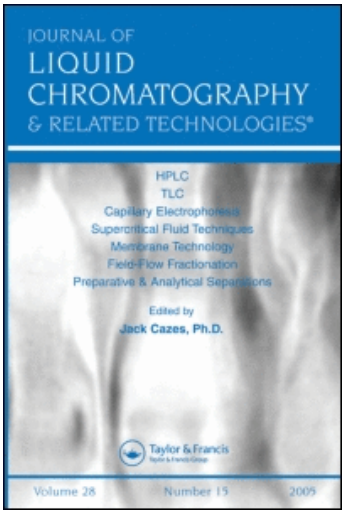

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LIQUID CHROMATOGRAPHIC ANALYSIS OF PENTOBARBITAL

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ABSTRACT

A method is described for a one step acetonitrile precipitation of serum or plasma and subsequent analysis of pentobarbital by reverse phase HPLC. The results of using two internal standards, N,N-Diethyl-m-toluamide and 5-(p-Methylphenyl)-5-phenylhydantoin are compared. Internal standard is added to serum (as little as 25 μL) and vortex-mixed with acetonitrile followed by centrifugation. An aliquote of the supernatant is analyzed on a C18 reverse phase column eluted with metanol/0.05 M $(NH_4)_2HPO_4$, pH 8/water (55/20/25, v/v/v). The effluent is monitored at 220 nm.

INTRODUCTION

Several disorders have been clinically treated using pentobarbital. These include Reye's syndrome (1), head injury (2) cerebral ischemia (3,4) and metabolic coma (5). Serious side effects can result from extended therapy or overdose making it necessary to have a quick plasma assay available. Pentobarbital has been assayed by GLC (6-8), however these procedures require both extraction and derivatization. HPLC drug screening procedures, which include pentobarbital, have been published (9-11) but no quantitative results were reported for pentobarbital. During the course of this study two quantitative HPLC methods have appeared (12,13) both utilizing extraction and reconcentration prior to analysis. The internal standard 5-(p-Methyl-phenyl)-5-phenylhydantoin has been used in the HPLC analysis of other barbiturates and

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drugs (11,14). We reported previously on the use of N,N-Diethyl-m-toulamide as an internal for acetaminopen and salicylate quantitation by HPLC (15).

In this paper we describe a quick one step precipitation procedure for the analysis of pentobarbital by reverse phase HPLC and compare the use of the aforementioned internal standards, which differ greatly in price.

MATERIALS AND METHODS

Instrumentation

A constant volume liquid chromatograph from Waters (Milford, MA.) consisting of a model 6000A solvent delivery system, a U6K sample injector and a model 450 detector (interfaced with a Waters 730 Data System) set at 220 nm was used. The detector was set at 0.04 for routine analysis. The column was a 4.5 x 150 mm prepacked 5 micron C18 from IBM Instruments (Wallingford, CT.).

Reagents

Methanol (MeOH) and acetonitrile (ACN) were HPLC grade obtained from Fisher Scientific (Detroit, MI.), as was $(NH_4)_2HPO_4$. Pentobarbital and internal standard (IS) 5-(p-Methylphenyl)-5-phenylhydantoin (5-MPPH) were obtained from The Anspec Co., Inc. (Ann Arbor, MI.). The IS N,N-Diethyl-m-toluamide was obtained from Chemical Dynamics Corp. (South Plainfield, NJ.).

A standard solution of pentobarbital was prepared by dissolving 40 mg in a liter of MeOH. This solution was found to be stable for up to two months at -20°C. Most of the experiments were carried out using a plasma standard solution prepared by dissolving 40 mg of pentobarbital in 2 ml MeOH and diluting this to a liter with drug free plasma obtained from our Blood Bank. This solution was then aliquoted in 500 µL amounts stored at -20°C until used. These aliquots have been stable for 3 months. The various dilutions were made from these aliquots by the addition of drug free plasma.

A stock IS solution (250 mg/L) of 5-(p-Methylphenyl)-5-phenylhydantoin was prepared in MeOH and is stable for about 1 month at 4°C. A 10-fold dilution working IS was prepared daily.

