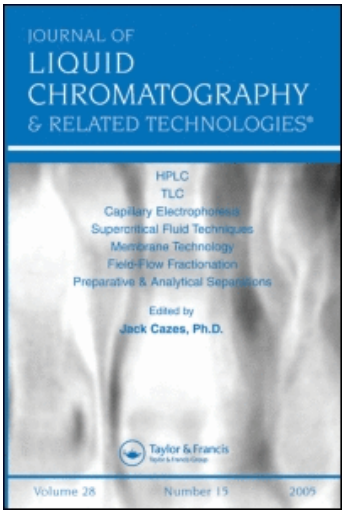

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Serum Pentobarbital Assay by Capillary Electrophoresis

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SERUM PENTOBARBITAL ASSAY BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

A simple and rapid assay for pentobarbital in serum based on capillary electrophoresis (CE) is described. Serum is deproteinized with acetonitrile then electrophoresed at 11 KV in 300 mmol/L borate buffer pH 8.5. This method demonstrates the potential of CE for a rapid and simple quantitation of many drugs and small molecules in serum.

Introduction

Pentobarbital is a short acting hypnotic barbiturate which is often administered to lower intracranial pressure in cerebral accidents. However, the serum level has to be regulated in a narrow therapeutic window to avoid toxicity (1-3). Several methods have been described for the assay of this

compound based on HPLC (4,5) or immunoassays (6,7). The HPLC methods are specific while the immunoassays are non-specific and subject to interferences by other barbiturates (7).

Capillary electrophoresis (CE) is a relatively new analytical technique (8,9) especially in the area of therapeutic drug monitoring (10). The separation can be accomplished based not just on charge, but also on size, hydrophobicity or stereospecificity. Here is a description of a simple and rapid assay for pentobarbital in serum based on capillary electrophoresis (CE). This method demonstrates the potential of CE for rapid and simple quantitation of many drugs and small molecules in serum.

Materials and Methods

Instrument:

An automated capillary electrophoresis instrument (Beckman Instruments, Palo Alto, CA) was set at 11 KV, 24 C and 254 nm. The capillary was 25 cm X 50 um (i.d.). The electrophoresis buffer was boric acid 300 mmol/L adjusted to pH 8.5 with NaOH. Samples were introduced by pressure injection for 10 sec.

Reagents

1. Stock standard: Sodium pentobarbital 1000 mg/L.
2. Working standard (50 mg/L): The stock standard is diluted in a pool of sera free of this drug.

3. Internal standard: 3-isobutyl-1-methyl-xanthine 80 mg/L in acetonitrile.

Procedure:

A-Acetonitrile Deproteinization:

Serum (100 µL) is vortex-mixed with 150 µL acetonitrile containing the internal standard for 15 sec and centrifuged for 1 min at 15,000 x g. Samples were introduced by pressure injection for 10 sec and electrophoresed at 11 KV in borate buffer 300 mmol/L adjusted to pH 8.5 with NaOH.

B-Chloroform Extraction:

Serum (100 µL) was added to 50 µL of buffer (phosphate buffer 100 mmol/L pH 6.2 containing 200 mg/L isobutyl methyl xanthine) and vortex-mixed with 1 mL of chloroform. After centrifugation for 1 min at 15,000 x g, the chloroform layer was evaporated and the residue reconstituted with 100 uL of 50% acetonitrile in water containing 5 g/L sodium chloride.

Calculation: The peak height ratio of pentobarbital to the internal standard was used for the calculation.

Results and Discussion

The separation of some common barbiturates using a 500 mmol/L borate buffer, pH 8.5 is illustrated in Fig 1. The high molarity of the buffer is needed to separate secobarbital from pentobarbital. However, this high concentration is not quite suitable for

