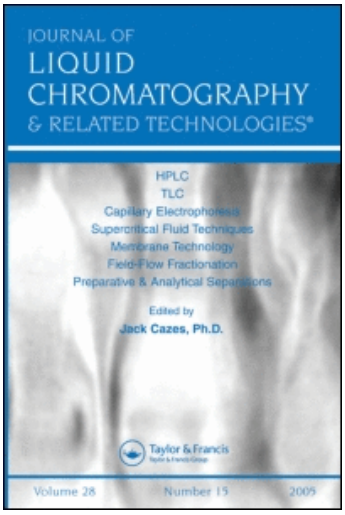

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CAPILLARY ELECTROPHORESIS DETERMINATION OF BUTALBITAL FROM SERUM USING SOLID PHASE EXTRACTION (SPE) AND ULTRAVIOLET DETECTION

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ABSTRACT

Butalbital is a sedative hypnotic that is used mainly in combination with analgesics in the treatment of tension headaches. This paper develops and validates a rapid and sensitive method for the determination of butalbital in serum using capillary electrophoresis (CE) and ultraviolet detection. The internal standard chosen for this assay was aprobarbital because it showed similar chemical structure, identical chromatographic/SPE characteristics, and was commercially available. The CE run buffer contained 50 mM sodium dihydrogen phosphate buffer at pH 9.0 containing 20 mM hydroxypropyl-β-cyclodextrin as a capillary zone electrophoresis (CZE) buffer additive.

An SPE method, using C_{18} Bond-Elut cartridges, was developed to recover the drug and internal standard from serum. The CE system consists of a 75 micron I.D., 52 cm length fused silica capillary maintained at a run voltage of 15 kV with sample detection performed at 254 nm. The limit of detection for butalbital was < 1 μg/mL from serum. Linear calibration curves generated in the concentration ranges of 1 to 60 μg/mL showed a coefficient of determination greater than 0.99. The precision and accuracy of the method calculated as RSD and error were 0.65-3.16% and 2.20-8.90%, respectively. Butalbital is commonly coadminisetred

with aspirin, acetaminophen, caffeine, and codeine for the treatment of tension headaches. The CE method baseline resolved these compounds from the butalbital and the internal standard. Therefore, there is a decreased chance for interference from commonly coadministered compounds in this assay.

INTRODUCTION

Barbiturates are sedative hypnotics used to relieve anxiety prior to surgical procedures. They are also used to manage elevated intracranial pressures and cerebral ischemia from head injuries, asphyxiation, and neurosurgical procedures. Barbiturates modify the mechanism of synaptic transmission rather than intraneuronal conduction.[1] Barbiturates reduce the excitability of the postsynaptic cell by altering the permeability of the cell membrane. Excitatory synaptic transmission is depressed by barbiturates, but inhibitory synaptic transmission is largely unaffected.[1]

Butalbital is a short-to-intermediate acting barbiturate with an approximate elimination half-life of 61 hours.[2] It is commonly used in combination products containing aspirin or acetaminophen, caffeine, and/or codeine. The major clinical application of butalbital is to treat tension headaches, although, it has been used to treat dental pain following oral surgery. Butalbital by itself has been used to treat preoperative anxiety in patients. At higher doses, butalbital can been used as a hypnotic.[3] Butalbital is known to cause drowsiness, dizziness, and other symptoms of central nervous system depression that may lead to respiratory depression. Tolerance to the drug has been observed even on short-term use. The drug may also lead to addiction and withdrawal symptoms may occur on cessation of administration. Butalbital has been known to cause toxicity on overdose. An elevated serum concentration in overdose patients may lead to coma or death. Butalbital dosage needs to be regulated in geriatric patients and patients with renal abnormalities because its metabolites are renally excreted.

The propensity of butalbital to be addictive, even on a short exposure, and the high toxicity of butalbital on overdose, necessitates its regulation in the patient community to which it is prescribed. This work is an attempt to develop a reliable method that would measure the serum levels of butalbital from chronic headache sufferers prescribed a combination of drugs that contain butalbital. This would aid in the prevention and treatment of an overdose of butalbital, either due to carelessness or conscious drug abuse on the part of the patient. In addition, this method was developed to aid in the treatment of patients who show signs of addiction due to chronic use of butalbital containing analgesics.

