Recommended methods for the Identification and Analysis of Barbiturates and Benzodiazepines under International Control
Photo credits:
UNODC Photo Library; UNODC/Ioulia Kondratovitch; Alessandro Scotti.
Recommended Methods for the Identification and Analysis of Barbiturates and Benzodiazepines under International Control

MANUAL FOR USE BY NATIONAL DRUG ANALYSIS LABORATORIES

UNIVERSAL NATIONS OFFICE ON DRUGS AND CRIME Vienna

UNIVERSAL NATIONS New York, 2012
Note

Operating and experimental conditions are reproduced from the original reference materials, including unpublished methods, validated and used in selected national laboratories as per the list of references. A number of alternative conditions and substitution of named commercial products may provide comparable results in many cases, but any modification has to be validated before it is integrated into laboratory routines.

Mention of names of firms and commercial products does not imply the endorsement of the United Nations.
Acknowledgements

The UNODC Laboratory and Scientific Section (LSS, headed by Dr. Justice Tettey), wishes to express its appreciation and thanks to Professor Alexander Gray, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, United Kingdom, for the preparation of the first draft of the present manual, and to Dr Pirjo Lillisunde, Head of Drug Laboratory, National Institute for Health and Welfare, Finland, for the expert review of the document. The preparation of this manual was coordinated by Dr. Iphigenia Naidis, staff member of LSS. The contribution of other UNODC staff is gratefully acknowledged.
# Contents

<table>
<thead>
<tr>
<th>Acknowledgements</th>
<th>iii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Background</td>
<td>1</td>
</tr>
<tr>
<td>Purpose and use of the manual</td>
<td>1</td>
</tr>
<tr>
<td>I. Barbiturates</td>
<td>3</td>
</tr>
<tr>
<td>1. Classifications and definitions</td>
<td>3</td>
</tr>
<tr>
<td>2. Description of pure compounds</td>
<td>4</td>
</tr>
<tr>
<td>3. Qualitative and quantitative analysis of materials containing barbiturates</td>
<td>5</td>
</tr>
<tr>
<td>3.1 Sampling</td>
<td>5</td>
</tr>
<tr>
<td>3.2 Extraction and sample preparation</td>
<td>6</td>
</tr>
<tr>
<td>3.3 Presumptive tests</td>
<td>7</td>
</tr>
<tr>
<td>3.4 Thin-layer chromatography (TLC)</td>
<td>9</td>
</tr>
<tr>
<td>3.5 Gas chromatography (GC) with flame ionization detector (FID)</td>
<td>11</td>
</tr>
<tr>
<td>3.6 Gas chromatography - mass spectrometry (GC-MS)</td>
<td>13</td>
</tr>
<tr>
<td>3.7 High performance liquid chromatography (HPLC)</td>
<td>16</td>
</tr>
<tr>
<td>3.8 Infrared spectroscopy</td>
<td>19</td>
</tr>
<tr>
<td>II. Benzodiazepines and related substances</td>
<td>21</td>
</tr>
<tr>
<td>1. Classifications and definitions</td>
<td>21</td>
</tr>
<tr>
<td>2. Description of pure compounds (see also annex II)</td>
<td>22</td>
</tr>
<tr>
<td>3. Qualitative and quantitative analysis of materials containing benzodiazepines</td>
<td>23</td>
</tr>
<tr>
<td>3.1 Sampling</td>
<td>23</td>
</tr>
<tr>
<td>3.2 Extraction and sample preparation</td>
<td>23</td>
</tr>
<tr>
<td>3.3 Presumptive tests</td>
<td>24</td>
</tr>
<tr>
<td>3.4 Thin-layer chromatography (TLC)</td>
<td>25</td>
</tr>
<tr>
<td>3.5 Gas chromatography (GC) with flame ionization detector (FID)</td>
<td>28</td>
</tr>
<tr>
<td>3.6 Gas chromatography - mass spectrometry (GC-MS)</td>
<td>30</td>
</tr>
<tr>
<td>3.7 High performance liquid chromatography (HPLC)</td>
<td>31</td>
</tr>
<tr>
<td>3.8 Infrared spectroscopy</td>
<td>35</td>
</tr>
</tbody>
</table>
References .......................................................... 37
Further reading ..................................................... 40

Annex
I. Description of barbiturates under international control .......... 42
II. Description of benzodiazepines under international control ....... 49
Introduction

Background

Barbiturates and benzodiazepines are used in medicine as anticonvulsants, anxiolytics, hypnotics, sedatives, skeletal muscle relaxants and tranquilizers. Currently, twelve barbiturates and thirty-five benzodiazepines are under international control. Those that have found their way into illicit use have mainly been diverted from the licit market.

Seizures of benzodiazepines and barbiturates increased by more than 50 per cent between 2005 and 2009 according to the World Drug Report 2011 [1]. The use of benzodiazepines is common among drug users, including substitution treatment clients.

The non-medical use of barbiturates, benzodiazepines and other sedatives together with opioids has been a commonly observed phenomenon in many regions. The existence of parallel markets is observed, where prescription drugs are sold outside the control of the health authorities as well as through illegally operating Internet pharmacies. Benzodiazepines, specifically aprazolam and diazepam, are among the most often diverted and abused psychotropic substances [2]. However, given the absence of information on overall drug use patterns, it is difficult to estimate the extent of non-medical prescription drug use worldwide.

Purpose and use of the manual

The present manual is one in a series of similar UNODC publications dealing with the identification and analysis of various types of drugs under international control. These manuals are the outcome of a programme pursued by UNODC since the early 1980s, aimed at the harmonization and establishment of recommended methods of analysis for national drug analysis laboratories.

The present manual combines and updates the two existing manuals on Recommended methods for testing barbiturate derivatives [3] and benzodiazepine derivatives [4] under international control, published in 1989 and 1989 respectively. It has been prepared taking into account developments in analytical technology with a view to
Recommended Methods for the Identification and Analysis of Barbiturates and Benzodiazepines

providing the basis for reliable forensic evidence on seized materials containing barbiturate or benzodiazepine derivatives. It is divided into two parts which provide analytical methodologies for the two types of substances with reference to validated techniques. Description of the individual barbiturates and benzodiazepines under international control is provided in two annexes.

In line with the overall objective of the series of UNODC publications, this manual suggests approaches that may assist drug analysts in the selection of methods appropriate to the sample under examination and provide data suitable for the purpose at hand, leaving room also for adaptation to the level of sophistication of different laboratories and the various legal needs.

Therefore, the methods described here should be understood as practical guidance, that is, minor modifications to suit local circumstances should normally not change the validity of the results. Not all methods described in this manual need to be applied to all samples suspected to consist of or contain barbiturates or benzodiazepines. The choice of the methodology and approach to analysis as well as the decision as to whether or not additional methods are required remain with the analyst and may also be dependent on the availability of appropriate instrumentation and the level of legally acceptable proof in the jurisdiction within which the analyst works. The reader should be aware that there are a number of other methods, including those published in forensic science literature, which may also produce acceptable results. However, any new method that is about to be used in a laboratory must be validated and/or verified prior to routine use.

Attention is also drawn to the importance of the availability to drug analysts of reference materials and literature on drugs of abuse and analytical techniques. Moreover, the analyst must of necessity keep abreast of current trends in drug analysis, consistently following current analytical and forensic science literature.

UNODC Laboratory and Scientific Section welcomes observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

Laboratory and Scientific Section
United Nations Office on Drugs and Crime
Vienna International Centre
P.O. Box 500
1400 Vienna
Austria
Fax: (+43-1) 26060-5967
E-mail: Lab@unodc.org

All manuals, as well as guidelines and other scientific-technical publications may be requested by contacting the address above.
I. Barbiturates

The abuse of barbiturates is widespread and it has often been found mixed with other substances of abuse such as heroin. The international nature of the illicit market means that any forensic laboratory may encounter a range of these compounds. However, virtually all of the barbiturates in the illicit market result from diversion from legitimate sources and there is no reported evidence of clandestine manufacture.

The twelve barbiturate derivatives under international control (1971 Convention on Psychotropic Substances) appear mainly as capsules and tablets. Some are marketed in other pharmaceutical forms such as elixirs, injectable solutions and sterile powders for injection. Pentobarbital sodium is available in some countries as rectal suppositories and barbital sodium is commonly sold in powder form. Barbiturates often occur as mixtures with other barbiturates (e.g. amobarbital/secobarbital), with other drugs (e.g. aspirin, caffeine, codeine, ephedrine, theophylline) and with attendant pharmaceutical excipients. This makes the isolation and identification of a specific barbiturate a considerable analytical challenge.

Analysts should be aware of the particular barbiturates commonly available in their area as well as the characteristics and methodologies for their identification and analysis. As barbiturate derivatives end up on the illicit market from diversion from legitimate sources, reference should be made to national pharmacopoeias and drug tablet and capsule identification guides for preliminary screening information.

1. Classifications and definitions

Barbiturates are drugs that act as central nervous system depressants, and, by virtue of this, they produce a wide spectrum of effects, from mild sedation to total anesthesia. Barbiturates are therapeutically used as anaesthetics, anticonvulsants, anxiolytics, hypnotics and sedatives. They are usually classified according to the duration of their clinical effects into “long-”, “intermediate-”, “short-” and “ultrashort-” acting compounds.

They are synthetic drugs derived from barbituric acid (figure 1 below), which is a synthetic condensation product of malonic acid and urea. They differ mainly in the substitution pattern at position-5 with some also including an N-methyl at N-1 (see
annex I). The most well known derivative, phenobarbitone (see annex I), has been used medicinally since 1912, mainly in the treatment of epilepsy.

Over 2,500 barbiturates have reportedly been synthesized with more than 50 of these presently marketed for clinical use throughout the world. Twelve of these are subject to international control under the Convention on Psychotropic Substances 1971 as follows:

Schedule. II: Secobarbital
Schedule. III: Amobarbital, butalbital, cyclobarbital and pentobarbital
Schedule. IV: Allobarbital, barbital, butobarbital, methylphenobarbital, phenobarbital, secbutabarbital and vinylbital

In recent years, there has been a significant downturn in prescribing and therefore the general availability of barbiturates owing to their side-effects and associated dependency problems. An exception is phenobarbitone which is still used as an anticonvulsant/anti-epileptic. Through the years, alternatives to barbiturates have been developed including methaqualone and mepchloraloned (Recommended methods for the identification and analysis of Methaqualone/Mepchloralone, ST/NAR/15/Rev.1), benzodiazepines and related compounds.

2. **Description of pure compounds**

The information related to each of the twelve barbiturates (allobarbital, amobarbital, barbital, butalbital, butobarbital, cyclobarbital, methylphenobarbital, phenobarbital, pentobarbital, secbutabarbital, secobarbital and vinylbital) currently under international control, can be found in the annex I. In addition, the Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control [5] (MLD, ST/NAR/1/Rev.2) provides information on the nomenclature of the barbiturate derivatives under international control. It also includes synonyms and different trade names for the relevant substances and provides information on their control status.
3. Qualitative and quantitative analysis of materials containing barbiturates

Generally, in attempting to establish the identity of a controlled substance in suspected material, the analytical approach must entail the determination of at least two uncorrelated parameters one of which should provide information on the chemical structure of the analyte (for example, IR, MS; or tandem methods such as GC-MS). It is recognized that the selection of these parameters in any particular case would take into account the drug involved and the laboratory resources available to the analyst. It is also accepted that unique requirements in different jurisdictions may dictate the actual practices followed by a particular laboratory.

When possible, three entirely different analytical techniques should be used, for example: colour tests, chromatography (e.g. TLC, GC or HPLC) and spectroscopy (e.g. IR or UV). Hyphenated techniques, such as gas chromatography – mass spectrometry (GC-MS), count as two parameters, provided the information from both techniques is used (i.e. retention time and mass spectral characteristics).

3.1 Sampling

Seizures of barbiturates and related substances may consist mainly of pharmaceutical drugs formulated as tablets, capsules, oral liquids or injectables. Sampling depends on the number and type of dosage units seized.

