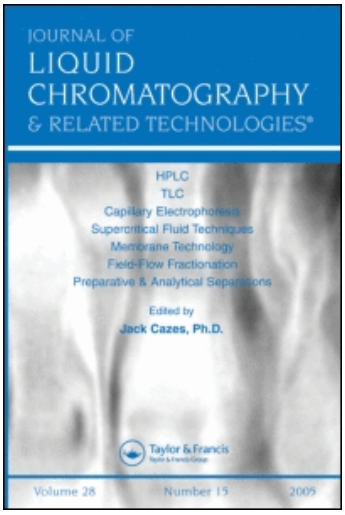

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HPLC METHOD WITH PRECOLUMN PHENACYLATION FOR THE ASSAY OF VALPROIC ACID AND ITS SALTS IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, accurate and reproducible HPLC method is described for the assay of valproic acid and its sodium salts in commercial dosage forms. The analyte and sodium caproate, the internal standard, were detected in the UV range after formation of the corresponding phenacyl ester derivatives with a mixture of phenacyl bromide-triethylamine in acetone. The ester derivatives were analyzed directly on a Microsorb-MV C18 column with a mobile phase composed of acetonitrile-methanol-water (50:20:30) and detection at 245 nm. At a flow rate of 2 mL/min, phenacyl caproate and phenacyl valproate eluted at about 4.5 min and 8.5 min, respectively. Peak height ratios were linearly related to amounts of valproic acid, or its equivalent in sodium valproate, in the range 30-750 μg ($r = 0.9997$). Based on peak height ratios, the RSD for a set of replicate injections was 1.10% ($n = 6$).

Recoveries of valproic acid or sodium valproate from spiked commercial dosage forms were in the range 101.0-102.6% of added (n = 2). The proposed HPLC method yielded assay values that were in good agreement with those obtained by the GC method for valproic acid in USP 23.

INTRODUCTION

Valproic acid (2-propylpentanoic acid) is a simple aliphatic carboxylic acid endowed with anticonvulsant properties that are found useful to suppress epileptic seizures while causing only minimal sedation and other effects in the central nervous system.[1] By the oral or rectal routes of administration, valproic acid is indicated for use as sole or adjunctive therapy to manage simple and complex absence seizures, mixed seizure types, and myoclonic seizures coexisting with tonic-clonic or absence seizures.[1-3]

As such, valproic acid is commercially available in the form of soft gelatin capsules; as the sodium salt it is formulated as a syrup; and as divalproex sodium (a 1:1 coordination compound with the sodium salt) it is marketed as a delayed release tablet.[3]

Owing to the widespread use and clinical importance of valproic acid and its salts, numerous analytical methods have been reported for monitoring their therapeutic circulating levels in human subjects.[4-22] Most of these methods have been based on either gas chromatography (GC)[4-9] or high performance liquid chromatography (HPLC);[10-20] but thin-layer chromatography,[19] enzyme immunoassay,[9,12] immunoassay,[21] and chemical ionization-mass spectrometry[22] have also been proposed. In contrast, methods for the assay of these drugs in pharmaceutical samples are fewer in number, and have relied on spectrophotometry,[23] isotachophoresis,[24] GC,[25] HPLC with conductivity[20] or fluorescence[26] detection, and potentiometry.[25,27] In USP 23, the assay of valproic acid in dosage forms entails a GC approach whereas that for the drug substance is accomplished by a nonaqueous potentiometric titration.[28]

The purpose of this communication is to describe a simple and rapid reverse phase HPLC method with UV detection for the determination of valproic acid and its salts in pharmaceutical products after precolumn derivatization to phenacyl esters. This HPLC method was found to be well suited for the analysis of the title drugs in both liquid and solid commercial dosage forms.

EXPERIMENTAL

Materials

Dosage forms

Various lots of valproic acid capsules (250 mg), sodium valproate syrup (250 mg/5 mL), and divalproex sodium tablets (250 and 500 mg) were obtained from local commercial sources.

Chemicals

Valproic acid, sodium valproate, sodium caproate (Sigma Chemical Co.) and phenacyl bromide (Fluka) were used as received. Triethylamine and acetone (J.T. Baker) were of analytical reagent grade. The acetonitrile, methanol and water (EM Science) were of HPLC grade.