Currently, gas chromatography-mass spectrometry (GC-MS) methods are widely used to study barbiturates in biological fluids.[4,5] Solid-phase microextraction, followed by GC-MS determination of barbiturates with high precision, was accomplished by Hall et al.[6] Analysis of butalbital containing products has been conducted using reverse phase liquid chromatography.[7] We have previously reported a method for quantitation of barbiturates from serum using liquid chromatography electrospray ionization-tandem mass spectrometry.[8] HPLC and GC have been the most widely used techniques for drug analysis. In recent years, capillary electrophoresis (CE) has gained in popularity due to its various advantages like the low sample volumes required for analysis, high efficiency, and ease of operation. These factors have made CE assays a powerful alternative to existing chromatographic methodologies.[9-15] Stewart and Haque have developed CE methods for the determination of butalbital from commercial tablets.[16-17] Forensic analysis of barbiturates was conducted by Ferslew et al. using capillary electrophoresis.[18] Cyclodextrin (CD) mediated capillary zone electrophoresis is a versatile, and probably the most well understood, separation principle to date. Although cyclodextrins are used predominantly in chiral separations, we have found them to be useful in separations that do not involve chiral compounds. Hence, hydroxypropyl-β-cyclodextrin has been employed as a buffer additive to aid in the CE separation of butalbital and the internal standard, aprobarbital, as described in the following sections.

EXPERIMENTAL

Reagents and Chemicals

Butalbital, aprobarbital, and hydroxypropyl-β-cyclodextrin were obtained from Sigma (St. Louis, MO, USA). Phosphoric acid (85%), sodium dihydrogen phosphate monohydrate, and ammonia solutions were obtained from J. T. Baker (Phillipsburg, NJ, USA). Drug free human serum was obtained from Biological Specialty (Colmar, PA, USA). C_8 and C_{18} solid-phase extraction cartridges of 1 mL capacity (100 mg/mL) were obtained from Varian Sample Preparation Products (Harbor City, CA, USA). All solutions were filtered through a 0.2 μm nylon filter (Acrodisc 13, Gelman Sciences, Ann Arbor, MI, USA).

Preparation of Stock and Standard Solutions

Individual stock solutions were prepared in 20% (v/v) methanol/water to give a concentration of 1.0 mg/mL of butalbital and the internal standard, aprobarbital. Appropriate volumes of the individual drug and internal standard were pipetted into a 2 mL volumetric flask and evaporated. Then 1 mL of serum was added and mixed well. A 50 mM buffer solution of sodium dihydrogen phosphate was prepared in double distilled, deionized water and adjusted to three

pH's 2.5, 7.0, and 9.0 using 100 mM sodium hydroxide and concentrated phosphoric acid. One mL of the buffer was added to drug spiked serum sample that was prepared earlier, in order to adjust it to the required pH. The samples buffered to the three pH's were used according to the different pH requirement of the SPE methods. The concentrations of the spiked samples were 1, 5, 15, 25, 40, and 60 μg/mL for the calibration curves and 10 and 30 μg/mL for the spiked unknowns.

Electrophoretic System

All CE experiments were performed using a P/ACE System 5000 (Beckman Inc., Fullerton, CA, USA) equipped with an UV detector. An uncoated fused silica capillary, total length 62 cm, effective length 52 cm, 75 μm I.D (Polymicro Technologies, Phoenix, AZ, USA), was used for analysis. The capillary was thermostated at 25°C and the voltage applied was 15 kV. The typical running current was approximately 100 μA. A 0.5 cm detection window was created by stripping the polyamide coating of the capillary. The detection was towards the cathodic end. The run buffer consisted of an aqueous solution of 50 mM phosphate buffer at a pH of 9.0 (adjusted with 100 mM sodium hydroxide) containing 20 mM hydroxypropyl-β-cyclodextrin. The analytes were monitored at a wavelength of 254 nm.

Electrophoretic Conditions

New capillaries were conditioned by rinsing with 1 M hydrochloric acid for 5 min, 0.1 M sodium hydroxide for 10 min, followed by 5 min each with water and run buffer solutions. The sample introduction was performed using vacuum injection (0.5 PSI) for 10 s. Before each analysis, the capillary was rinsed for 2 min, first with 0.1 M sodium hydroxide, and then, 2 min with the run buffer solution.

