Journal of Pharmacological and Toxicological Methods 76 (2015) 38–42

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

Injectable sodium pentobarbital: Stability at room temperature

Sydney M. Priest, Timothy P. Geisbuhler *

A. T. Still University of Health Sciences, Department of Physiology, Kirksville, MO 63501, United States

ARTICLE INFO

Article history:
Received 4 June 2015
Received in revised form 22 July 2015
Accepted 23 July 2015
Available online 31 July 2015

Keywords:
Pentobarbital
Nembutal
Stability
High-pressure liquid chromatography

ABSTRACT

Introduction: Sodium pentobarbital (Nembutal) is a barbiturate used in research as an anesthetic in many animal models. The injectable form of this drug has lately become difficult to procure and prohibitively expensive. Due to this lack of availability, researchers have begun to compound injectable sodium pentobarbital from so-called "nonpharmaceutical" pentobarbital. Some oversight agencies have objected to this practice, claiming a lack of quality control and degradation of the drug. We sought with this study to establish both: 1) a protocol for the preparation of injectable sodium pentobarbital, and 2) standard operating procedures to monitor the quality of the preparation and degradation of the drug over time.
Methods: Our preparation consists of a mixture of sodium pentobarbital in alkaline aqueous solution, propylene glycol, and ethanol. Pentobarbital content in this preparation was assayed by high-pressure liquid chromatography (HPLC). We also assayed pentobarbital content over time in preparations of various ages up to 6 years old.
Results: We determined that the drug degraded at a maximum of 0.5% per year in our preparation (alkaline water/propylene glycol/ethanol) when stored in the dark at room temperature. A yellow discoloration developed after about 2 years, which we have arbitrarily determined disqualifies the preparation from use as an anesthetic. Attempts to spectroscopically assay this discoloration were not successful.
Chemicals: Pentobarbital sodium (CID: 14075609)

1. Introduction

Compounds which exert sedative (reduce anxiety with no effect on motor function) or hypnotic (encourage a near-normal state of sleep) effects have been known since antiquity. These compounds have a variety of different chemistries; alcohols (ethanol), ethers (diethyl ether, paraldehyde) and various plant extracts (opium, cannabis, valerian) have all been used at various times as sedatives/hypnotics. The use of barbiturates for this purpose began with the discovery in 1903 that barbital (diethylbarbituric acid) was effective at putting dogs to sleep (Fischer & Von Mering, 1903). Bayer and Co. marketed barbital as veronal in 1904 for use as an anesthetic. By modifying the side chains and other ring substituents, other barbiturates have been developed which are longer- or shorter-acting, or have other specific properties related to anesthesia that make them desirable in specific circumstances [see (Lopez-Munoz, Ucha-Udabe, & Alamo, 2005) for a review].

Sodium pentobarbital was synthesized and introduced as an oral anesthetic by Abbott Laboratories in 1930. Injectable forms were introduced later, and there were even suppositories for use in children fearful of examination. The drug also enjoyed a period of popularity as a treatment for schizophrenia and anxiety. Today, sodium pentobarbital is used clinically as a pre-operative sedative, in certain forms of severe insomnia, and for the emergency management of seizures (Hobbs, Rall, & Verdoorn, 1996).

In research, pentobarbital is chiefly used as an anesthetic in many animal models. An overdose of pentobarbital produces severe CNS depression, which results in death (Trevor & Way, 1982). In the research laboratory and veterinary clinic, then, pentobarbital is used in euthanasia as well as anesthesia. Indeed, until recently pentobarbital was used in the execution of criminals in the United States, either alone or as part of a "lethal triad" including pancuronium bromide and potassium chloride [see Zimmers et al. (2007) or the Wikipedia entry http://en.wikipedia.org/wiki/Pentobarbital for a discussion of this use].

Clinical use of barbiturates has been largely supplanted by benzodiazepines for many reasons. These include a better therapeutic index (difference between a therapeutic and a lethal dose), lower risk of interactions with other drugs based on biotransformation, slow elimination rates, and lower addictive potential. This has led most manufacturers to discontinue making sodium pentobarbital. A single Danish supplier (Lundbeck) is now the sole approved source of injectable pentobarbital in the United States. Lack of availability has driven the price of a single 50 mL bottle of injectable pentobarbital to over $1000 US, a price which renders it "logistically unavailable" according to the National Institutes of Health (NIH). This agency has therefore allowed the use of "nonpharmaceutical" pentobarbital to prepare the injectable product with the approval of the Institutional Animal Care and Use Committee. Other oversight agencies which regularly inspect animal facilities [Association for the Assessment and Accreditation of Laboratory Animal

* Corresponding author at: A. T. Still University, Department of Physiology, 800 West Jefferson Street, Kirksville, MO 63501, United States.
E-mail address: tgeisbuhler@atsu.edu (T.P. Geisbuhler).

http://dx.doi.org/10.1016/j.vascn.2015.07.012
1056-8719/

Care (AAALAC), the United States Department of Agriculture (USDA)] have objected to this practice, claiming that the drug degrades over time and that quality control would suffer. While this might be true of sodium pentobarbital dissolved in water alone, we doubted that it was the case in solutions stabilized with propylene glycol.

We decided, therefore, to establish procedures for 1) preparing the injectable pentobarbital, 2) assaying its concentration by methods (other than weight) available on-site, and 3) assessing suitability of near-expired bottles for continued use, based on degradation of the drug. We also retained "expired" bottles of the injectable pentobarbital for an assay of pentobarbital degradation over time. We found that our procedures created a safe, controlled environment for the use of this drug. We also found that, contrary to the concerns of inspecting agencies, the drug in our hands does not degrade substantially over time.