Reagents

Phenacyl bromide solution

A solution of phenacyl bromide in acetone was prepared to contain about 12.8 mg/mL. This solution was stable for at least 3 weeks when stored in an amber glass bottle and in the refrigerator.

Triethylamine solution

A solution of freshly distilled triethylamine in acetone was prepared to contain about 10 mg/mL. This solution was stable for at least 3 weeks when stored in an amber glass bottle and in the refrigerator.

Internal standard solution

It was prepared by dissolving sodium caproate, previously dried to constant weight, in acetone-water (45:55) to a concentration of about 400 μg/mL. This solution was diluted with acetone to a final concentration of about 200 μg/mL.

Sample Preparations

Valproic acid standard preparation

A solution of valproic acid in acetone was prepared to contain about 2.5 mg/mL of valproic acid.

Sodium valproate standard preparation

An accurately weighed quantity of sodium valproate, previously dried to constant weight and equivalent to 250 mg of valproic acid, was transferred to a 100 mL volumetric flask, and dissolved in, and diluted with, acetone-water (45:55) to volume. This solution contained the equivalent of 2.5 mg/mL of valproic acid.

Capsules

The contents of 10 capsules were squeezed into a 200 mL volumetric flask after puncturing each capsule with a pair of scissors. Then each capsule was cut in half and dropped into the flask. The scissors were rinsed with acetone, and the rinsings were quantitatively collected in the flask. After diluting with acetone to volume, and mixing, 20.0 mL of the solution was transferred to a 100 mL volumetric flask, to be diluted with acetone-water (45:55) to volume, and mixed. This solution contained about 2.5 mg/mL of valproic acid.

Syrups

An accurately measured volume of syrup (5 mL) was transferred to a 100 mL volumetric flask, diluted with acetone-water (45:55) to volume, and mixed. This solution contained the equivalent of about 2.5 mg/mL of valproic acid.

Tablets

A group of 20 tablets was accurately weighed, and reduced to a fine powder with the aid of an electric mill. A portion of powder, equivalent to 250 mg of valproic acid, was transferred to a 100 mL volumetric flask, mixed with about 50 mL of acetone-water (45:55), and sonicated for about 10 min.

After diluting with acetone-water (45:55) to volume, and mixing, a portion of the suspension was centrifuged at 4000 rpm for 5 min. The clear solution contained the equivalent of about 2.5 mg/mL of valproic acid.

Derivatization Method

A 1.0 mL volume of sample preparation was transferred to a 10 mL volumetric flask, diluted with acetone to volume, and mixed. To a glass test tube with a Teflon-lined screw cap, 1.0 mL of diluted sample preparation, 1.0 mL of internal standard solution, 50 μL of phenacyl bromide solution, and 50 μL of triethylamine solution, were added in succession. After mixing with gentle swirling, and stoppering tightly, the mixture was heated to 50°C for 2 hr on a heating block. After allowing to cool to room temperature, a portion of the reaction mixture was injected into the liquid chromatograph.

HPLC Method

Apparatus

An isocratic HPLC system was used, consisting of a Series 10 liquid chromatograph and LC 90 UV spectrophotometric detector (Perkin-Elmer), connected to a ChromJet integrator (Spectra-Physics). Samples were introduced through a high pressure injection valve fitted with a 50 μL sample loop (Rheodyne).

Chromatographic conditions

Analyses were performed at ambient temperature on a Microsorb-MV C18, 25 cm x 4.6 mm i.d., 5 μm, column (Rainin). The mobile phase was a mixture of acetonitrile-methanol-water (50:20:30), filtered and degassed prior to use, and flowing at the rate of 2 mL/min. The detection wavelength was 245 nm.

Calculations

The quantity of valproic acid, or the equivalent in sodium valproate, in the dosage form analyzed was calculated from one of the following equations:

$$\text{mg/tablet} = (R_1/R_2) \times C \times (W/S)$$

$$\text{mg/capsule} = (R_1/R_2) \times C$$

$$\text{mg/mL syrup} = (R_1/R_2) \times (C/V)$$

where R_1 and R_2 are the detector response ratios (i.e.,, peak height of analyte/peak height of internal standard) for the sample preparation and standard preparation, respectively; C is the concentration of the standard preparation, mg; W is the average tablet weight, mg; S is the quantity of powdered tablet taken for the assay, mg; and V is the volume of syrup taken for the assay, mL.