Assay Procedure

Different SPE methods were studied for optimizing the recovery of butalbital and the internal standard, aprobarbital. The cartridges studied were Bond-Elut C_{18} and C_8 at acidic, neutral, and basic pH's of 2.5, 7.0, and 9.0, respectively. The cartridges were pretreated using 3 mL of methanol and then with 3 mL of phosphate buffer at one of the above pH's. The spiked serum samples (buffered to the same pH as the SPE cartridge was conditioned) containing butalbital and the internal standard, aprobarbital, were added to the cartridge and allowed to flow down under low vacuum. The cartridge was not allowed to dry between the pretreatment and sample application steps. The column was washed with 3 mL of the buffer and allowed to dry for 5 min. The analytes were

then eluted with 3 mL of methylene chloride. The eluant was evaporated using a nitrogen stream. The samples were reconstituted in 1 mL of 30% (v/v) methanol/water mixture, filtered using a 0.2 μm nylon filter, and vacuum injected into the capillary for 10 seconds. The absolute recoveries were calculated by comparing the drug peak heights in the spiked serum samples to the unextracted stock solutions that were injected directly into the electrophoretic system. The drug concentration used to study the absolute recovery was 30 μg/mL. Calibration curves were constructed using a concentration range of 1-60 μg/mL. Linear regression analysis of concentration versus Drug/Internal Standard peak height ratios gave slope and intercept data for each analyte. These were used to calculate the concentration of unknown analytes in serum samples.

RESULTS AND DISCUSSION

The chemical structures of butalbital and the internal standard aprobarbital are shown in Figure 1. Baseline resolution (Rs=1.83) of the drug and the internal standard was achieved using 20 mM hydroxypropyl-β-cyclodextrin as a CZE buffer additive. Aprobarbital was chosen as an internal standard because it is a drug that is rarely used, showed similar chemical structure to butalbital, chromatographic characteristics and is also commercially available. Cyclodextrins have been used for the separation of chiral barbiturates in our

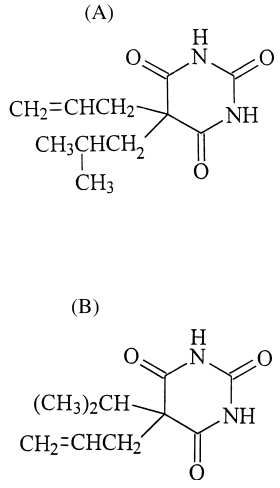

Figure 1. The chemical structures of (A) butalbital and (B) aprobarbital.

laboratory.[19,20] Since cyclodextrins have been shown to have high affinity for barbiturates, it was believed that they would be useful for the separation of achiral barbiturates as well.

Cyclodextrins separate compounds using the phenomenon of host guest complexation where a transient complex is formed between the CD and analyte. The affinity of the CD for the analyte is due to hydrophobic interactions between the CD cavity and analyte, and the hydrogen bonding of the functional groups on the analyte with the hydroxyl groups in the CD ring.[21] Cyclodextrins act as pseudo stationary phases, thereby, imparting chromatographic partitioning effects in addition to the normal electrophoretic separation that takes place in CZE. Cyclodextrins as buffer additives in CZE do not affect assay sensitivity, since at the ultraviolet wavelength of 254 nm they possess negligible absorbance. The separation efficiencies were studied at pH's 7.5, 8.0, and 9.0, and were found to be highest at pH 9.0. Migration times were also not significantly affected by increasing the concentration of the buffer. The analyte peak shape symmetries were found to be best at 50 mM phosphate buffer and the migration time was approximately 8 min. Counter current flow of analyte and CD was effected by negatively charging the analyte and moving it against the flow of the CD. Migration time reproducibility was high with less than 0.5% RSD (n=9) for both peaks.

To decrease the detection limit and to reduce band broadening, the sample was prepared in a lower conductivity solvent (methanol/water) than the electrolyte solution. When a voltage of 15 kV is applied across the capillary, a greater field develops across the sample plug. This field causes the ions to move faster. When the ions reach the buffer they slow down due to the reduced field to which they are subjected, thereby resulting in analyte stacking within a narrow zone of the capillary.[22,23]

The SPE recoveries were studied using Bond-Elut C_8 and C_{18} cartridges at pH's of 2.5, 7.0, and 9.0. The recoveries were found to be optimal at a pH of 9.0 using C_{18} SPE cartridges. The sample clean up was effective and no matrix related background noise was observed. The recoveries of butalbital and the internal standard, aprobarbital were found between 98-99%. Table 1 shows the % recoveries for butalbital and aprobarbital from C_8 and C_{18} SPE cartridges at pH's 2.5, 7.0, and 9.0. Figure 2A shows the electropherogram of blank serum and Figure 2B shows the electropherogram of serum spiked with butalbital and the internal standard, aprobarbital.