2. Methods

2.1. Preparation of sodium pentobarbital

An appropriate amount of sodium pentobarbital was weighed into a glass vessel (see Table 1) and suspended in distilled water with stirring (sodium pentobarbital will not dissolve completely in water at neutral pH, instead producing a suspension). Dry NaOH (1–2 pellets) was added, so that the pH of the solution was 12 to 12.5. The pH was then adjusted to approximately 9.8 (i.e., until the drug barely stayed in solution) by adding 10 M HCl dropwise. The volume of the drug solution was then adjusted such that it was 50% of the final product volume (see Table 1). An appropriate amount of propylene glycol (40% of total volume) was added with stirring, followed by an appropriate amount of ethanol (10% of total volume). The solution was stirred until homogeneous and completely dissolved. As we desired a sterile, injectable product, the solution was filter sterilized into a sterile bottle using a 0.22 μm porosity nylon filter. The solution was aseptically transferred to sterile glass serum bottles, sealed with a capper and stored in a locked drawer at room temperature until use.

2.2. Aging of sodium pentobarbital

Our laboratory uses injectable sodium pentobarbital routinely for anesthesia in non-survival rodent surgery. This protocol has been approved by AAALAC, USDA and the American Veterinary Medicine Association. Serum bottles of injectable sodium pentobarbital are routinely marked with the date of manufacture of the mixture, which was used as a benchmark for subsequent studies. As the pentobarbital was used, a small amount was reserved for the aging study. Each aliquot was stored in a locked drawer at room temperature (that is, normal storage) until needed for analysis. At intervals of a few weeks to several months, each aliquot was assayed for pentobarbital and its degradation products by high-pressure liquid chromatography (HPLC) as described below.

2.3. HPLC of sodium pentobarbital

High performance liquid chromatography (HPLC) was used to analyze the sodium pentobarbital preparations. The basic machine was a Hitachi L6200 pump equipped with a Hitachi L4200 variable-wavelength UV–Vis detector. A Hitachi D2500 integrator was used to quantitate detector output. The injectable drug was diluted 1:1000 with deionized water (nominally 50 μg/mL); 200 μL was then applied to a Supelco Discovery C-18 column (Supelco, St. Louis, MO), and assayed using two separate methods.

Table 1
Amounts of reagents used to prepare different volumes of injectable sodium pentobarbital (Na pentobarbital). EtOH = ethanol.

	Volume final product			
	50 mL	100 mL	150 mL	200 mL
Na pentobarbital (mg)	2500	5000	7500	10,000
Propylene glycol (mL)	20	40	60	80
EtOH (mL)	5	10	15	20
Water + drug (mL)	25	50	75	100

2.4. Analysis, Method 1

The sodium pentobarbital preparation was analyzed by HPLC, essentially as described by Morley and Elrod (1997). Briefly, elution was isocratic (1 mL/min) using a buffer consisting of 10 mM phosphate buffer (pH = 3.5)/acetonitrile (72:28). Detection was by UV detector set at 214 nm, using a Hitachi integrator.

2.5. Analysis, Method 2

The sodium pentobarbital preparation was analyzed by HPLC, essentially as described by Reif, Kaufmann, DeAngelis, and Frankhouser (1986). An acetate buffer was prepared using 6.8 g of sodium acetate trihydrate and 5.12 mL of 80% acetic acid per liter of water (final pH = 4.65). This buffer and a liter of HPLC-grade methanol were degassed separately, then mixed together to form an elution buffer of acetate buffer (45%) and methanol (55%). Pentobarbital and its products were eluted isocratically (1.0 mL/min) from the C-18 column with this elution buffer. Detection was by UV detector at 230 nm, using a Hitachi integrator.

2.6. Spectroscopy of injectable pentobarbital

The sodium pentobarbital preparation was examined at several dilutions using a Hitachi model U2000 ultraviolet–visible range spectrophotometer and quartz cuvettes. The mixture was scanned at wavelengths from 200 nm to 750 nm.

2.7. Materials

Sodium pentobarbital was purchased as dry powder from Sigma Chemical Company (St, Louis, MO). Methanol and acetonitrile were HPLC-grade (Fisher Scientific, St. Louis, MO). All other reagents were reagent-grade or better.

3. Results

The formulation for sodium pentobarbital described above produced a crystal clear solution indistinguishable from the commercially prepared product. The drug we prepared (nominally at 50 mg/mL) was effective at producing anesthesia in rats at the same levels of commercially prepared product previously used. The preparation even showed a tendency to turn yellow after about two years, as we have observed with the purchased injectable pentobarbital.

We adapted the HPLC method of Morley and Elrod (1997) for routine quantitative analysis. The major peak in our product (presumably pentobarbital) eluted at 14–15 min, in good agreement with the results of the published method (Fig. 1). No attempt was made to identify small peaks at 11 and 24 min. [Morley and Elrod (1997) suggest three such impurities, and even 2-mercaptobenzothiazole has been suggested as a contaminant (residue from the rubber septum (Garcia, 2004))].

Analysis of our aged samples suggests that large-scale degradation is not a feature of our mixture (Fig. 2). In no case was loss of pentobarbital greater than 0.5%/year, and usually much less. This result prompted us to use another HPLC method to assay our pentobarbital samples, one which was developed to observe degradation products. The method of Reif et al. (1986) was adapted for this purpose; a sample chromatogram of our sample is shown in Fig. 3. Two peaks appeared with the solvent front (one is shown in Fig. 3). These appeared somewhat sporadically,