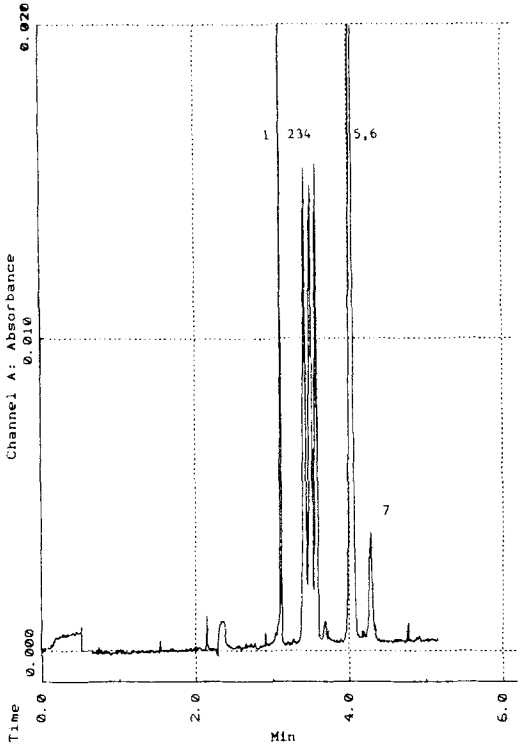

Fig 1 Separation of different barbiturates using a borate buffer, 500 mmol/L, pH 8.5 (1= Internal standard, 2= Pentobarbital, 3= Secobarbital, 4= Amobarbital, 5= Phenobarbital, 6= butabarbital and 7= contamination).

routine use since the current is high leading to a relatively long analysis time. The buffer also tends to crystallize. Since secobarbital is not administered to the patient with pentobarbital, we elected to use 300 mmol/L borate buffer. At this lower buffer concentration, the secobarbital peak is not well resolved from pentobarbital but it can be detected.

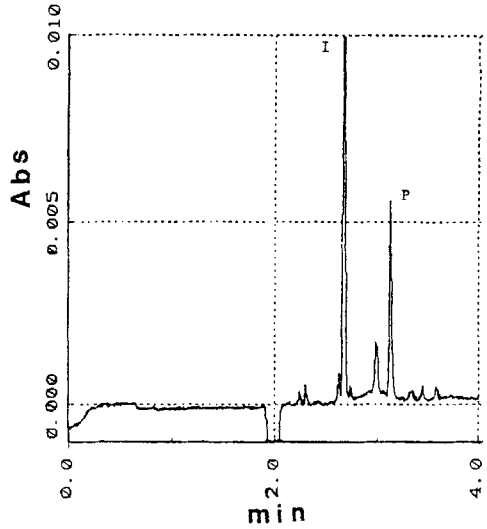

Fig 2. Electropherogram, using acetonitrile deproteinization of serum of a patient on pentobarbital (P) 48 mg/L using borate buffer, 300 mmol/L, pH 8.6; (Internal standard =I).

The analysis time with the lower buffer concentration is faster (about 4 min) with less tendency for the buffer to crystallize. Common drugs such as theophylline, carbamazepine, phenytoin, caffeine and other barbiturates do not interfere in the analysis.

At pH 8.5 pentobarbital has an absorption maximum at 240 nm which is about 3 times higher than that at 254 nm, the wavelength of the filter used. The electropherograms of serum samples using acetonitrile deproteinization are clean at 254 nm, Fig 2. The internal standard and pentobarbital have migration

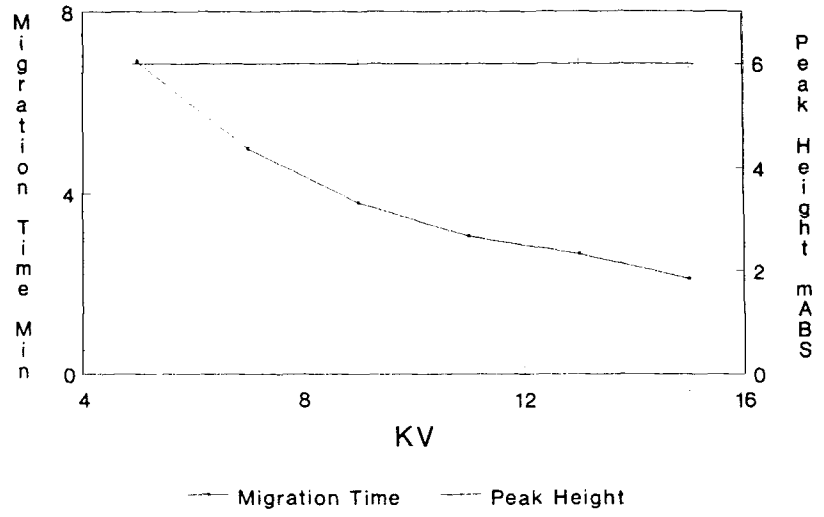

Fig 3. Effect of voltage on migration time and peak height using borate buffer, 300 mmol/L, pH 8.6.

times of about 2.5 and 3 min respectively with a slight difference between the samples deproteinized and those extracted in chloroform. Aqueous standards have also slightly different migration times. Thus the standards were prepared in a pool of sera free from this drug. This also eliminates any differences in recovery between the patient samples and the standards. The analysis can be completed in less than 5 min. A further increase in sensitivity and slightly cleaner electropherograms can be obtained by extraction with chloroform. However, the extraction step is not as suitable for routine use as the acetonitrile deproteinization.