The principal reason for a sampling procedure is to permit an accurate and meaningful chemical analysis. Because most methods, qualitative and quantitative, used in forensic drug analysis laboratories require very small aliquots of material, it is vital that these small aliquots be representative of the bulk from which they have been drawn. They should conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or by regional or international organizations. The use of an approved sampling system also helps to preserve valuable resources and time by reducing the number of determinations needed. The Guidelines on Representative Drug Sampling [6] provides the general aspects of qualitative sampling of multi-unit samples. However, it is recognized that there may be situations where, for legal reasons, the normal rules of sampling and homogenization cannot be followed.

Having chosen the materials to be examined, the analyst must identify the specific drug present and quantify the amount of the drug present if this is required. The full physical description of the exhibit should be carried out and, if from a licit source, should be identified by reference to the appropriate databases and the identity confirmed. The analyst should bear in mind that if the dosage forms appear to be from an illicit source, i.e. not recognizable as a pharmaceutical product, then a full chemical analysis of the material including extraction of the drug, presumptive tests, screening and confirmatory tests will be required.
3.2 Extraction and sample preparation

Extraction techniques for substances in tablets, capsules, aqueous solutions (for injection), syrups, residue from syringes should be mentioned and reference made to relevant pharmacopoeias. General aspects, e.g. solubility for the relevant group of substances, acid or salt concentration of the medicament in the licit preparation, should be considered (see annex I).

Virtually all of the barbiturates encountered in illicit traffic appear in the form of tablets, capsules and bulk powder diverted from legitimate sources. They are present as the free acid or as the sodium or calcium salt (see annex I).

For qualitative analysis

Both the free acids and the salts are soluble in methanol and this is the solvent of choice for sample preparation for presumptive or qualitative analysis.

Method

Triturate a quantity of finely powdered tablet or capsule contents or bulk drug powder with a small amount of methanol sufficient to obtain a solution containing approximately 1 to 20 mg/ml of barbiturate. The extract may be used directly or filtered and evaporated to dryness under a stream of nitrogen.

For quantitative analysis

(a) Capsules and tablets containing barbiturates in the free acid form

Method

Combine the contents of a representative number of capsules or tablets as determined by the sampling procedure. Transfer an accurately weighed amount of the capsule or tablet contents, equal to the full weight of one or more tablets or capsules to a suitably sized volumetric flask and make up to volume with ethyl acetate. The extract may be used directly or an aliquot removed, filtered and evaporated to dryness under a stream of nitrogen.

(b) Capsules and tablets containing barbiturates in the salt form

Method

Dissolve or suspend an accurately weighed amount of the representative sample of barbiturate as determined by the sampling procedure above, in an appropriate
Tablet and capsule identification guides

As a first test, analysts should refer to national identification guides for presumptive identification of the barbiturate products commonly available in their country. Some useful guides [7, 8, 9, 10] include pictorial representations of legitimate capsule and tablet forms to assist in identification.

Colour tests

It must be stressed that a positive result from a colour test is only a presumptive indication of the possible presence of a barbiturate derivative. Nevertheless, the colour test suggested below [11, 12] is very useful because all compounds in the barbiturate class react in a similar manner and very few other drugs cross-react to give the same colour with the test reagents. However, no information as to which particular barbiturate is present can be obtained with this colour test.

(a) Dille-Koppanyi test

<table>
<thead>
<tr>
<th>Solution 1:</th>
<th>Dissolve 0.1g cobaltous acetate tetrahydrate in 100 ml absolute methanol, then add 0.2 ml glacial acetic acid.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution 2:</td>
<td>Mix 5 ml isopropylamine with 95 ml absolute methanol.</td>
</tr>
</tbody>
</table>

Method

Three drops of solution 1, followed by three drops of solution 2 are added to a small amount of the suspected sample on a test plate. It is recommended to separately perform negative controls and use reference material of the suspected barbiturate for positive results.

Results

A purple colour indicates the presence of a barbiturate but does not indicate a specific barbiturate.
(b) Koppanyi-Zwikker test

This is a modification of the Dille-Koppany test.

<table>
<thead>
<tr>
<th>Solution 1:</th>
<th>Prepare a 1 per cent w/v solution of cobalt nitrate in ethanol, then add 10µl of pyrrolidine</th>
</tr>
</thead>
</table>

**Method**

The sample to be tested is dissolved in 1ml of ethanol on a test plate to which one drop of solution 1 is added and agitated.

**Results**

Barbiturates give a blue-violet colour. However, a number of other imide and sulphonamide-type drugs also react.

**Note:** The colours described for positive results of the above presumptive tests are subjective judgements due to individual perception of colours. Because of this subjective aspect of colour evaluation, it is necessary for each analyst to test appropriate reference standards in order to ensure that the colour of each test result can be recognized. Similarly, it is advisable to carry out a blank test without the target substance to ensure familiarity with the colour of the reagent. When performed properly, a negative colour test is generally quite reliable in establishing the absence of a target compound. However, positive results are only presumptive indications of the possible presence of a compound. Many other compounds, often harmless and uncontrolled by national legislation or international treaties, may give similar colours with a given colour test reagent. Therefore, it is mandatory for the analyst to confirm a positive colour test for any legally controlled compound by the use of additional laboratory analysis. To eliminate the possibility of a false positive result due to a contaminated spot plate, it may be advantageous to place the reagent onto the spot plate, and then add a small quantity of the sample to the reagent.

**Salt determination**

For quantitative purposes, it is necessary to know whether the barbiturate is present as a free acid or in a salt form. Test (a) is mainly applicable to bulk powder. As excipients in tablets and capsules may interfere with the observation, test (b) may be more appropriate for those preparations.
I. Barbiturates

(a) Solubility

Place small amounts of the suspect material in each of two test tubes. Add several drops of water to the first test tube and several drops of ethyl acetate to the second. Observe in which solvent the material dissolves. Free acids are soluble in organic solvents such as ethyl acetate, but are insoluble in water. The salt forms of the barbiturates are readily soluble in water, but are insoluble in ethyl acetate. Other organic solvents such as ether and chloroform may substitute the ethyl acetate.

(b) pH determination

Place a small amount (ca.10-20 mg) of the suspected barbiturate in a test tube and add 1 ml of water. Determine the pH. A pH greater than 8.0 indicates that the barbiturate is present as the sodium or calcium salt.

3.4 Thin-layer chromatography (TLC)

TLC is a commonly used technique for the separation and identification of illicitly manufactured drugs. It is inexpensive, rapid, sensitive (sub-milligram quantities of analyte required), flexible in the selection of both the stationary and mobile phase and amenable to a wide variety of substances, in base and salt form, ranging from the most polar to non-polar materials. A variety of visualization techniques can be used. However, in many countries it is not accepted as a single technique for drug identification.

TLC plates (stationary phases)

Coating: Silica gel G with a layer thickness of 0.25 mm and containing an inert indicator, which fluoresces under UV light wavelength 254 nm (Silica gel GF254).

Typical plate sizes: 20 x 20 cm; 20 x 10 cm; 10 x 5 cm (the latter should be used with the 10 cm side vertical with the TLC tank).

Plates prepared by the analyst must be activated before use by placing them into an oven at 120°C for at least 10 to 30 minutes. Plates are then stored in a grease-free desiccator over blue silica gel. Heat activation is not required for commercially available coated plates.

Solvent systems (mobile phases, by volume)

System A: Ethyl acetate, methanol, 25 per cent ammonia solution (85 : 10 : 5 v/v/v)

System B*: Chloroform, acetone (80 : 20 v/v)

* Note: Due to the carcinogenic potential of chloroform this system should only be used with appropriate safety precautions and ventilation.
**Preparation of solutions to be applied to the TLC plate**

Extract the material using the method outlined in section 3.2. Prepare a solution of the sample in methanol at a concentration of approximately 5 mg/ml. All standard solutions should be prepared at a concentration of 5 mg/ml in methanol. Apply 1 to 2 µl of the sample and standard solutions to the plate.

**Visualization**

The plates must be dried prior to visualization. This can be done at 120°C for 5 minutes in an oven or by using a hot air blower.

**Visualization methods**

A. **UV light at 254 nm**

First observe the plate under short wavelength UV light (254nm). Expose the plate to concentrated ammonia vapours and observe again under UV light at the same wavelength.

B. **Mercuric chloride-diphenylcarbazone spray reagent**

Dissolve 0.1g of diphenylcarbazone in 50 ml of ethanol to produce solution A. Dissolve 1g of mercuric chloride in 50 ml of ethanol to produce solution B. The two solutions (A and B) should be freshly prepared and mixed just before use. Barbiturates give blue-violet spots on a pink background. The detection limit is about 1-5µg.

**Note:** Mercuric chloride—diphenylcarbazone is the most sensitive spray reagent among the many tested for the detection of barbiturates. However, the use of mercury salts cannot be recommended because of environmental concerns. Detection by visualization method 1 is sufficient, but should the use of this reagent still be required, the spraying procedure must be performed with special care to guard against harmful mercury vapours.

**Interpretation**

After visualization, mark spots (e.g. with a pencil) and calculate retardation factor (Rf) values.

\[ Rf = \frac{\text{Migration distance: from origin to centre of spot}}{\text{Development distance: from origin to solvent front}} \]
**Results (expressed as Rf x 100)**

<table>
<thead>
<tr>
<th>COMPOUNDS</th>
<th>Developing system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Allobarbital</td>
<td>31</td>
</tr>
<tr>
<td>Amobarbital</td>
<td>40</td>
</tr>
<tr>
<td>Barbital</td>
<td>33</td>
</tr>
<tr>
<td>Butalbital</td>
<td>44</td>
</tr>
<tr>
<td>Butobarbital</td>
<td>39</td>
</tr>
<tr>
<td>Cyclobarbital</td>
<td>35</td>
</tr>
<tr>
<td>Methylphenobarbital</td>
<td>41</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>44</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>29</td>
</tr>
<tr>
<td>Secbutabarbital</td>
<td>44</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>42</td>
</tr>
<tr>
<td>Vinybital</td>
<td>40</td>
</tr>
</tbody>
</table>

**Analytical notes**

Rf values are not always reproducible due to small changes in plate composition and activation, in solvent systems, tank saturation or development distance. Therefore, the Rf values provided are indications of the chromatographic behaviour of the substances listed.

### 3.5 Gas chromatography (GC) with flame ionization detector (FID) [13, 14]

The GC instrument of choice for routine analytical work is the narrow bore capillary gas chromatograph, using columns with an internal diameter of between 0.2 and 0.32 mm. It is recognized that there are laboratories that, for a variety of reasons, may wish to maintain a packed column system. For those laboratories, a method
Recommended Methods for the Identification and Analysis of Barbiturates and Benzodiazepines using packed columns was described in the earlier edition of this manual (ST/NAR/18) and is not included in this revised version.

**Capillary column technique without derivatization**

<table>
<thead>
<tr>
<th>Operating conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column: 25m x 0.35mm: film thickness 0.52µm or narrower</td>
</tr>
<tr>
<td>Phase: Fused silica, chemically bonded and cross-linked methylsilicone or methylphenylsilicone, such as OV-1, SE-30, SE-54 or equivalent</td>
</tr>
<tr>
<td>Carrier: Nitrogen at 1ml/min (or helium)</td>
</tr>
<tr>
<td>Injector: Split/splitless, 275°C</td>
</tr>
<tr>
<td>Split ratio: 20:1</td>
</tr>
<tr>
<td>Oven: Isothermal at 200°C or programmed from 200-260°C at 4°C/min</td>
</tr>
<tr>
<td>Detector: FID 275°C, hydrogen 35 ml/min, air 350 ml/min</td>
</tr>
<tr>
<td>Internal standard: n-alkanes</td>
</tr>
<tr>
<td>Injection: 1µl</td>
</tr>
</tbody>
</table>

**Sample preparation**

Prepare internal standard, drug standard and sample solution at concentrations of 1 mg/ml. Use the sample extract obtained as described in section 3.2. Inject successively 1µl of the sample and standard solutions into the gas chromatograph.

