of diazepam content. Interestingly, the second-highest diazepam amount (19.30 mg) was recorded in 2006, when the lowest diazepam content of 0.52 mg (0.30%) was also recorded. A possible explanation could be that the samples tested from 2019 were mainly street tablets, which are more likely to be counterfeit products. This can become problematic when people dose their intake based on previous administration or re-dose too quickly when they do not experience the desired effects.

In 2018, the lowest tablet analyzed weighed 60 mg, containing 3.45 mg of diazepam, whereas the highest tablet, recorded in 2014, weighed 350 mg with a diazepam content of 5.67 mg. This shows there is no strong linear correlation between total tablet weight and diazepam content.

In recent years, the active diazepam content has shown a significant decrease, ranging from 7.60 mg in 2022 to 0.82 mg in 2023. Another possible reason for the decline, as shown in **Fig. 6**, could be the impact of the COVID-19 pandemic, during which all public events and festivals were canceled, hence the illegal drug market stagnated. The lack of demand led to revenue drops for illicit drug manufacturers and operational difficulties.[40] The quality of the illegal drugs could have been impacted by more prominent dilution. However, it should be noted that the relatively small number of samples included per year, with no samples analyzed from 2021, does not provide a representative overview, and no reliable over-time trends have been established yet. A list of all samples included in the testing batch analysis, alongside their diazepam content, can be found in the Supporting Information (**Table S5**).

Unlike excipients and diluents, which are inactive substances, adulterants are prone to cause or increase harm to individuals who use the falsified drugs.[41] Moreover, drug adulterations can occur both unintentionally and intentionally, the latter often involving substances such as caffeine, paracetamol, and diphenhydramine, which are commonly seen mixed with illicit drugs.[42]

Caffeine is the most used psychoactive stimulant drug worldwide[43] promoting alertness and alleviating fatigue.[44] During the 1960s, it was categorized as a drug adulterant because of its legal status, widespread availability, and low cost.[42] This study found that caffeine was added to the street samples. Although not confirmed, the aim could be to maximize profit. However, this combination has contradictory effects: caffeine produces stimulation, the benzodiazepines exert amnestic, anxiolytic, hypnotic and sedative effects,[45,46] which are akin to alcohol.[43] Co-consumption may attenuate the perceived effects of benzodiazepines, which might ultimately boost their abuse, hence supporting illicit market demand. Moreover, the counteracting effects could provide an illusion of sobriety, leading users to believe they are still conscious and responsible for their decisions. Individuals may experience intermittent episodes of confusion, with fluctuating levels of consciousness. Such perceptual distortions can mislead recreational users into escalating their intake in pursuit of "euphoria," thereby increasing the risk of overdose.[47,48]

Paracetamol was identified as a common adulterant during the 1980s, primarily owing to its accessibility and low cost—paralleling the reasons associated with caffeine

adulteration.[42] Its presence is most noted in heroin samples. Although it was previously detected in benzodiazepine street samples, no drug interactions between paracetamol and benzodiazepines have been reported. Mulley *et al.* attempted to study the influence of paracetamol on diazepam in four volunteers. In two females, a delayed excretion of diazepam was observed, but no other alterations to the diazepam and the desmethyldiazepam metabolite were detected in the blood samples.[49] A study investigating the benzodiazepine deaths in the UK emphasized the need for analytical techniques that can detect low concentrations and combinations of benzodiazepines with other drugs, finding paracetamol was present in 4 out of 35 cases.[50]

Diphenhydramine is an antihistamine commonly administered to relieve allergic symptoms such as eczema, hay fever, reactions to insect bites or stings, and urticaria.[51] Because it produces sedative effects comparable to those of benzodiazepines, it is sometimes used for the short-term management of insomnia.[52] The compound has also been detected in illicit formulations containing fentanyl, cocaine, phencyclidine, opioids, and methamphetamine, where its presence may contribute to increased overall toxicity. Moreover, based on its pharmacodynamic synergism, if added to street benzodiazepine tablets or taken concomitantly, an increase in the sedative effects and impact on psychomotor abilities can be expected.[53]

Therefore, this study confirms that benzodiazepine street tablets can contain cutting agents, and their presence poses more risks to users. Furthermore, it is of paramount importance that the qNMR method is both selective and specific in order to quantify the amount of diazepam when mixed with other substances.

## CONCLUSIONS

The escalating misuse of prescription drugs is an increasing global concern.[54] Benzodiazepines are frequently co-administered with other substances, such as alcohol, cocaine, amphetamine, and heroin, especially in party and festival settings.[7, 8] Furthermore, harm reduction efforts often rely on user perceptions, which may differ significantly from the actual substance composition and dosage, thereby increasing the risk of harm.[31, 55]

A quantification method for diazepam using a benchtop NMR instrument with an internal calibrant was successfully developed and validated. The developed method demonstrated strong specificity and selectivity, achieving an LOD of 0.14 mg/mL and an LOQ of 0.46 mg/mL, even in the presence of common cutting agents, excipients, and structurally related analogues. However, the characteristic diazepam peaks overlapped with the peaks of certain analogues (such as diclazepam and phenazepam) and a cutting agent (caffeine). Under these circumstances, accurate quantification requires initial qualitative NMR analysis and comparison with individual compound spectra from selectivity testing. Precision, accuracy and recovery rates were evaluated, with all results conforming to the UNODC acceptance criteria. Among the 100 samples analyzed, diazepam content ranged from 0.52 mg (0.30%) to 26.25 mg (15.72%). Variability in street sample analysis underscores the importance of continuous market monitoring and tracking of emerging trends. The method validation in this study demonstrated that benchtop qNMR is a highly effective and suitable approach to analyze diazepam content in street tablet samples.

Future studies should include a larger number of street samples to expand the testing batch and allow for a within-batch variation assessment. Finally, additional experiments should be performed to increase the sensitivity of the method, which would extend the applicability to other benzodiazepine analogues, such as etizolam or alprazolam, commonly found in lower-strength tablets (maximum 2 mg dose). Preliminary investigations conducted beyond the scope of this study indicate that these lower-dose analogues could be reliably quantified by increasing the number of scans. The development of a method that broadens the scope of benzodiazepine analogues will be the focus of future investigations.

## DISCLOSURES

AFGK is employed by Nanalysis Scientific C orp. T he other authors declare no conflicts of interest..

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