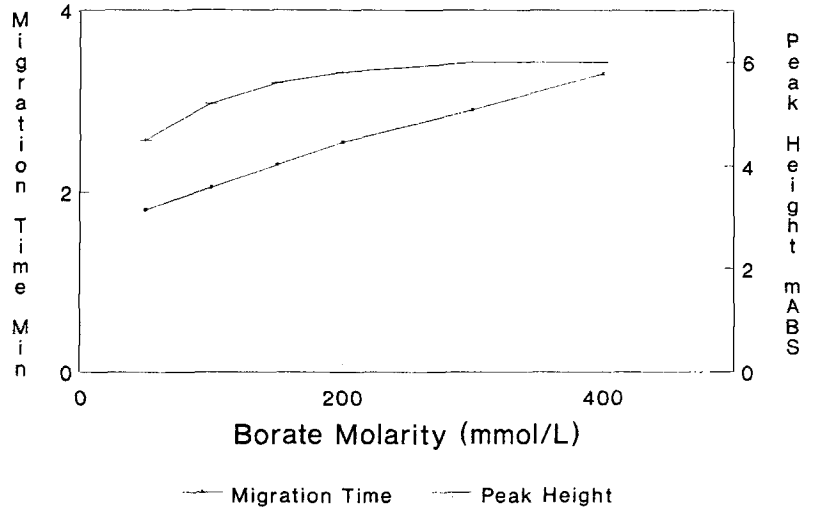

Fig 4. Effect of buffer concentration on migration time and peak height.

Increasing the voltage decreased the migration time (analysis time) without a change in the peak height, Fig 3. Increasing the buffer molarity increased the migration time with slight increase in peak height, Fig 4. The higher molarity of the buffer improved the separation in general due to decreased band diffusion. Increasing the pH increased both the peak height and also the retention time, Fig 5. At a pH higher than 8.5 the secobarbital and pentobarbital coeleute together.

The test is linear between 10 - 100 mg/L , Fig 6. The within-run CV for migration time is 2.3 % (n=12, mean 3.05 and for peak height 3% (n= 12, mean= 5.0) .

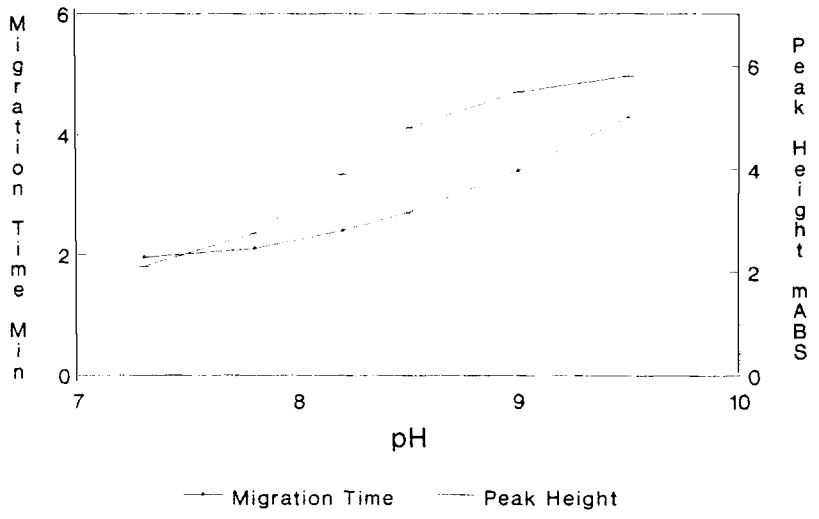

Fig 5. Effect of pH on migration time and peak height.