**Results**

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Retention indices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SE-30</td>
</tr>
<tr>
<td>Allobarbital</td>
<td>1575</td>
</tr>
<tr>
<td>Amobarbital</td>
<td>1695</td>
</tr>
<tr>
<td>COMPOUND</td>
<td>Retention indices</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td></td>
<td>SE-30</td>
</tr>
<tr>
<td>Barbital</td>
<td>1465</td>
</tr>
<tr>
<td>Butalbital</td>
<td>1642</td>
</tr>
<tr>
<td>Butobarbital</td>
<td>1642</td>
</tr>
<tr>
<td>Cyclobarbital</td>
<td>1946</td>
</tr>
<tr>
<td>Methylphenobarbital</td>
<td>1875</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>1719</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>1934</td>
</tr>
<tr>
<td>Secbutabarbital</td>
<td>1635</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>1770</td>
</tr>
<tr>
<td>Vinylbital</td>
<td>1712</td>
</tr>
</tbody>
</table>

**Note:** All of these methods should include the use of appropriate standards. Many analysts prefer to derivatize barbiturates using some form of methylating agent, but many consider it unreliable for quantitative analysis.

### 3.6 Gas chromatography - mass spectrometry (GC-MS) [13, 15]

GC-MS is one of the most commonly used techniques for the identification and quantitation of forensic drug samples. As a “hyphenated” technique, it combines the separation power of a GC with the analyte specificity of a spectroscopic technique, providing highly specific spectral data on individual compounds in a complex mixture often without prior separation.

**Preparation of sample solutions**

The same method of sample preparation described in section 3.5 can be adopted.
Recommended Methods for the Identification and Analysis of Barbiturates and Benzodiazepines

**GC-MS operating conditions**

- **Column:** 30m x 0.25mm; film thickness 0.25µm
- **Phase:** Fused silica, chemically bonded and cross-linked methylsilicone or methylphenylsilicone, such as DB-1, OV-1, SE-30, SE-54 or equivalent
- **Carrier:** Helium at 1ml/min; constant flow (or constant pressure)
- **Injector:** Split/splitless, 250-300°C (as appropriate)
- **Oven:** Isothermal at 200°C or programmed from 200-260°C at 4°C/min (same as for capillary GC analysis described above or equivalent)
- **Detector:** Ionization mode: EI mode, 70 eV
  - Transfer line temp: 280°C (or appropriate)
  - Ion source temp: 230°C (or appropriate)
  - Scan parameters: TIC (SIM if required), scan range: to 500amu

**Results**

Identification is accomplished by comparing the retention time and mass spectrum of the analyte with that of a reference standard. All compounds identified by GC-MS and reported by the analyst must be compared to a current mass spectrum of the appropriate reference standard, preferably obtained on the same instrument, operated under identical conditions.

**Note:** Most spectrometers have their own mass spectral reference libraries that may be commercial or developed by the operator. However, these should be used for reference purposes only. Direct comparison of the GC-MS characteristics of the sample under test should be compared with those of an authentic sample derived under the same conditions.

**Gas chromatography - mass spectrometry (GC-MS) for selected barbiturates**

A GC-MS method for selected barbiturates including allobarbital, amobarbital, barbital, pentobarbital, phenobarbital and secobarbital is presented below. The method may be adapted for quantitative analysis of these barbiturates [16, 17, 18]
I. Barbiturates

Preparation of barbiturate standard and sample solutions

(a) Barbiturate standard solution

Weigh an appropriate amount of standard barbiturates into a volumetric flask to obtain a final concentration of approximately 0.1mg/ml of each compound. Dilute to volume with methanol. This stock solution is stable for at least three months when stored at -20°C.

(b) Barbiturate sample solution

Weigh an appropriate amount of sample into a volumetric flask so that the final barbiturate concentration is approximately that of the standard solution. Dilute to volume with methanol. Depending on the provenance of the sample, any undissolved solid particulates can be removed by filtration.

Operating conditions

| Column | HP-5MS, 0.25µm film, fused silica capillary, 30m x 0.25mm. i.d. |
| Column temperature | 100°C for 1min, then 100-280° at 15°C min-1, hold 280°C for 3min. |
| Injection temp | 250°C |
| Ion source temp | 280°C |
| Injection mode | Splitless |
| Mobile phase | Helium at 50kPa |
| Detector | EI mode 70eV; scanning range m/z 50 – 500 |

Results

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Approx. retention times of barbiturates (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbital</td>
<td>8.00</td>
</tr>
<tr>
<td>Allobarbital</td>
<td>8.98</td>
</tr>
<tr>
<td>Amobarbital</td>
<td>9.90</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>10.15</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>10.55</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>11.90</td>
</tr>
</tbody>
</table>
3.7 High performance liquid chromatography (HPLC) [3]

HPLC is one of the major separation techniques commonly used in forensic drug analysis. Reversed phase chromatography is recommended for the analysis of barbiturates. There are a large variety of stationary and mobile phases available to the analyst. The following methods are provided as a guide. The most commonly encountered and versatile column is a bonded octadecyl silica column (C18). Column length, diameter, particle size, pore size and carbon load should be considered before final selection of the column.

Preparation of barbiturate standard and sample solutions

(a) Barbiturate standard solution

Weigh an appropriate amount of standard barbiturates into a volumetric flask to obtain a final concentration of approximately 1 mg/ml of each compound. Dilute to volume with methanol. This stock solution is stable for at least three months when stored at -20°C.

(b) Barbiturate sample solution

Weigh an appropriate amount of sample obtained by one of the extraction procedures outlined in section 3.2 into a volumetric flask, dissolve in and make up to volume with methanol to produce a final barbiturate concentration of 1 mg/ml. Depending on the provenance of the sample, any undissolved solid particulates can be removed by filtration.

### Method 1

<table>
<thead>
<tr>
<th>Column:</th>
<th>250 mm x 4.6 mm ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packing material:</td>
<td>Octadecyl-silica HPLC grade, 5µm (Spherisorb 5 ODS-2 or equivalent)</td>
</tr>
<tr>
<td>Mobile phase:</td>
<td>Acetonitrile: water (30:70 by volume)</td>
</tr>
<tr>
<td>Flow rate:</td>
<td>0.9 ml/min.</td>
</tr>
<tr>
<td>Detection:</td>
<td>UV at 220 nm or diode array detection (DAD).</td>
</tr>
<tr>
<td>Injection volume:</td>
<td>1-5µl</td>
</tr>
<tr>
<td>Quantitation:</td>
<td>By peak area, external standard method</td>
</tr>
</tbody>
</table>
Method 2

Column: 150 mm x 4.6 mm ID

Packing material: Octadecyl-silica HPLC grade, 5µm

(ODS-Hypersil, or equivalent)

Mobile phase A: 0.1M sodium dihydrogen phosphate buffer: methanol (60:40 by volume; adjust pH to 3.5 units with phosphoric acid)

Mobile phase B: 0.1M sodium dihydrogen phosphate buffer: methanol (60:40 by volume; adjust pH to 8.5 units with sodium hydroxide solution)

Flow rate: 2.0 ml/min (low flow values should be considered with shorter columns and smaller particle sizes).

Detection: UV at 216 nm

Injection volume: 1-5µl by syringe or loop injection.

Quantitation: By peak area, external standard method.

Results

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Capacity ratios (k’ values)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mobile phase A</td>
<td>Mobile phase B</td>
</tr>
<tr>
<td>Allobarbital</td>
<td>1.35</td>
<td>2.46</td>
</tr>
<tr>
<td>Amobarbital</td>
<td>4.86</td>
<td>10.91</td>
</tr>
<tr>
<td>Barbital</td>
<td>0.60</td>
<td>1.11</td>
</tr>
<tr>
<td>Butalbital</td>
<td>2.90</td>
<td>6.17</td>
</tr>
<tr>
<td>Butobarbital</td>
<td>2.56</td>
<td>5.43</td>
</tr>
<tr>
<td>Cyclobarbital</td>
<td>2.56</td>
<td>5.25</td>
</tr>
<tr>
<td>Methylphenobarbital</td>
<td>5.72</td>
<td>7.27</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>4.63</td>
<td>10.96</td>
</tr>
</tbody>
</table>
### COMPOUND Capacity ratios ($k'$ values)

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mobile phase A</td>
<td>Mobile phase B</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>1.94</td>
<td>3.09</td>
</tr>
<tr>
<td>Secbutabarbital</td>
<td>2.24</td>
<td>4.89</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>6.81</td>
<td>16.28</td>
</tr>
<tr>
<td>Vinylbital</td>
<td>4.86</td>
<td>10.40</td>
</tr>
</tbody>
</table>

The following HPLC method [17, 19], involving the use of an internal standard, may be adopted for the quantification of the selected barbiturates allobarbital, amobarbital, barbital, cyclobarbital, metharbital, pentobarbital, phenobarbital and secobarbital.

**Method**

- **Column 1:** 2µm TSK gel Super-ODS C$_{18}$-reverse phase, 100 x 4.6 mm.
- **Column 2:** 5µm Hypersil-ODS C$_{18}$-reverse phase, 100 x 4.6 mm.
- **Column temperature:** 23°C
- **Injection:** 2µl
- **Mobile phase:** 30 per cent acetonitrile, 70 per cent (8 mM KH$_2$O$_3$)
- **Flow rate:** 0.4 ml/min
- **UV wavelength:** 215 nm
- **Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (MPPH)
- **Quantitation:** By peak area ratio, internal standard method
Results

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Retention times of barbiturates (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column 1</td>
</tr>
<tr>
<td>Barbital</td>
<td>3.8</td>
</tr>
<tr>
<td>Allobarbital</td>
<td>5.1</td>
</tr>
<tr>
<td>Metharbital</td>
<td>5.9</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>6.1</td>
</tr>
<tr>
<td>Cyclobarbital</td>
<td>7.1</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>10.6</td>
</tr>
<tr>
<td>Amobarbital</td>
<td>10.9</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>14.2</td>
</tr>
</tbody>
</table>

3.8 Infrared spectroscopy [12]

Theoretically, each substance has a unique infrared spectrum and this method would permit the unequivocal identification of any barbiturate but would not distinguish between enantiomers.

For powders, considered from prior chromatographic analysis to be reasonably pure, the infrared spectrum may be run directly in a KBr disk for comparison with the spectra of the barbiturate free acids or salts. However, with licit pharmaceutical tablet and capsule samples separation of individual barbiturates from excipients and their isolation in pure form is essential. For tablets, capsules and powders suspected to be mixtures, the extraction procedures outlined in section 3.2. above may be used to isolate the barbiturate as the free acid.

An additional difficulty in comparing infrared spectra arises from the existence of polymorphic forms of some barbiturates giving rise to differences in the infrared spectra. Interestingly, the very act of grinding a barbiturate sample to prepare, for example a halide disk or nujol mull, may result in a change of crystalline form. To overcome this difficulty, analysts should subject the standard barbiturate to the same manipulations as the sample. This should convert the standard to the same crystalline form as the sample and give good comparative infrared spectra.
II. Benzodiazepines and related substances

The abuse or misuse of benzodiazepines is internationally widespread which means that any forensic laboratory may encounter a range of these compounds. In general, benzodiazepines encountered in the illicit market are diverted from legitimated sources. The benzodiazepines, specifically aprazolam and diazepam, are among the most often diverted and abused psychotropic substances [2]. In a few cases, combination products such as chlordiazepoxide-amitriptyline and chlordiazepoxide-clidinium bromide also appear on the illicit market.

Analysts should be aware of the particular benzodiazepines commonly available in their area as well as the characteristics and methodologies for their identification and analysis. As benzodiazepine derivatives end up on the illicit market from diversion from legitimate sources, reference should be made to national pharmacopoeias and drug tablet and capsule identification guides for preliminary screening information. Reference can also be made to the Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control [5] (MLD, ST/NAR/1/Rev.2) which provides information on the nomenclature of the substances under international control, includes a listing of many brand names for the controlled benzodiazepines and provides information on their control status.