The IS N,N-Diethyl-m-toluamide (50 mg/L) was also prepared in MeOH and has been stable at room temperature for several months.

The mobile phase consisted of MeOH/0.05 M $(NH_4)_2$ PO_4, pH 8/H_2O (55/20/25 v/v/v).

Procedure

Add 200 µL of ACN and 50 µL of IS to 100 µL of serum or plasma in a 10 x 75 mm glass test tube. Vortex-mix for 15 sec., followed by a 2 min. centrifugation (2500 r.p.m.). An aliquot of the supernatant (usually 20 µL) was injected into the chromatograph and the column eluted with mobile phase at a flow rate of 1.3 mL/min. We have scaled this procedure down to using 25 µL of sample with no observed differences.

Quantification of the samples was carried out by the Waters 730 Data System following calibration with a 20 µg/mL standard plasma solution. Calculations were performed using the peak height calibration mode.

For routine analysis of patient samples the 20 µg/mL standard solution is used to calibrate the Data Module and high and medium level controls (Utak Lab, Saugus, CA.) are used as a procedure control.

RESULTS AND DISCUSSION

Figure 1 shows several typical HPLC chromatograms obtained using our described procedure. Figure 1A is the drug free plasma. Figure 1B is our plasma standard solution containing 20 µg/mL pentobarbital (RT = 6.21) and N,N-Diethyl-m-toluamide (RT = 9.58) as IS. Figure 1C is the plasma standard solution containing 10 µg/mL pentobarbital (RT = 6.21) and 5-(p-Methylphenyl)-5-phenylhydantoin (RT = 8.04) as IS. Figure 1D is the Utak control containing

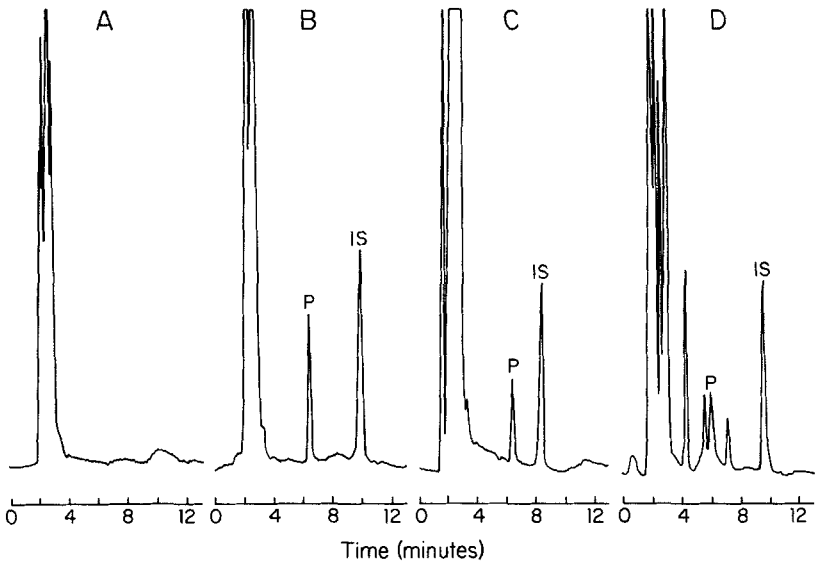

Figure 1. Typical chromatograms, obtained using the procedure given herein. (A) drug-free plasma; (B) control serum containing 20 μg/mL pentobarbital (P) and NNDET internal standard (IS); (C) control serum containing 10 μg/mL pentobarbital and 5-MPPH as IS; (D) Utak control containing 12 μg/mL pentobarbital and NNDEMT as IS. Other components include phenobarbital (RT = 3.10), butabarbital (RT = 4.38), amobarbital (RT = 5.68) and secobarbital (RT = 7.18).

12 μg/mL pentobarbital (RT = 6.18) and N,N-Diethyl-m-toluamide (RT = 9.51) as IS.

Figure 2 shows standard curves which were obtained by manual measurements and plotting peak height ratios (pentobarbital/IS) vs concentration of pentobarbital. As can be seen the response is essentially linear over the entire range tested (5 μg/mL to 40 μg/mL) using either IS.