Butalbital is administered in combination with analgesics such as aspirin and acetaminophen, in addition to other drugs, such as caffeine and codeine. These co-administered compounds were, therefore, studied in the presence of butalbital using the same method. The CE methods baseline resolved butalbital

Table 1

The Intra- and Inter-Day Precision and Accuracy (% Error) of the SPE-CE Method for Butalbital from Serum

SPE Sorbent	pH	% Recovery Aprobarbital	Butalbital
C_8	2.50	69.08	73.42
C_8	7.00	95.47	69.32
C_8	9.00	77.94	85.62
C_{18}	2.50	76.85	89.49
C_{18}	7.00	70.41	81.79
C_{18}	9.00	98.16	99.96

and internal standard peaks from these co-administered compounds and, hence, interference from aspirin, acetaminophen, caffeine, and codeine would not be observed while analyzing patient samples. This study was conducted in order to establish the reliability of this method in studying serum levels of butalbital when administered in combination with these drugs. Figure 3A shows the separation of butalbital, aprobarbital, and codeine. Figure 3B shows the separation of the butalbital, aprobarbital, and aspirin. Figure 3C shows the separation of the butalbital, aprobarbital, caffeine, and acetaminophen. As shown in Figure 3C, the co-administered drugs, caffeine and acetaminophen, elute with the electroosmotic flow (EOF). This was due to the strongly basic nature of these drugs. At a pH of 9.0, caffeine and acetaminophen would be totally uncharged and therefore elute with the EOF.

The range of concentrations (1-60 μg/mL) encompasses the typical therapeutic serum drug concentration of butalbital. The calibration curve showed a good linearity in this range (1-60 μg/mL) for butalbital. The coefficient of determination was 0.999 (n=3). The representative linear regression equations obtained for butalbital was y = 46.72x-6.83, where y and x were concentration and drug to internal standard peak height ratios, respectively. The intra-day precision and accuracy (n=3) for butalbital, as expressed by RSD and error, was 1.84-2.07% and 7.73-8.90%, respectively. The inter-day precision and accuracy over three days (n=9), expressed by RSD and error, were 0.65-3.16% and 2.20-7.50%, respectively. Table 2 gives inter- and intra-day precision and accuracy values of this method. The methods limit of detection was found to be < 1.0 μg /mL at a signal to noise (S/N) ratio of 3/1.

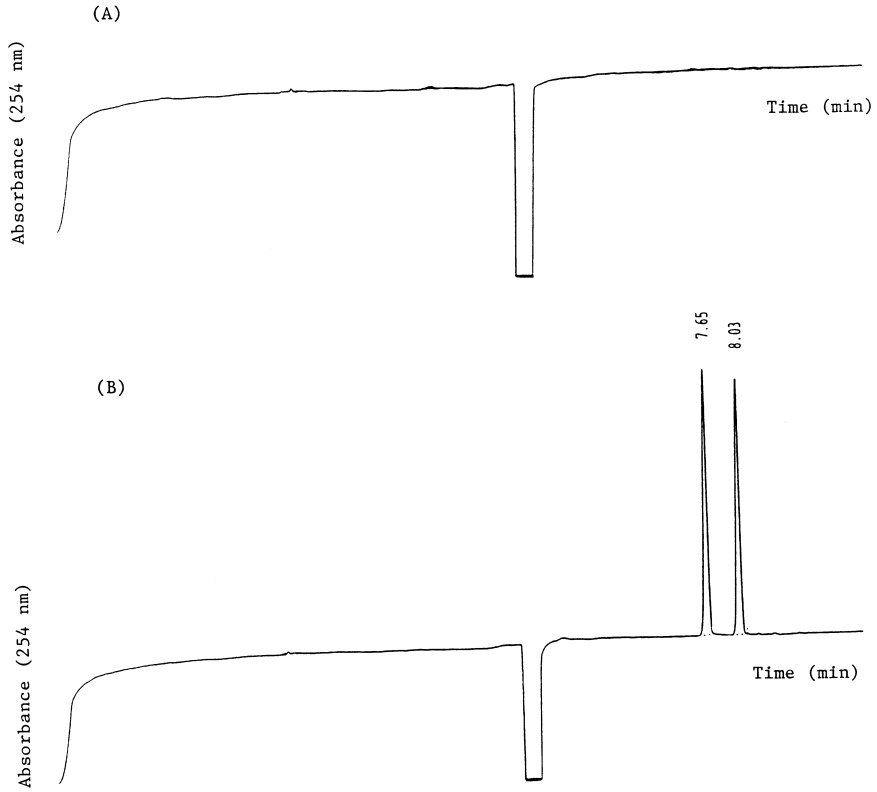

Figure 2. Typical chromatogram of (A) blank serum extract and (B) serum spiked with internal standard aprobarbital (7.65 mins) and butalbital (8.03 mins) on a 57 cm, 75 μm I.D. fused silica capillary. The run buffer contained 20 mM hydroxypropyl–β–cyclodextrin in 50 mM phosphate buffer (pH 9.0) with detection at 254 nm. The capillary was thermostated at 25°C and run voltage was 15 kV.