1. Classifications and definitions

Benzodiazepines enhance the effect of the neurotransmitter gamma-aminobutyric and are subsequently used therapeutically as tranquilizers, hypnotics, anticonvulsants and centrally acting muscle relaxants. Numerous benzodiazepines have reportedly been synthesized and over fifty of these are marketed for clinical use throughout the world. Out of the marketed benzodiazepines, thirty-five are subject to international control under the 1971 Convention on Psychotropic Substances.
The classical benzodiazepines are based on a 5-aryl-1,4-benzodiazepine structure (figure 2, below).

![Basic 5-phenyl-1,4-benzodiazepine skeleton](image)

Some of the compounds that are classified alongside them are, strictly speaking, not benzodiazepines. For example, brotizolam (figure 3) (see annex II) is a thienotriazolo-1,4-diazepine. Additionally, some compounds such as clobazam (figure 4) are actually 1,5-benzodiazepines.

![Brotizolam](image)
![Clobazam](image)

The benzodiazepines are formulated mainly as capsules and tablets. However some are available in other pharmaceutical forms such as injectable solutions. Diazepam, the most traded and widely available benzodiazepine, can be found as capsules, tablets, aqueous or polyethyleneglycol solutions for injection, syrups and suppositories.

2. **Description of pure compounds [5, 21]**

(see also annex II)

The information related to each of the thirty-five benzodiazepines currently under international control, can be found in the annex II. In addition, the *Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International*
Control [5] (MLD, ST/NAR/1/Rev.2) provides information on the nomenclature of the benzodiazepine derivatives under international control. It also includes synonyms and different trade names for the relevant substances and provides information on their control status.

3. Qualitative and quantitative analysis of materials containing benzodiazepines

Generally, in attempting to establish the identity of a controlled drug in suspected material, the analytical approach must entail the determination of at least two uncorrelated parameters. It is recognized that the selection of these parameters in any particular case would take into account the drug involved and the laboratory resources available to the analyst. It is also accepted that unique requirements in different jurisdictions may dictate the actual practices followed by a particular laboratory.

When possible, three entirely different analytical techniques should be used, for example: colour tests, chromatography (e.g. TLC, GC or HPLC) and spectroscopy (e.g. IR or UV). Hyphenated techniques, such as gas chromatography – mass spectrometry (GC-MS), count as two parameters, provided the information from both techniques is used (i.e. retention time and mass spectral characteristics).

3.1 Sampling

Similar to barbiturates, seizures of benzodiazepines may consist mainly of pharmaceutical substances formulated as tablets, capsules, oral liquids or injectables and should be sampled depending on the number and type of dosage units seized. The Guidelines on Representative Drug Sampling [6] provides the general aspects of qualitative sampling of multi-unit samples. Having chosen the materials to be examined, the analyst must identify the specific drug present and quantify the amount of the drug present, if this is required. The full physical description of the exhibit should be carried out and, if from a licit source, identified by reference to the appropriate databases and the identity confirmed. The analyst should bear in mind that if the dosage forms appear to be from an illicit source, i.e. not recognizable as a pharmaceutical product, then a full chemical analysis of the material including extraction of the drug, presumptive tests, screening and some confirmatory test will all be required.

3.2 Extraction and sample preparation

Virtually all of the benzodiazepines encountered in the illicit traffic appear in the form of tablets, capsules, liquid preparation and bulk powder diverted from
legitimate sources. They are usually present as the free base or as the hydrochloride, mesilate or other salt. However, some also have carboxylic acid functionalities and may be presented as potassium salts, e.g. chlorazepate. All the benzodiazepines are generally soluble in methanol (see annex II) The analyst should refer to the relevant formularies or pharmacopoeias for the published content of the drug.

**For qualitative analysis**

Both the free base/acid and the salts are soluble in methanol and this is the solvent of choice for sample preparation for presumptive or qualitative analysis.

**Method**

Triturate a quantity of finely powdered tablet or capsule contents or bulk drug powder with a small amount of methanol sufficient to obtain a solution containing approximately 1 to 20mg/ml of the benzodiazepine. The extract may be used directly or filtered and evaporated to dryness under a stream of nitrogen.

**For quantitative analysis**

**Method**

Combine the contents of a representative number of capsules or tablets as determined by a sampling procedure. Transfer an accurately weighed amount of the capsule or tablet contents, equal to the full weight of one or more tablets or capsules to a suitably sized volumetric flask and dilute to volume with methanol to give a final concentration of approximately 1 to 20mg/ml.

### 3.3. Presumptive tests

**Tablet and capsule identification guides [7, 8, 9, 10]**

As a first test, analysts should refer to national identification guides for presumptive identification of the benzodiazepine products commonly available in their country. Some useful guides include pictorial representations of legitimate capsule and tablet forms to assist in identification. For example, capsules and tablets may be identified using TICTAC [7] (www.tictac.org) or other available pharmaceutical identification databases.
II. Benzodiazepines and related substances

Colour tests [11, 22]

With such a large group of drugs containing diverse chemical functionality, no one colour test is specific for this class of drug. Subsequently, specific colour tests are not recommended although the Zimmerman test is often applied.

Zimmerman test

Solution 1: Prepare a solution containing 2,4-dinitrobenzene (1 per cent w/v) in methanol.

Solution 2: Prepare a 15 per cent potassium hydroxide aqueous solution.

Method

On a micro-test plate, place sufficient quantity of the sample to be tested, add one drop of solution 1 followed by one drop of solution 2, and mix. A red-purple to pink colour indicates the probable presence of a benzodiazepine. A reference material of the suspected drug should be tested at the same time.

This test is not specific to this class of compounds. Analysts are therefore advised to use a combination of TLC and colour development after spraying with selected reagents as a presumptive test.

3.4 Thin-layer chromatography (TLC)

Benzodiazepines form a diverse group of chemicals; however, the following three thin-layer chromatographic systems, when used in combination, give good separations for a number of benzodiazepines. System C is a general system recommended also for the identification and analysis of cocaine, opium and amphetamine/methamphetamine.

TLC plates (stationary phases)

Coating: Silica gel G with a layer thickness of 0.25 mm and containing an inert indicator, which fluoresces under UV light wavelength 254 nm (Silica gel GF254).

Typical plate sizes: 20 x 20 cm; 20 x 10 cm; 10 x 5 cm (the latter should be used with the 10 cm side vertical with the TLC tank).

Plates prepared by the analyst must be activated before use by placing them into an oven at 120°C for at least 10 to 30 minutes. Plates are then stored in a
Recommended Methods for the Identification and Analysis of Barbiturates and Benzodiazepines

grease-free desiccator over blue silica gel. Heat activation is not required for commercially available coated plates.

**Solvent systems (mobile phases, by volume)**

System A*: Chloroform: acetone (80:20, v/v)

System B*: Chloroform, methanol (90:10 v/v)

System C: Cyclohexane: toluene: diethylamine (75:15:10 v/v/v)

*Due to the carcinogenic potential of chloroform, solvent systems A and B should only be used with appropriate safety precautions and ventilation.

**Preparation of solutions to be applied to the TLC plates**

**Powder:** Prepare a solution at a concentration of 5 mg/ml in methanol.

**Capsules:** Remove the contents of a representative sample of capsules and prepare a solution containing the equivalent of approximately 5 mg/ml of the drug in methanol.

**Tablets:** Grind a representative number of tablets to a fine powder and prepare a solution containing the equivalent of approximately 5mg/ml of the drug in methanol.

**Aqueous solutions:** Spot directly or use a concentration of 5mg/ml if the concentration of the drug is known.

**Standard solutions:** All solutions made at a concentration of 5mg/ml in methanol.

Apply 2µl of a 5mg/ml solution of the drug in methanol to the plate.

**Visualization**

The plates must be dried prior to visualization. This can be done at 120°C for 5 minutes in an oven or using a hot air blower. It is important for proper colour development that all traces of diethylamine be removed from the plate.

**Visualization methods**

A. UV light at 254 nm

B. 2N H₂SO₄/heat/observe under UV light at 366 nm

C. Acidified potassium iodoplatinate reagent
II. Benzodiazepines and related substances

Spray reagent

Acidified potassium iodoplattinate reagent: Dissolve 0.25g of platinic chloride and 5g of potassium iodide in sufficient water to produce 100 ml. This is potassium iodoplattinate reagent; for the acidified version add 5ml of concentrated hydrochloric acid to 100 ml of iodoplattinate solution.

Method

First observe the plate under short wave UV light. Spray with 2N H₂SO₄ and heat in an oven at 80°C for 5 minutes. Observe the fluorescent spots under long UV light (366nm). The plate may then be oversprayed with the acidified iodoplattinate reagent. All benzodiazepines give purple coloured spots when oversprayed with the reagent (alkaloid positive reaction). Other alternative methods are described in the literature [23, 24, 25, 26].

Results

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Rf x 100 values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>System</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>1</td>
</tr>
<tr>
<td>Bromazepam</td>
<td>3</td>
</tr>
<tr>
<td>Camazepam</td>
<td>11</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>2</td>
</tr>
<tr>
<td>Clobazam</td>
<td>11</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>0</td>
</tr>
<tr>
<td>Clorazepic acid*</td>
<td>4</td>
</tr>
<tr>
<td>Clotiazepam</td>
<td>34</td>
</tr>
<tr>
<td>Cloxazolam</td>
<td>13</td>
</tr>
<tr>
<td>Delorazepam</td>
<td>7</td>
</tr>
<tr>
<td>Diazepam</td>
<td>30</td>
</tr>
<tr>
<td>Estazolam</td>
<td>1</td>
</tr>
<tr>
<td>Ethyl loflazepate</td>
<td>1</td>
</tr>
<tr>
<td>Fludiazepam</td>
<td>29</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>16</td>
</tr>
<tr>
<td>Flurazepam</td>
<td>38</td>
</tr>
<tr>
<td>Halazepam</td>
<td>21</td>
</tr>
<tr>
<td>Haloxazolam</td>
<td>13</td>
</tr>
<tr>
<td>Ketazolam**</td>
<td>12,30</td>
</tr>
</tbody>
</table>
### Table: Rf x 100 values for Benzodiazepines

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>System</th>
<th>Rf x 100 values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loprazolam</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>Lormetazepam</td>
<td>9</td>
<td>63</td>
</tr>
<tr>
<td>Medazepam</td>
<td>45</td>
<td>72</td>
</tr>
<tr>
<td>Nimetazepam</td>
<td>12</td>
<td>45</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>1</td>
<td>42</td>
</tr>
<tr>
<td>Nordazepam*</td>
<td>5</td>
<td>44</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>Oxazolam</td>
<td>4, 15, 19***</td>
<td>69</td>
</tr>
<tr>
<td>Pinazepam</td>
<td>27</td>
<td>81</td>
</tr>
<tr>
<td>Prazepam</td>
<td>35</td>
<td>79</td>
</tr>
<tr>
<td>Temazepam</td>
<td>10</td>
<td>63</td>
</tr>
<tr>
<td>Tetrazepam</td>
<td>32</td>
<td>74</td>
</tr>
<tr>
<td>Triazolam</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

*Clorazepic acid converts to nordazepam.

**Ketazolam decomposes to diazepam.

***Multiple spots.

---

### 3.5 Gas chromatography (GC) with flame ionization detector (FID) [12, 28, 29, 30, 31]

Although gas chromatography can be recommended as a suitable method for the analysis of most benzodiazepines, several of them, particularly the 3-hydroxyderivatives, undergo thermal degradation and rearrangements. Chlordiazepoxide, cloxazolam, lormetazepam, haloxazolam, oxazolam, ethyl loflazepate and temazepam yield multiple peaks. Similarly while clorazepic acid gives a single peak having a retention index value corresponding to nordazepam, the commercial product which is the dipotassium salt can not be analysed. Ketazolam gives a single peak corresponding to diazepam. Loprazolam does not elute from the GC columns under the experimental conditions described in this manual.