Recovery and Linearity

Recoveries were assessed by comparing data obtained from a 100 μL aliquot of pentobarbital standards in MeOH to data obtained from drug free plasma which was adjusted to various levels of pentobarbital. These results are given in Table I. Essentially complete recovery was obtained up to a concentration

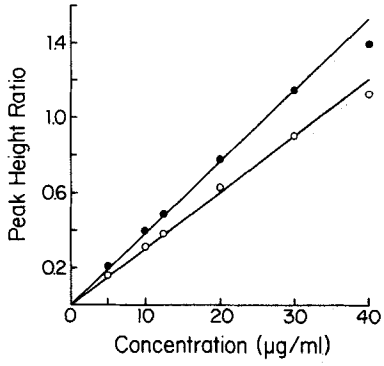

Figure 2. Pentobarbital standard curves obtained by manual calculation of peak height ratios, (●) with 5-MPPH as the IS and (○) with NNDEMT as IS. Each point is the average of 5 determinations.

of 30 µg/mL with slightly higher values being obtained using NNDEMT as IS. While the values obtained for the 40 µg/mL level are acceptable they were lower than the others, again NNDEMT gave higher recoveries. The lower recoveries of the uppel level pentobarbital explains why these levels don't fall exactly on the standard curve (Figure 2).

Accuracy and Precision

The within-run accuracy and precision was evaluated by applying the method 7 times to our plasma standards containing 5 µg/mL and 20 µg/mL of pentobarbital. The results are given in Table II. As can be seen there were slight but insignificant differences between the two internal standards. Day to day precision was assessed by assaying the 20 µg/mL plasma standard over a period of a week. The overall average of the mean coefficient of variation was 6.32% using NNDEMT as IS and 6.75% using 5-MPPH as IS.

Application

Table III shows some typical results from assays of various commercial serum controls, with and without added pentobarbital, and patient samples.

TABLE I

Recovery of Pentobarbital From Plasma.

Comparison Using Two Internal Standards

Concentration	NNDEMT	5-MPPH
µg/mL	%Recovery*	% Recovery*
5	102.1 (5.7)	98.4 (6.3)
10	99.5 (4.9)	96.3 (5.8)
30	97.3 (4.3)	93.4 (4.9)
40	89.0 (3.9)	80.3 (4.6)

*Values given as mean (S.D.), n = 5.

TABLE II

Accuracy and Precision of Pentobarbital Assay

	5 µg/mL		20 µg/mL	
Measured Values*	NNDEMT	5-MPPH	NNDEMT	5-MPPH
Concentration	5.12	4.87	20.34	19.43
S.D.	0.23	0.26	1.37	1.34
C.V.	3.3	3.4	6.78	6.69
S.E.M.	0.08	0.09	0.52	0.51

*n = 7 in all cases

TABLE III

Results of Assaying Various Samples

Sample	µg/mL Pentobarbital Expected	Found NNDEMT	Found 5-MPPH
Ortho I[1]	0	0	0
Ortho I + 10 µg/mL	10	10.19 9.97	9.84 9.76
Ortho II[1]	0	0	0
Ortho II + 10 µg/mL	10	9.94 10.06	9.23 9.56
Tox Control Mid[2]	4	3.96 4.25	3.94 3.87
Tox Control High[2]	12	11.53 11.87	11.23 11.57
Patient 1 Patient 2 Patient 3	6.2[3] 12.8 15.6	6.01 12.32 15.73	5.81 11.94 14.87

[1]Ortho Diagnostics, Raritan, NJ.. [2]Utak Lab., Saugus, CA. [3]Assayed by BioScience Laboratories, Farmington Hills, MI.

Our 20 µg/mL plasma standard was used to calibrate the Data Module. As would be expected from the previous discussed results either IS gave comparable results with 5-MPPH yielding consistently slightly lower pentobarbital levels. Table IV lists some commonly used drugs which have been shown not to interfere with the present assay for pentobarbital.

Based on the assessed accuracy and precision, linearity and simplicity this present method is easily applicable to a clinical laboratory. The small sample size requirements make this an especially useful method to a pediatric service laboratory.