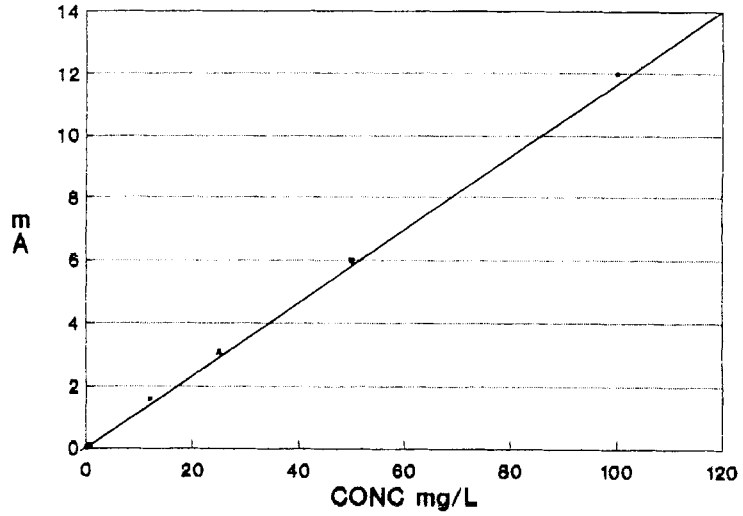

Fig 6. Detector response to different concentrations of pentobarbital.

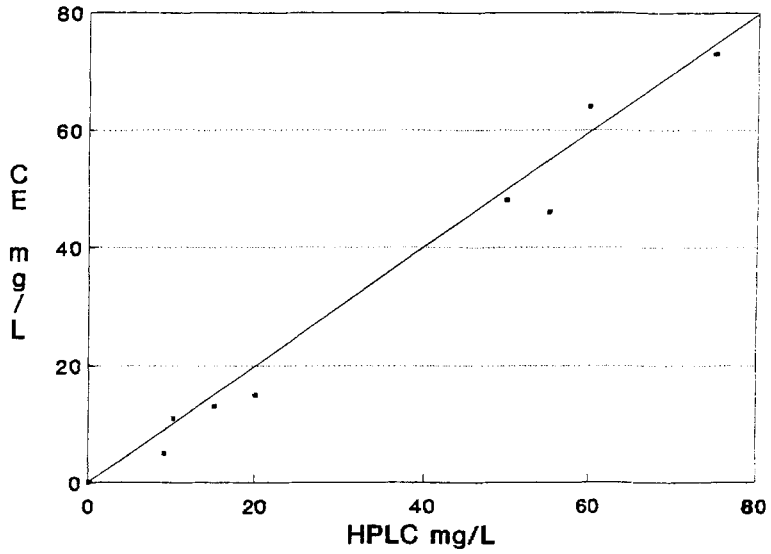

Fig 7. Comparison of serum levels measured by CE and HPLC (5); (CE = 0.98 HPLC = 3.2 , r = 0.96 , n = 9).

However, when the calculation of these two variables is performed as a ratio to the internal standard the CV drops to 0.8 and 1.4, respectively. A comparison of serum assays by CE and HPLC (5) is illustrated in Fig 7 (r = 0.96).

The main advantage of CE over HPLC is the simplicity of the instrument, especially the avoidance of using a packed column, and the extremely low operating cost. This method illustrates that acetonitrile deproteinization is a practical method for preparation of samples for CE, and in addition to that, CE has a good potential as an analytical

technique for therapeutic drug monitoring especially for those drugs which lack an immunoassay.

References

1-Yatsue FM. Pharmacologic protection against ischemic brain damage. Neurol Clin. 1, 37, 1983.

2-Rockoff MA, Marshall LF and Shapiro HM. High-dose barbiturate therapy in humans: A clinical review of 60 patients. Ann Neurol. 6, 194, 1979.

3-Marshall LF, Shapiro HM, Rausscher A and Kauffman NM. Pentobarbital therapy for intracranial hypertension in metabolic coma. Crit Care Med. 6, 1, 1978.

4-Gupta RN , Smith PT and Eng F. Liquid chromatographic determination of pentobarbital in plasma with the use of a resin column and alkaline mobile phase. Clin Chem. 28, 1772, 1982.

5-Shihabi ZK, Dyer RD and Scaro J. Serum injection on the HPLC column for pentobarbital assay. J Liq Chromatog. 10, 10663, 1987.

6-Shihabi ZK and Smith BL. Serum pentobarbital quantitation by fluorescence polarization immunoassay. Clin Chem. 33, 1026, 1987.

7-Earl R, Sobeski L, Timko D and Markin R. Pentobarbital quantification in the presence of phenobarbital by fluorescence polarization immunoassay. Clin Chem. 37, 1774, 1991.

8-Jorgeson JW and Lukacs KD.Capilllary zone electrophoresis. Sci. 222, 266, 1983.

9-Gordon MJ, Huang X, Pentoney SL and Zare RN. Sci. 242, 224, 1988.

10-Shihabi ZK. Clinical applications of capillary electrophoresis. Ann Clin Lab SCi. 22, 398 1992.