Retention indices for benzodiazepines have been shown to be dependent on column temperature. Values should be checked before use by analysing few reference standards of known retention indices. As with the barbiturates, GC methods involving the use of packed columns have not been included in this manual. Laboratories using packed columns are referred to the previous manual on benzodiazepines [4].
II. Benzodiazepines and related substances

Capillary column technique

Operating conditions

Column: 25m x 0.25 mm: film thickness 0.25µm
Phase: Fused silica, chemically bonded and cross-linked methylsilicone, such as BP-1, DB-1 or equivalent
Carrier: Nitrogen at 1ml/min (or helium)
Injector: Split/splitless, 275°C
Split ratio: 20:1
Oven: 250°C
Detector: FID 275°C, hydrogen 35 ml/min, air 350 ml/min
Injection: 1µl

Preparation of solutions

Solutions of the standards and samples are prepared in methanol at a concentration of 1 mg/ml.

Results

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Retention indices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BP-1 Capillary</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>2936</td>
</tr>
<tr>
<td>Bromazepam</td>
<td>2626</td>
</tr>
<tr>
<td>Camazepam</td>
<td>2954</td>
</tr>
<tr>
<td>Chlordiazepoxide*</td>
<td>2815 (2531, 2646)</td>
</tr>
<tr>
<td>Clobazam</td>
<td>2582</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>2833</td>
</tr>
<tr>
<td>Clorazepic acid*</td>
<td>2521 (2783)</td>
</tr>
<tr>
<td>Clotiazepam</td>
<td>2485</td>
</tr>
<tr>
<td>Cloxazolam*</td>
<td>2344 (2604)</td>
</tr>
<tr>
<td>Delorazepam</td>
<td>2537</td>
</tr>
</tbody>
</table>
### COMPOUND | Retention indices
--- | ---
BP-1 Capillary
Diazepam | 2477
Estazolam | 2893
Ethyl loflazepate | 2925 (2878)
Fludiazepam | 2403
Flunitrazepam | 2633
Flurazepam | 2795
Halazepam | 2314
Haloxazolam* | 2634 (2518, 2272)
Ketazolam* | 2475
Loprazolam* | Does not elute
Lorazepam | 2448
Lormetazepam* | 2703 (2946)
Medazepam | 2287
Nimetazepam | 2693
Nitrazepam | 2755
Nordazepam | 2522
Oxazepam | 2374
Oxazolam | 2514 (2534, 2212)
Pinazepam | 2551
Prazepam | 2676
Temazepam | 2613 (2828)
Tetrazepam | 2463
Triazolam | 3025

* Thermally unstable.
( ) Minor peaks in brackets

### 3.6 Gas chromatography - mass spectrometry (GC-MS)

GC-MS is one of the most commonly used techniques for the identification and quantitation of forensic drug samples. As a “hyphenated” technique, it combines the
II. Benzodiazepines and related substances

separation power of a GC with the analyte specificity of a spectroscopic technique, providing highly specific spectral data on individual compounds in a complex mixture of compounds often without prior separation.

**Preparation of solutions**

Solutions of the standards and samples are prepared in methanol at a concentration of 1 mg/ml

<table>
<thead>
<tr>
<th>GC-MS operating conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column:</strong> 10-15 m x 0.32 or 0.53 mm ID; film thickness 1.5-3µm</td>
</tr>
<tr>
<td><strong>Phase:</strong> Fused silica, chemically bonded and cross-linked methylsilicone, e.g. DB-1 or equivalent</td>
</tr>
<tr>
<td><strong>Oven</strong> Temp. prog., 4 min at 135°, 13°/min to 200°, 6°/min to 312°, 6 min final hold (or similar programmes)</td>
</tr>
<tr>
<td><strong>Carrier gas:</strong> Helium, 1 ml/min; constant flow (or constant pressure)</td>
</tr>
<tr>
<td><strong>Injector:</strong> Split/splitless, 250-300°C as appropriate</td>
</tr>
<tr>
<td><strong>Detector:</strong> Ionization mode: EI mode, 70 eV</td>
</tr>
<tr>
<td>Transfer line temp: 280°C (or appropriate)</td>
</tr>
<tr>
<td>Ion source temp: 230°C (or appropriate)</td>
</tr>
<tr>
<td>Scan parameters: TIC (SIM if required), scan range: to 500amu</td>
</tr>
</tbody>
</table>

**Results**

Identification is accomplished by comparing the retention time and mass spectrum of the analyte with that of a reference standard. All compounds identified by GC-MS and reported by the analyst must be compared to a current mass spectrum of the appropriate reference standard, preferably obtained on the same instrument, operated under identical conditions.

3.7 **High performance liquid chromatography (HPLC)**

[31, 32, 33, 34, 35]

Because of the diversity of chemical structure among benzodiazepines, the use of a single HPLC method to separate all possible compounds is difficult. Nevertheless, the following methods are recommended for the qualitative and quantitative analysis of all benzodiazepine derivatives under international control. The selection of column
and solvent system by national laboratories will depend upon the particular benzodiazepines commonly available in that country.

**Preparation of standard and sample solutions**

**Benzodiazepine standard solution**

Weigh an appropriate amount of the benzodiazepine standard into a volumetric flask, dissolve in and make up to volume with 50 per cent aqueous methanol to produce a solution of concentration 1mg/ml.

**Benzodiazepine sample solution**

Weigh an appropriate amount of the sample obtained by one of the extraction methods outlined in section 3.2 into a volumetric flask, dissolve in and make up to volume with 50 per cent v/v aqueous methanol to produce a final benzodiazepine concentration of approximately 1mg/ml. Depending on the provenance of the sample, any undissolved particulates can be removed by filtration.

<table>
<thead>
<tr>
<th>Method</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column:</strong></td>
<td>250 mm by 5 mm ID</td>
</tr>
<tr>
<td><strong>Packing material:</strong></td>
<td>Octadecyl-silica HPLC 5µm diameter (ODS-Hypersil or equivalent)</td>
</tr>
<tr>
<td><strong>Mobile phase:</strong></td>
<td>A. Methanol: water: phosphate buffer (0.1M), (55:25:20, v/v/v), pH 7.25</td>
</tr>
<tr>
<td></td>
<td>B. Methanol: water: phosphate buffer (0.1M), (70:10:20, v/v/v), pH 7.67</td>
</tr>
<tr>
<td>The phosphate buffer (0.1M) is prepared by dissolving sodium dihydrogen-phosphate dehydrate (14.35g, 0.092 mol) and disodium hydrogen phosphate (1.14g, 0.008 mol) in 1000ml water.</td>
<td></td>
</tr>
<tr>
<td><strong>Flow rate:</strong></td>
<td>1.5 ml/min</td>
</tr>
<tr>
<td><strong>Detector:</strong></td>
<td>UV at 240nm or DAD</td>
</tr>
<tr>
<td><strong>Injection volume:</strong></td>
<td>20µl</td>
</tr>
<tr>
<td><strong>Quantitation:</strong></td>
<td>By peak areas, external standard method</td>
</tr>
</tbody>
</table>
**Results**

Capacity ratios, k’ values for a selection of benzodiazepines in the two mobile phases are provided below:

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Mobile Phases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>3.35</td>
</tr>
<tr>
<td>Bromazepam</td>
<td>1.63</td>
</tr>
<tr>
<td>Camazepam</td>
<td>11.80</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>6.68</td>
</tr>
<tr>
<td>Clobazam</td>
<td>4.14</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>2.92</td>
</tr>
<tr>
<td>Clorazepic acid*</td>
<td>8.22</td>
</tr>
<tr>
<td>Clotiazepam</td>
<td>17.91</td>
</tr>
<tr>
<td>Cloxazolam</td>
<td>15.22</td>
</tr>
<tr>
<td>Delorazepam</td>
<td>7.20</td>
</tr>
<tr>
<td>Diazepam</td>
<td>10.41</td>
</tr>
<tr>
<td>Estazolam</td>
<td>4.22</td>
</tr>
<tr>
<td>Ethyl loflazepate</td>
<td>13.71</td>
</tr>
<tr>
<td>Fludiazepam</td>
<td>7.91</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>3.34</td>
</tr>
<tr>
<td>Flurazepam</td>
<td>12.98</td>
</tr>
<tr>
<td>Halazepam</td>
<td>18.92</td>
</tr>
<tr>
<td>Haloxazolam</td>
<td>11.62</td>
</tr>
<tr>
<td>Ketazolam</td>
<td>10.78</td>
</tr>
<tr>
<td>Loprazolam</td>
<td>6.42</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>5.16</td>
</tr>
<tr>
<td>Lormetazepam</td>
<td>7.19</td>
</tr>
<tr>
<td>Medazepam</td>
<td>41.46</td>
</tr>
<tr>
<td>Nimetazepam</td>
<td>3.91</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>3.22</td>
</tr>
<tr>
<td>Nordazepam</td>
<td>8.97</td>
</tr>
</tbody>
</table>
### Capacity ratios, k’ values

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Mobile Phases</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>Oxazepam</td>
<td>5.42</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>Oxazolam**</td>
<td>22.43, 25.14</td>
<td>3.51, 3.87</td>
<td></td>
</tr>
<tr>
<td>Pinazepam</td>
<td>12.82</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>Prazepam</td>
<td>29.99</td>
<td>4.28</td>
<td></td>
</tr>
<tr>
<td>Temazepam</td>
<td>6.80</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td>Tetrazepam</td>
<td>25.82</td>
<td>4.25</td>
<td></td>
</tr>
<tr>
<td>Triazolam</td>
<td>5.18</td>
<td>1.13</td>
<td></td>
</tr>
</tbody>
</table>

* Converts to Nordazepam during chromatography.
** Two peaks due to equilibrium mixture of Z and E isomers [36]

---

**Liquid chromatography - mass spectrometry (LC-MS)**

This method represents a rapid, simple and highly sensitive procedure for the simultaneous analysis of fourteen benzodiazepines [37, 38, 39, 40, 41, 42] using a triple quadrupole MS in ESI-mode. Samples of suitable concentration were prepared from the following deuterated internal standards, D5-diazepam, D5-temazepam, D5-alprazolam, D4-clonazepam, as well as unlabelled standards: alprazolam, bromazepam, chlordiazepoxide, clonazepam, diazepam, flunitrazepam, flurazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, temazepam and triazolam. The limit of quantification of most benzodiazepines using this method is approximately 0.5 ng/ml.

### LC conditions

<table>
<thead>
<tr>
<th>Instrument:</th>
<th>Agilent 1200 Series RRLC; 6410 LC Triple Quadrupole Masspectrometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column:</td>
<td>ZORBAX Eclipse XDB C18 2.1 x 50 mm x 1.8 µm</td>
</tr>
<tr>
<td>Column temperature:</td>
<td>35°C</td>
</tr>
<tr>
<td>Injection volume:</td>
<td>5 µL</td>
</tr>
<tr>
<td>Solvent flow rate:</td>
<td>Time (minutes) Flow rate (ml/min)</td>
</tr>
<tr>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>6.5</td>
<td>0.2</td>
</tr>
<tr>
<td>8</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
</tr>
<tr>
<td>Post time:</td>
<td>4.5 min</td>
</tr>
<tr>
<td>Mobile phase:</td>
<td>Acetonitrile: 20 mM ammonium formate pH = 8.6 (50:50 by vol.)</td>
</tr>
</tbody>
</table>
II. Benzodiazepines and related substances

**MS Conditions**

- **Operation:** Electrospray ESI positive mode
- **Gas temperature:** 300°C
- **Gas flow (N2):** 6 L/min
- **Nebulizer pressure:** 15 psi (pressure of 30 to 40 psi recommended)
- **Capillary voltage:** 4,500 V

### 3.8 Infrared spectroscopy

In some countries, confirmation of identity by spectroscopic means may be required. Theoretically each substance has a unique infrared spectrum and this method should permit the unequivocal identification of any benzodiazepine. However, with licit samples, the lack of solubility in chloroform of certain benzodiazepine derivatives and the isolation of the drug in a pure form, free from pharmaceutical excipients, place limitations on this method. The following procedure is recommended. Other methods utilizing column or preparative thin-layer chromatography may also be employed. Because of solvate formation and polymorphism, it is recommended that an authentic pharmaceutical formulation be carried through the same extraction procedure and its spectrum compared with that of the sample.