TABLE IV

Some Common Drugs Not Interfering with Pentobarbital Assay

Acetominophen	Methyprylone
Amobarbital	Mephobarbital
Aprobarbital	Meprobamate
Barbital	Phenobarbital
Butabarbital	Propoxyphene
Diazepam	Salicylate
Diphenylhydantoin	Secobarbital
Methaqualone	

Both of the IS investigated appeared to work equally well. The decision as to which one to use may be based on economic mattaers. The cost of 100 g of NNDEMT is $6.50 whereas the same amount of 5-MPPH would be over 3000 times this amount.

REFERENCES

1. Frewen, T.C., Swedlow, D.B., Watcha, M., Raphaely, R.C., Godinez, R.I., Heiser, M.S., Kettrick, R.G., and Bruce, D.A.: Outcome in severe Reye syndrome with early pentobarbital coma and hypothermia. J. Pediatr. 100, 663(1982).

2. Marshall, L.F., Smith, R.W., and Shapiro, H.M.: The outcome with aggressive treatment in severe head injuries. Part II: Acute and chronic barbiturate administration in the management of head injury. J. Neurosurg. 50, 26(1979).

3. Breivik, H., Safar, P., Sands, P., Fabritius, R., Lind, B., Lust., P., Mullie, A., Orr, M., Renck, H., and Snyder, J.V.: Clinical feasibility trials of barbiturate therapy after cardiac arrest. Crit. Care Med. 6, 228(1978).

4. Belopavlovic, M., and Buchthal, A.: Barbiturate therapy in the management of cerebral ischeaemia. Anesthaesia 35, 271(1980).

5. Marshall, L.F., Shapiro, H.M., Rauscher, A., and Kaufman, N.M.: Pentobarbital therapy for intracranial hypertension in metabolic coma. Crit. Care Med. 6, 1(1978).

6. Fiereck, E.A., and Tiety, N.W.: A gas-chromatographic method for separating and measuring barbiturates and glutethimide in blood. Clin. Chem. 17, 1024(1971).

7. Greeley, R.H.: New approach to derivatization and gas-chromatographic analysis of barbiturates. Clin. Chem. 20, 192(1974).

8. Hulshoff, A., VanDerHouwen, O., and Barends, D.M.: The determination of pentobarbital and other barbiturates in plasma by gas-liquid chromatography with on-column and pre-column butylation. Anal. Chim. Acta 105, 139(1979).

9. Kabra, P.M., Stafford, B.E., and Marton, L.J.: Simultaneous measurement of phenobarbital, phenytoin, primidone, ethosuximide, and carbamazepine in serum by high-pressure liquid chromatography. Clin. Chem. 23, 1284(1977).

10. Gill, R., Lopes, A., and Moffat, A.C.: Analysis of barbiturates in blood by high-performance liquid chromatography. J. Chromatog. 226, 117(1981).

11. Kabra, P.M., Koo, H.Y., and Marton, L.J.: Hypnotics and Sedatives. In "Liquid Chromatography in Clinical Analysis" Kabra, P.M. and Marton, L.J., eds. Humana Press, Clifton, N.J. 1981, p. 223.

12. Shin, G.K., and Nemoto, E.M.: Simple, rapid and sensitive reversed-phase high-performance liquid chromatographic method for thiopental and pentobarbital determination in plasma and brain tissue. J. Chromatog. 227, 207(1982).

13. Gupta, R.N., Smith, P.T. and Eng, F.: Liquid-chromatographic determination of pentobarbital in plasma with use of a resin column and an alkaline mobile phase. Clin. Chem. 28, 1772(1982).

14. Kabra, P.M., Gotelli, G., Stanfill, R., and Marton, L.J.: Simultaneous measurement of phenobarbital, diphenylhydantoin, and primidone in blood by high-pressure liquid chromatography. Clin. Chem. 22, 824(1976).

15. Ferrell, W.J., and Goyette, G.W.: Analysis of acetaminophen and salicylate by reverse phase HPLC. J. Liquid Chrom. 5, 93(1982).