CONCLUSION

The CE assay described herein is sensitive and suitable for simultaneous determination of butalbital from serum in the presence of co-administered compounds such as aspirin, acetaminophen, caffeine, and codeine. The SPE method provides excellent sample clean up with no endogenous interferences and almost complete recovery. This method also shows good linearity, precision, and accuracy within the linear range of 1-60 μg/mL. The method was found to be rapid, reproducible, and sensitive. This method would be a good alternative

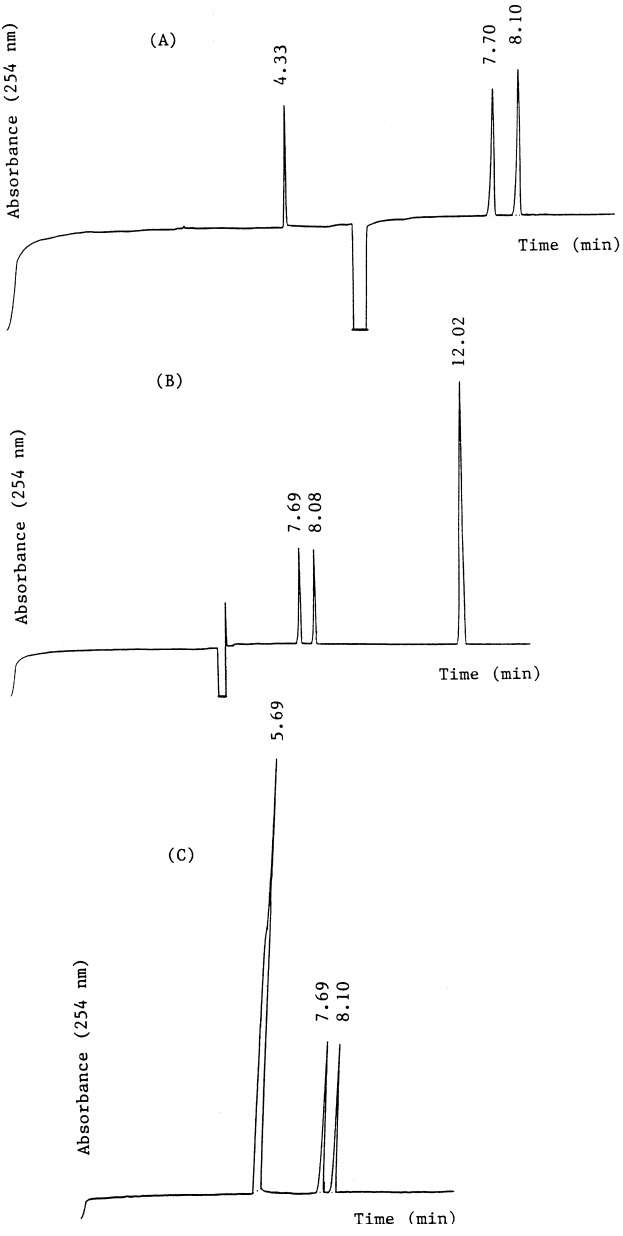

Figure 3. The separation of (A) codeine (4.33 mins), aprobarbital (7.70 mins) and butalbital (8.10 mins) (B) aprobarbital (7.69 mins) and butalbital (8.08 mins) and aspirin (12.02 mins) (C) caffeine and acetaminophen eluting with the EOF (5.69 mins), aprobarbital (7.69 mins) and butalbital (8.10 mins) on a 57 cm, 75 μm I.D. fused silica capillary. The run buffer contained 20 mM hydroxypropyl–β–cyclodextrin in 50 mM phosphate buffer (pH 9.0) with detection at 254 nm. The capillary was thermostated at 25°C and run voltage was 15 kV.

Table 2

Accuracy and Precision of Butalbital Added to Serum

	Conc. added (μg/mL)	Conc. found (μg/mL)	RSD (%)	Error (%)
Intra-Day (n=3)				
Butalbital	10	10.89 ± 0.20	1.84[a]	8.90
	30	32.32 ± 0.67	2.07	7.73
Inter-Day (n=9)				
Butalbital	10	10.75 ± 0.07	0.65[b]	7.50
	30	30.66 ± 0.97	3.16	2.20

[a] Mean ± S.D., based on n=3 for intra-day assay. [b] Mean ± S.D., based on n=9 for inter-day assay.

to the current HPLC and GC-MS methods. The CE assay developed here would be a useful method for the study of serum drug levels in patients administered analgesics that contain butalbital.

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