**Isolation of pure drugs from sample**

(a) Benzodiazepines as the hydrochloride salt

Chlordiazepoxide, flurazepam and medazepam are marketed as hydrochloride salts. Triturate the powders from the capsule contents with 1 to 2ml of methanol. Filter, collect the extract and evaporate to dryness. Induce crystallization and run the infrared spectrum of the resulting benzodiazepine salt by the KBr disk method.

(b) Benzodiazepine free base

Suspend a portion of the powder or crushed tablet contents containing approximately 10mg of drug in 1ml of 0.1M NaOH solution and extract with CHCl₃. Evaporate the CHCl₃ to dryness and induce crystallization.

Other standard methods can be used depending on instrumentation available. The IR spectrum should be compared to a standard sample prepared/treated in the same way as the test material.
References


7. The Identification CD-ROM for Tablets And Capsules, TICTAC. Available at: www.tictac.org


Further reading

Further reading


Annex I. Description of barbiturates under international control

**Allobarbital**

5,5-diallylbarbituric acid; 5,5-di-2propenyl-2,4,6 (1H,3H, 5H) pyrimidinetrione

Sch. IV (1971)

- **Empirical formula:** \( C_{10}H_{12}N_{2}O_{3} \)
- **CAS No.:** 52-43-7
- **MWt:** 208.2
- **MPt:** 171 – 173º C
- **Physical appearance:** White crystalline powder
- **Solubility:** Soluble in aqueous alkali
- **Infrared data:** Principal peaks at wavenumbers 1687, 1315, 925, 1219, 847, 1640 cm\(^{-1}\) (KBr disk) [3, 12]
Amobarbital (amobarbital sodium)

5-ethyl-5-isopentylbarbituric acid; 5-ethyl-5-(3-methylbutyl)-2, 4,6(1H, 3H, 5H)-pyrimidinetrione

Sch. III (1971)

Empirical formula: \( \text{C}_{11} \text{H}_{18} \text{N}_{2} \text{O}_{3} (\text{C}_{11} \text{H}_{17} \text{N}_{2} \text{NaO}_{3}) \)
CAS No.: 57-43-2 (64-43-7)
MWt: 226.3 (248.3)
MPt: 157º C (after conversion to free acid using method of Ph Eur)

Physical appearance: Free acid is a white crystalline powder; sodium salt is a white hygroscopic granular powder.

Solubility: Soluble in aqueous alkali.

Infrared data: Principal peaks at wavenumbers 1725, 1696, 1758, 1317, 1240, 850 cm\(^{-1}\) (KBr disk) [3, 12]

Barbital (barbital sodium)

5,5-diethylbarbituric acid; 5,5-diethyl-2, 4,6 (1H, 3H, 5H)-pyrimidinetrione

Sch. IV (1971)

Empirical formula: \( \text{C}_{8} \text{H}_{12} \text{N}_{2} \text{O}_{3} (\text{C}_{8} \text{H}_{11} \text{N}_{2} \text{NaO}_{3}) \)
CAS No.: 57-44-3 (144-02-5)
MWt: 184.2 (206.2)
MPt: 188 – 192º C (247ºC)

Physical appearance: Free acid is a white crystalline powder (available as sodium salt).

Solubility: Soluble in alcohol, boiling water and in aqueous alkali.

Infrared data: Principal peaks at wavenumbers 745, 1593, 725, 1495, 690, 1275 cm\(^{-1}\) (KBr disk) [3, 12]
**Butalbital**

5-(2-methylpropyl)-5-(2-propenyl)-barbituric acid

Sch. III (1971)

Empirical formula: $C_{11}H_{16}N_2O_3$

CAS No.: 77-26-9

MWt: 224.3

MPt: 138.5°C

Physical appearance: Free acid is a white crystalline powder

Solubility: Soluble in alcohol, acetone, ether, chloroform, boiling water and in aqueous alkali.

Infrared data: Principal peaks at wavenumbers 1690, 1720, 1740, 1310, 1290, 1200 cm$^{-1}$ (KBr disk) [3, 12]

---

**Butobarbital**

5-butyl-5-ethylbarbituric acid

Sch. IV (1971)

Empirical formula: $C_{10}H_{16}N_2O_3$

CAS No.: 77-28-1

MWt: 212.3

MPt: 126°C

Physical appearance: Free acid is a white crystalline powder

Solubility: Soluble in alcohol, ether and in aqueous alkali

Infrared data: Principal peaks at wavenumbers 1696, 1727, 1760, 1310, 1290, 1215 cm$^{-1}$ (KBr disk) [3, 12]
Cyclobarbital (cyclobarbital calcium)

5-(1-cyclohexen-1-yl)-5-ethylbarbituric acid
Sch. III (1971)

Empirical formula: $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_3$ ([C$_{12}$H$_{15}$N$_2$O$_3$)$_2$Ca]
CAS No.: 52-31-3 (143-76-0)
MWt: 236.3 (510.6)
MPt : 171 – 174º C (>300º C)
Physical appearance: Free acid is white crystalline powder that slowly decomposes (calcium salt is slightly yellowish crystalline powder)
Solubility: Soluble in 1 to 5 ethanol and 1 to 20 chloroform and ether
Infrared data: Principal peaks at wavenumbers 1693, 1725, 1745, 1300, 1210, 830 cm$^{-1}$ (KBr disk) [3, 12]

Methylphenobarbital

5-ethyl-1-methyl-5-phenylbarbituric acid
Sch. IV (1971)

Empirical formula: $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_3$
CAS No.: 115-38-8
MWt: 246.3
MPt : 178º C
Physical appearance: Colourless crystals or white crystalline powder,
Methylphenobarbital (continued)

Solubility: Soluble in hot water, soluble 1 in 40 in chloroform, soluble in aqueous alkali
Infrared data: Principal peaks at wavenumbers 1707, 1684, 1754, 720, 1298, 1050 cm⁻¹ (KBr disk) [3, 12]

Pentobarbital (pentobarbital calcium; pentobarbital sodium)

5-ethyl-5-(1-methylbutyl)-barbituric acid
Sch. III (1971)

\[
\begin{align*}
\text{Empirical formula:} & \quad C_{11}H_{18}N_2O_3 \quad ([C_{11}H_{17}N_2O_3]_2Ca; C_{11}H_{17}N_2NaO_3) \\
\text{CAS No.:} & \quad 76-74-4 \quad (7563-42-0; \ 57-33-0) \\
\text{MWt:} & \quad 226.3 \quad (490.6; \ 248.3) \\
\text{MPt:} & \quad 131º C \quad (\text{after conversion to free acid using method of Ph Eur}) \\
\text{Physical appearance:} & \quad \text{Free acid is a white crystalline powder (sodium salt, white hygroscopic powder)} \\
\text{Solubility:} & \quad \text{Soluble in acetone, alcohol, chloroform, ether and methanol and in aqueous alkali. (calcium salt sparingly soluble in water and other solvents; sodium salt soluble in alcohol and water from which it decomposes gradually)} \\
\text{Infrared data:} & \quad \text{Principal peaks at wavenumbers 1685, 1719, 1744, 1315, 1218, 845 cm}^{-1} \ (\text{KBr disk}) \ [3, 12]
\end{align*}
\]
Annex I. Description of barbiturates under international control

Phenobarbital (phenobarbital sodium)
5-ethyl-5-phenylbarbituric acid
Sch. IV (1971)

Empirical formula: \( \text{C}_{12}\text{H}_{12}\text{N}_{2}\text{O}_{3} \) (\( \text{C}_{12}\text{H}_{11}\text{N}_{2}\text{NaO}_{3} \))
CAS No.: 50-06-6 (57-30-7)
MWt: 232.2 (254.2)
MPT : 176º C (after conversion to free acid using method of Ph Eur)
Physical appearance: Free acid is a white crystalline powder that exhibits polymorphism; sodium salt, white hygroscopic powder
Solubility: Soluble in alcohol and in aqueous alkali (sodium salt is soluble in alcohol and CO\(_2\)-free water, almost insoluble in dichloromethane)
Infrared data: Principal peaks at wavenumbers 1712, 1684, 1670, 1770, 1310, 1300 cm\(^{-1}\) (KBr disk) [3, 12]

Secbutabarbital (secbutabarbital sodium)
5-ethyl-5-(1methylpropyl)-barbituric acid
Sch. IV (1971)

Empirical formula: \( \text{C}_{10}\text{H}_{16}\text{N}_{2}\text{O}_{3} \) (\( \text{C}_{10}\text{H}_{15}\text{N}_{2}\text{NaO}_{3} \))
CAS No.: 125-40-6 (143-81-7)
MWt: 212.2 (234.2)
MPT : 165 – 168º C
Physical appearance: Free acid is a white microcrystalline powder (sodium salt is a white powder)
Solubility: Soluble in alcohol, chloroform, ether and in aqueous alkali (sodium salt is soluble in alcohol and water, almost insoluble in ether)
Infrared data: Principal peaks at wavenumbers 1675, 1760, 1317, 1303, 1230, 853 cm\(^{-1}\) (KBr disk) [3, 12]
Secobarbital (secobarbital sodium)

5-(1-methylpropyl)-5-(2-propenyl)-barbituric acid
Sch. II (1971)

Empirical formula: \( C_{12}H_{18}N_2O_3 \) (\( C_{12}H_{17}N_2O_3\)Na)
CAS No.: 76-73-3 (309-43-3)
MWt: 238.3 (260.3)
MPt: 100º C
Physical appearance: Free acid is a white microcrystalline powder (sodium salt is a white powder)
Solubility: Soluble in alcohol, chloroform, ether and in aqueous alkali (sodium salt is soluble in alcohol and water, almost insoluble in ether)
Infrared data: Principal peaks at wavenumbers 1559, 1648, 1690, 1298, 1270, 925 cm\(^{-1}\) KBr disk) [3, 12]

Vinylbital

5-ethenyl-5-(1-methylbutyl)-barbituric acid
Sch. IV (1971)

Empirical formula: \( C_{11}H_{16}N_2O_3 \)
CAS No.: 2430-49-1
MWt: 224.3
MPt: 91º C
Physical appearance: White crystalline powder
Solubility: Soluble in alcohol and aqueous alkali
Infrared data: Principal peaks at wavenumbers 1692, 1730, 1750, 1318, 1220, 1630 cm\(^{-1}\) (KBr disk) [3, 12]
Annex II. Description of benzodiazepines under international control

Alprazolam

8-chloro-1-methyl-6-phenyl-4H-s-[1,2,4]triazolo[4,3-a][1,4] benzodiazepine

Sch. IV (1971)

Empirical formula: \( \text{C}_{17}\text{H}_{13}\text{ClN}_{4} \)

CAS No.: 28981-97-7

MWt: 308.8

MPt: 228 – 228.5°C (from ethil acetate)

Physical appearance: White crystalline (polymorphic) powder

Solubility: Soluble in dichloromethane and chloroform, sparingly soluble in acetone and ethanol, practically insoluble in water

Infrared data: Principal peaks at wavenumbers 1490, 1610, 697, 1316, 1540, 827 cm\(^{-1}\) (KBr disk). Three polymorphic forms may occur. [4, 12]
Bromazepam

7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one

Sch. IV (1971)

Empirical formula: $C_{14}H_{10}BrN_3O$
CAS No.: 1812-30-2
MWt: 316.2
MPt: 237 – 238.5º C
Physical appearance: White or yellowish crystalline powder
Solubility: Sparingly soluble in dichloromethane and ethanol, almost insoluble in water
Infrared data: Principal peaks at wavenumbers 1685, 825, 750, 802, 1315, 1230 cm\(^{-1}\) (KBr disk) [4], [12]

Brotizolam

2-bromo-4-(o-chlorophenyl)-9-methyl-6H-thieno[3,2-f]-s-triazolo[4,3-a][1,4] diazepine

Sch. IV (1971)

Empirical formula: $C_{15}H_{10}BrClN_4S$
CAS No.: 57801-81-7
MWt: 393.7
MPt: 212 – 214º C
Physical appearance: White or yellowish powder
Solubility: Practically insoluble in water, slightly soluble in ethanol and methanol
Annex II. Description of benzodiazepines under international control

Camazepam

7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one dimethylcarbamate (ester)

Sch. IV (1971)

![Chemical structure of Camazepam]

Empirical formula: \( C_{19}H_{18}ClN_3O_5 \)
CAS No.: 36104-80-0
MWt: 371.8
MPt: 173 – 174º C
Physical appearance: White crystalline powder
Solubility: Soluble in ethanol, moderately soluble in water
Infrared data: Principal peaks at wavenumbers 1680, 1718, 1193, 1318, 1110, 703 cm\(^{-1}\) [4, 12]

Chlordiazepoxide (chlordiazepoxide hydrochloride)

7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-2-4-oxide;
7-chloro-N-methyl-5-phenyl-3H-1,4-benzodiazepin-2-amine 4-oxide

Sch. IV (1971)

![Chemical structure of Chlordiazepoxide]

Empirical formula: \( C_{16}H_{14}ClN_3O \) (\( C_{16}H_{14}ClN_3O.HCl \))
CAS No.: 58-25-3 (438-41-5)
MWt: 299.8 (336.2)
Chlordiazepoxide (chlor Diazepoxide hydrochloride) (continued)

MPt: 236 – 236.5º C (212 – 218º C [decomp.])
Physical appearance: Almost white or pale yellow crystalline (polymorphic) powder, sensitive to sunlight
Solubility: Sparingly soluble in ethanol, slightly soluble in chloroform, almost insoluble in water; (hydrochloride soluble in water, sparingly soluble in ethanol, almost insoluble in chloroform and ether)
Infrared data: Principal peaks at wavenumbers 1625, 760, 1260, 1590, 850 cm\(^{-1}\) (KBr disk) [4, 12]

Clobazam

7-chloro-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4 (3H,5H)-dione

Sch. IV (1971)

\[
\begin{array}{c}
\text{Cl} \quad \text{N} \\
\text{O} \\
\text{O} \\
\end{array}
\]

Empirical formula: C\(_{16}\)H\(_{13}\)ClN\(_2\)O\(_2\)
CAS No.: 22316-47-8
MWt: 300.7
MPt: 180 – 182º C
Physical appearance: Almost white crystalline powder,
Solubility: Sparingly soluble in ethanol, slightly soluble in water, freely soluble in acetone, chloroform and dichloromethane
Infrared data: Principal peaks at wavenumbers 1684, 1664, 1490, 764, 845 cm\(^{-1}\) (KBr disk) [4, 12]
**Clonazepam**

5-\((o\text{-chlorophenyl})\)-1,3-dihydro-7-nitro-2\(\text{H}\)-1,4-benzodiazepin-2-one

Sch. IV (1971)

![Clonazepam structure](image)

Empirical formula: \(C_{15}H_{10}ClN_3O_3\)
CAS No.: 1622-61-3
MWt: 315.7
MPt: 238 – 240° C
Physical appearance: White to yellowish crystalline powder,
Solubility: Sparingly soluble in ethanol and methanol, fairly soluble in acetone,
Practically insoluble in water
Infrared data: Principal peaks at wavenumbers 1685, 1610, 748, 1255, 1578, 1532 cm\(^{-1}\) (KBr disk) [4, 12]

**Clorazepate (clorazepate dipotassium)**

7-chloro-2,3-dihydro-2-oxo-5-phenyl-1\(\text{H}\)-1,4-benzodiazepine-3-carboxylic acid

(Potassium (3RS)-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1\(\text{H}\)-1,4-benzodiazepine-3-carboxylate with potassium hydroxide (1:1)

Sch. IV (1971)

![Clorazepate structure](image)

Empirical formula: \(C_{16}H_{11}ClN_2O_3\) (\(C_{16}H_{11}ClK_2N_2O_4\))
CAS No.: 23887-31-2 (57109-90-7)
MWt: 314.7 (408.9)
MPt: 238 – 240° C
Physical appearance: Off-white to light yellow crystalline powder,
Clorazepate (clorazepate dipotassium) (continued)

Solubility: Freely soluble in water, slightly soluble in ethanol, practically insoluble in chloroform, dichloromethane and ether
Infrared data: Principal peaks at wavenumbers 1597, 1548, 1300, 702, 1230, 830 cm\(^{-1}\) (dipotassium clorazepate, KBr disk) [8], [18, 44]

Clotiazepam

5-(o-chlorophenyl)-7-ethyl-1,3-dihydro-1-methyl-2\(H\)-thieno[2,3-e]-1,4-diazepin-2-one

Sch. IV (1971)

![Chemical structure of Clotiazepam]

Empirical formula: \(\text{C}_{16}\text{H}_{15}\text{ClN}_{2}\text{OS}\)
CAS No.: 33671-46-4
MWt: 318.8
MPt: 105 – 106° C
Physical appearance: Colourless crystalline powder
Solubility: Practically insoluble in water, fairly soluble in ethanol, chloroform, dichloromethane and ether
Infrared data: Principal peaks at wavenumbers 1675, 752, 1506, 1324, 834, 2968 cm\(^{-1}\) (KBr disk) [4]

Cloxazolam

10-chloro-11b-(o-chlorophenyl)-2,3,7,11b-tetrahydrooxazolo[3,2-d][1,4]benzodiazepin-6(5H)-one

Sch. IV (1971)

![Chemical structure of Cloxazolam]
Annex II. Description of benzodiazepines under international control

Delorazepam

7-chloro-5-(o-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one

Sch. IV (1971)

Empirical formula: \( C_{15}H_{10}Cl_2N_2O \)
CAS No.: 24166-13-0
MWt: 305.2
MPt: 198 – 199º C
Physical appearance: White crystalline powder
Solubility: Freely soluble in glacial acetic acid, slightly soluble in chloroform, ethanol and ethyl acetate, almost insoluble in water
Infrared data: Principal peaks at wavenumbers 1692, 1485, 749, 1325, 2937, 3188 cm\(^{-1}\) (KBr disk) [4]

Diazepam

7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one

Sch. IV (1971)

Empirical formula: \( C_{17}H_{14}Cl_2N_2O_2 \)
CAS No.: 24166-13-0
MWt: 349.2
MPt: 202 – 204º C (decomp.)
Physical appearance: White crystalline powder
Solubility: Freely soluble in glacial acetic acid, slightly soluble in chloroform, ethanol and ethyl acetate, almost insoluble in water
Infrared data: Principal peaks at wavenumbers 1673, 1485, 1399, 754, 2885, 3194 cm\(^{-1}\) (KBr disk) [4]
**Diazepam (continued)**

Empirical formula: $C_{16}H_{13}ClN_2O$
CAS No.: 439-14-5
MWt: 284.8
MPt: 131 – 135º C
Physical appearance: White or almost white crystalline powder, injection, oral solution, rectal solution, tablets (protect from light)
Solubility: Soluble in ethanol (1:25), chloroform (1:2) and ether (1:39), almost insoluble in water
Infrared data: Principal peaks at wavenumbers 1681, 1313, 705, 840, 1125, 740 cm$^{-1}$ (KBr disk) [4, 12]

**Estazolam**

8-chloro-6-phenyl-4H-s-triazolo [4,3-a][1,4] benzodiazepine

Sch. IV (1971)

![Chemical Structure of Estazolam]

Empirical formula: $C_{16}H_{11}ClN_4$
CAS No.: 29975-16-4
MWt: 294.7
MPt: 227 – 233º C
Physical appearance: White crystalline powder
Solubility: Soluble in dichloromethane and chloroform, sparingly soluble in acetone and ethanol, almost insoluble in water
Infrared data: Principal peaks at wavenumbers 1498, 1529, 1617, 827, 696, 3108 cm$^{-1}$ (KBr disk) [44]
**Ethyl loflazepate**

Ethyl 7-chloro-5-(o-fluorophenyl)-2,3-dihydro-2-oxo-1H-1,4-benzodiazepine-3-carboxylate

Sch. IV (1971)

![Structural formula of Ethyl loflazepate](image)

**Empirical formula:** $C_{18}H_{14}ClFN_2O_3$

**CAS No.:** 29177-84-2

**MWt:** 360.8

**MPt:** 193 – 194º C

**Physical appearance:** Off-white to light yellow crystalline powder

**Solubility:** Freely soluble in water, slightly soluble in ethanol, practically insoluble in chloroform, dichloromethane and ether

**Infrared data:** Principal peaks at wavenumbers 1737, 1688, 1483, 1212, 753, 3269 cm$^{-1}$ (KBr disk) [4]

**Fludiazepam**

7-chloro-5-(o-fluorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one

Sch. IV (1971)

![Structural formula of Fludiazepam](image)

**Empirical formula:** $C_{16}H_{12}ClFN_2O$

**CAS No.:** 3900-31-0

**MWt:** 302.7

**MPt:** 88 – 92º C

**Physical appearance:** White crystalline powder, injection, oral solution, rectal solution, tablets (protect from light)

**Solubility:** Soluble ethanol and methanol, almost insoluble in water

**Medical use:** Anxiolytic

**Infrared data:** Principal peaks at wavenumbers 1676, 1483, 827, 1452, 749, 1613 cm$^{-1}$ (KBr disk) [4]
**Flunitrazepam**

5-(2-fluorophenyl)-1,3-dihydro-1-methyl-7-nitro-2H-1,4-benzodiazepin-2-one

Sch. IV (1971)

![Flunitrazepam structure](image)

Empirical formula: \( \text{C}_{16}\text{H}_{12}\text{FN}_{3}\text{O}_{3} \)

CAS No.: 1622-62-4

MWt: 313.3

MPt: 166 – 167º C

Physical appearance: White or yellowish crystalline powder (light sensitive)

Solubility: Soluble in acetone, chloroform (1:3), slightly soluble in alcohol, almost insoluble in water.

Infrared data: Principal peaks at wavenumbers 1697, 1620, 1490, 1528, 1107, 783 cm\(^{-1}\) (KBr disk) [4, 12]

---

**Flurazepam (flurazepam monohydrochloride; dihydrochloride)**

7-chloro-1-[2-(dimethylamino)ethyl]-5-(o-fluorophenyl)-1,3-dihydro-2\(H\)-1,4-benzodiazepin-2-one

Sch. IV (1971)

![Flurazepam structure](image)

Empirical formula: \( \text{C}_{21}\text{H}_{23}\text{ClFN}_{3}\text{O} \) (\( \text{C}_{21}\text{H}_{23}\text{ClFN}_{3}\text{O}.\text{HCl} \) & \( \text{C}_{21}\text{H}_{23}\text{ClFN}_{3}\text{O}.2\text{HCl} \))

CAS No.: 17617-23-1 (36105-20-1 MonoHCl; 1172-18-5 DiHCl)

MWt: 387.9 (424.3; 460.8)
MPt: 77 – 82º C (212º C decomp. DiHCl)
Physical appearance: White or yellowish crystalline powder and capsules (protect from light)
Solubility: Soluble in acetone, slightly soluble in alcohol, almost insoluble in water
Infrared data: Principal peaks at wavenumbers 1672, 1613, 1316, 1211, 1171, 1100 cm\(^{-1}\) (KBr disk) [4, 12]

**Halazepam**

7-chloro-1,3-dihydro-5-phenyl-1-(2,2,2-trifluoroethyl)- 2\(H\)-1,4-benzodiazepin-2-one
Sch. IV (1971)

Empirical formula: C\(_{17}\)H\(_{12}\)ClF\(_3\)N\(_2\)O
CAS No.: 23092-17-3
MWt: 352.8
MPt: 164 – 166º C
Physical appearance: White or yellowish crystalline powder (protect from light)
Solubility: Soluble in acetone, slightly soluble in alcohol, almost insoluble in water
Infrared data: Principal peaks at wavenumbers 1688, 1702, 1169, 1325, 698,1261 cm\(^{-1}\) (KBr disk) [44]
Haloxazolam

10-bromo-11b-(o-fluorophenyl)-2,3,7,11b-tetrahydroazolo[3,2d][1,4]benzodiazepin-6(5H)-one

Sch. IV (1971)

Empirical formula: \( \text{C}_{17}\text{H}_{14}\text{BrFN}_{2}\text{O}_{2} \)
CAS No.: 59128-97-1
MWt: 377.2
MPt: 185º C
Physical appearance: White crystalline powder
Solubility: Slightly soluble in chloroform, ethanol and ethyl acetate, sparingly soluble in water
Infrared data: Principal peaks at wavenumbers 1688, 1484, 764, 1417, 2887, 3201 cm\(^{-1}\) (KBr disk) [4]

Ketazolam

11-chloro-8,12b-dihydro-2,8-dimethyl-12b-phenyl)-4H-[1,3]oxazino[3,2d][1,4]benzodiazepin-4,7 (6H)-dione

Sch. IV (1971)

Empirical formula: \( \text{C}_{20}\text{H}_{17}\text{ClN}_{2}\text{O}_{3} \)
CAS No.: 27223-35-4
MWt: 368.8
MPt: 182 – 183.5º C
Physical appearance: White crystalline powder
Solubility: Slightly soluble in chloroform, ethanol and ethyl acetate, sparingly soluble in water
Infrared data: Principal peaks at wavenumbers 1675, 820, 1105, 1195, 1308, 1242 cm\(^{-1}\) (KBr disk) [4, 12]
Loprazolam (loprazolam mesilate)

6-[(o-chlorophenyl)-2,4-dihydro-2-[(4-methyl-1-piperazinyl)methylene]-8-nitro-1H-imidazo [1,2-a][1,4] benzodiazepin-1-one

Sch. IV (1971)

Empirical formula: C_{23}H_{21}ClN_{6}O_{3} (mesilate hydrate C_{23}H_{21}ClN_{6}O_{3}·CH_{4}O_{3}·S·H_{2}O)

CAS No.: 61197-73-7 (70111-54-5 anhydrous)

MWt: 464.9 (579.0)

MPt: 214 – 215º C (mesilate 242 – 245º C [decomp.], crystals from CH_{2}Cl_{2} 205-210º C, anhydrous)

Physical appearance: White crystalline powder

Solubility: Mesilate: practically insoluble in ether, soluble about 1 in 200 in ethanol, soluble 1 in 100 in water

Infrared data: Principal peaks at wavenumbers 1628, 1610, 1045, 1182, 1192, 1518 cm^{-1} (KBr disk) [4, 12]

Lorazepam

7-chloro-5-(o-chlorophenyl)-1,3-dihydro-3-hydroxy- -2H-1,4-benzodiazepin-2-one

Sch. IV (1971)
Lorazepam (continued)

Empirical formula: \( \text{C}_{15}\text{H}_{10}\text{Cl}_{2}\text{N}_{2}\text{O}_{2} \)

CAS No.: 846-49-1

MWt: 321.2

MPt: 166 – 168° C

Physical appearance: White or almost white crystalline (polymorphic) powder

Solubility: Sparingly soluble in alcohol and dichloromethane, practically insoluble in water

Infrared data: Principal peaks at wavenumbers 1685, 1149, 1317, 1120, 1605, 826 cm\(^{-1}\) (KBr disk) [4, 12]

Lormetazepam and enantiomer

7-chloro-5-(o-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2H-1,4-benzodiazepin-2-one

Sch. IV (1971)

Empirical formula: \( \text{C}_{16}\text{H}_{12}\text{Cl}_{2}\text{N}_{2}\text{O}_{2} \)

CAS No.: 848-75-9

MWt: 335.2

MPt: 209 – 211° C (decomp.)

Physical appearance: White crystalline (polymorphic) powder

Solubility: Freely soluble in chloroform, slightly soluble in ethanol and methanol, practically insoluble in water

Infrared data: Principal peaks at wavenumbers 1682, 1153, 1121, 1315, 1610, 843 cm\(^{-1}\) (KBr disk) [4, 12]
**Medazepam**

7-chloro-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepine

Sch. IV (1971)

![Medazepam structure](image)

**Empirical formula:** $C_{16}H_{15}ClN_2$

**CAS No.:** 2898-12-6

**MWt:** 270.8

**MPt:** 95 – 97º C (crystals from ether and petroleum ether)

**Physical appearance:** White to greenish yellow crystalline powder

**Solubility:** Soluble in chloroform, ethanol and methanol, practically insoluble in water

**Infrared data:** Principal peaks at wavenumbers 1610, 1178, 1298, 1255, 815 cm$^{-1}$ (KBr disk) [4, 12]

---

**Midazolam**

8-chloro-6-(o-fluorophenyl)-1-methyl-4H-imidazo[1,5-a][1,4]benzodiazepine

Sch. IV (1971)

![Midazolam structure](image)

**Empirical formula:** $C_{18}H_{13}ClF\text{N}_3$

**CAS No.:** 59467-70-8

**MWt:** 325.8

**MPt:** 158 – 160º C

**Physical appearance:** White or yellowish crystalline powder (protect from light)

**Solubility:** Soluble in acetone, ethanol and methanol, insoluble in water

**Infrared data:** Principal peaks at wavenumbers 1608, 820, 767, 1310, 1210, 995 cm$^{-1}$ (KBr disk) [12]
**Nimetazepam**

1,3-dihydro -1-methyl-7-nitro- 5-phenyl -2H-1,4-benzodiazepin-2-one

Sch. IV (1971)

![Nimetazepam structure](image)

Empirical formula: $C_{16}H_{13}N_3O_3$
CAS No.: 2011-67-8
MWt: 295.3
MPt: 156.5 – 157.5º C
Physical appearance: Pale yellow crystalline powder (protect from light)
Solubility: slightly soluble in alcohol, almost insoluble in water
Infrared data: Principal peaks at wavenumbers 1685, 1335, 1610, 781, 1523, 701 cm$^{-1}$ (KBr disk) [4]

**Nitrazepam**

1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-one

Sch. IV (1971)

![Nitrazepam structure](image)

Empirical formula: $C_{15}H_{11}N_3O_3$
CAS No.: 146-22-5
MWt: 281.3
MPt: 224 – 226º C
Physical appearance: Yellow, crystalline powder, sensitive to light.
Solubility: Soluble in acetone and ethyl acetate, chloroform (1 in 45), slightly soluble in alcohol (1 in 120), soluble 1 in 900 of ether, almost insoluble in water
Infrared data: Principal peaks at wavenumbers 1690, 1610, 698, 1536, 745, 784 cm$^{-1}$ (KBr disk) [4, 12]
**Nordazepam**

7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one

Sch. IV (1971)

![Nordazepam molecule]

**Empirical formula:** $C_{15}H_{11}ClN_2O$

**CAS No.:** 1088-11-5

**MWt:** 270.7

**MPt:** 216 – 217º C (crystals from acetone)

**Physical appearance:** White to pale yellow crystalline powder

**Solubility:** Slightly soluble in chloroform and ethanol, practically insoluble in water

**Infrared data:** Principal peaks at wavenumbers 1680, 1602, 820, 738, 790 cm$^{-1}$ (KBr disk) [8, 44]

---

**Oxazepam and enantiomer**

7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4-benzodiazepin-2-one.

Sch. IV (1971)

![Oxazepam molecule]

**Empirical formula:** $C_{15}H_{11}ClN_2O_2$

**CAS No.:** 604-75-1

**MWt:** 286.7

**MPt:** 204 – 206º C (crystals from alcohol)

**Physical appearance:** White to pale yellow crystalline powder (sometimes found as hemi-succinate or succinate salts)

**Solubility:** Soluble in dioxan, slightly soluble in chloroform (1 IN 270) and ethanol (1 in 220), practically insoluble in water.

**Infrared data:** Principal peaks at wavenumbers 1687, 1706, 693, 830, 1136, 1123 cm$^{-1}$ (KBr disk) [4, 12]
**Oxazolam**

10-chloro-2,3,7,11b-tetrahydro-2-methyl-11b-phenyloxazolo[3,2-d][1,4]benzodiazepin-6(5H)-one oxazolazepam

Sch. IV (1971)

Empirical formula: $\text{C}_{18}\text{H}_{17}\text{ClN}_{2}\text{O}_{2}$

CAS No.: 27167-30-2

MWt: 328.8

MPt: 186 – 188º C

Physical appearance: White crystalline powder

Solubility: Soluble in chloroform, slightly soluble in ethanol, practically insoluble in water

Infrared data: Principal peaks at wavenumbers 1684, 1486, 1197, 698, 2929, 3058 cm$^{-1}$ (KBr disk) [4]

**Pinazepam**

7-chloro-1,3-dihydro-5-phenyl-1-(2-propynyl)-2H-1,4-benzodiazepin-2-one.

Sch. IV (1971)

Empirical formula: $\text{C}_{18}\text{H}_{13}\text{ClN}_{2}\text{O}$

CAS No.: 52463-83-9

MWt: 308.8

MPt: 140 – 142º C

Physical appearance: White crystalline powder

Solubility: Soluble in chloroform, slightly soluble in ethanol, practically insoluble in water

Infrared data: Principal peaks at wavenumbers 679, 1405, 1314, 706, 1484, 3296 cm$^{-1}$ (KBr disk) [4]
**Prazepam**

7-chloro-1-cyclopropylmethyl-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one  
Sch. IV (1971)

![Prazepam molecule](image)

Empirical formula: $\text{C}_{19}\text{H}_{17}\text{ClN}_{2}\text{O}$  
CAS No.: 2955-38-6  
MWt: 324.8  
MPt: 145 – 146º C (crystals from methanol)  
Physical appearance: White or almost white crystalline powder  
Solubility: Freely soluble in dichloromethane, slightly soluble in alcohol, practically insoluble in water  
Infrared data: Principal peaks at wavenumbers 1667, 1316, 740, 694, 1602, 704 cm$^{-1}$ (KBr disk) [4, 12]

**Temazepam and enantiomer**

7-chloro-1,3-dihydro -3-hydroxy-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one  
Sch. IV (1971)

![Temazepam and enantiomer molecule](image)

Empirical formula: $\text{C}_{16}\text{H}_{13}\text{ClN}_{2}\text{O}_{2}$  
CAS No.: 846-50-4  
MWt: 300.7  
MPt: 156 – 159º C  
Physical appearance: White or almost white crystalline powder  
Solubility: Freely soluble in dichloromethane sparingly soluble in alcohol, practically insoluble in water  
Infrared data: Principal peaks at wavenumbers 1687, 1670, 1112, 1603, 705, 1150 cm$^{-1}$ (KBr disk) [4, 12]
**Tetrazepam**

7-chloro-5-(cyclohexen-1-yl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one  
Sch. IV (1971)

![Tetrazepam structure](image)

**Empirical formula:** $\text{C}_{16}\text{H}_{17}\text{ClN}_2\text{O}$  
**CAS No.:** 10379-14-3  
**MWt:** 288.8  
**MPt:** 144º C  
**Physical appearance:** Light yellow or yellow crystalline powder  
**Solubility:** Freely soluble in dichloromethane, soluble in alcohol, practically insoluble in water  
**Infrared data:** Principal peaks at wavenumbers 1678, 1602, 825, 1132, 1310, 800 cm$^{-1}$ (tetryzoline hydrochloride, KBr disk) [4, 12]

**Triazolam**

8-chloro-6-(o-chlorophenyl)-1-methyl-4H-[1,2,4]-s-triazolo[4,3-a][1,4] benzodiazepine  
Sch. IV (1971)

![Triazolam structure](image)

**Empirical formula:** $\text{C}_{17}\text{H}_{12}\text{Cl}_2\text{N}_4$  
**CAS No.:** 28911-01-5  
**MWt:** 343.2  
**MPt:** 233 – 235º C  
**Physical appearance:** White, pale yellow to tan crystalline powder  
**Solubility:** Soluble in chloroform, sparingly soluble in ethanol, almost insoluble in water  
**Infrared data:** Principal peaks at wavenumbers 761, 842, 1618, 1003, 1310, 827 cm$^{-1}$ (tetryzoline hydrochloride, KBr disk) [4, 12]