RESULTS AND DISCUSSION

Ionization of a saturated fatty acid followed by reaction of the carboxylate anion with a phenacyl halide in an aprotic solvent is a chemical reaction that has been widely used for the prechromatographic formation of ester derivatives with excellent UV light absorbing properties.[10-18,29-33] In general, phenacyl esters can be obtained either by (a) forming an electron-deficient carboxylate ion with the aid of an inorganic base prior to alkylation in the presence of a crown ether;[7,11,13,14,16,31] or (b) direct alkylation following abstraction of the acidic proton with an organic base[10,12,15,18] or alkali fluoride.[32] In the present study, the phenacylation of valproic acid, its sodium salt, and the internal standard, was carried out essentially as described by Borch[33] for the derivatization of long chain fatty acids. This direct approach requires neither the strict anhydrous conditions nor the tedious and time-consuming evaporation step of those methods where the free acid is neutralized with an aqueous alkali prior to alkylation,[31] and in which solvation might slow down the rate of alkylation by decreasing nucleophilicity.[29] Moreover, the use of an amine such as triethylamine represents a more convenient alternative to the more expensive and hygroscopic crown ether catalysts.[31]

Preliminary studies with various aromatic alkyl bromides, including phenacyl, 4-bromophenacyl, 4-nitrophenacyl, 4-methoxyphenacyl, and 4-phenylphenacyl, indicated that phenacyl bromide was the most suitable one in view of the shorter retention times of the esters obtained with this reagent. The formation of potentially bothersome thermal degradation products was minimized by conducting the derivatization at the relatively low temperature of 50°C, and the addition of only a slight excess of reagents insured good reproducibility and minimal peak tailing. Typically, 1.73 μmol of analyte and 1.45 μmol of internal standard were reacted with 3.20 μmol of alkyl halide and 4.94 μmol of triethylamine. The reaction mixture was analyzable without the need for a purification or isolation step since no interfering peaks were observed in the chromatograms. The derivatives were stable in solution and at ambient temperature for at least two weeks when stored in tightly capped test tubes and away from light.

Table 1

Results of Recovery of Valproic Acid, or its Equivalent in Sodium Valproate, from Commercial Dosage Forms by Proposed HPLC Method[a]

	Amount of Valproic Acid Found, as % of Added			
Matrix	**Run 1**	**Run 2**	**Mean**	**SD**
Capsule, 250 mg[b]	101.1	101.0	101.1	0.05
Syrup, 250 mg/5mL[c]	102.8	102.5	102.6	0.15
Tablet, 250 mg[b]	100.4	101.7	101.1	0.67

[a] All experiments were conducted at a spike concentration of valproic acid, or its equivalent in sodium valproate, equal to 50% of the declared amount.
[b] Spiked with valproic acid.
[c] Spiked with sodium valproate.

Figure 1 shows the time course of the derivatization reaction for valproic acid and sodium caproate, the internal standard. For both compounds, the reaction rate became nearly constant after 2 hr of heating at 50°C. An evaluation of detector response as a function of detection wavelength indicated that, although peak heights were maximal at 245 nm, comparable analytical results could be obtained at wavelengths in the range 210-254 nm. On the other hand, detector responses dropped significantly at ≥ 260 nm.

The linearity of the proposed HPLC method was verified by serially diluting a stock solution of valproic acid in acetone with the same solvent, mixing each dilution with the internal standard solution, and subjecting the mixtures to derivatization. Peak height ratios were found to be linearly related to amounts of derivatized valproic acid in the approximate range of 30-750 μg. A similar linearity study was also conducted on the internal standard but keeping the concentration of valproic acid constant. Line equations for valproic acid and sodium valproate were $y = 0.00328x + 0.0124$ ($r = 0.9997$) and $y = 0.00486x - 0.00102$ ($r = 0.9999$), respectively, with both lines passing through the origin. Recommended reaction conditions were set to analyze 250 μg of drug and 200 μg of internal standard in a total volume of reaction mixture of about 2.1 mL.

The reproducibility of the method was assessed on the basis of peak height ratios for a set of 6 replicate injections of a standard phenacylated valproic acid preparation containing 250 μg/mL of analyte. The RSD value was 1.10%. As

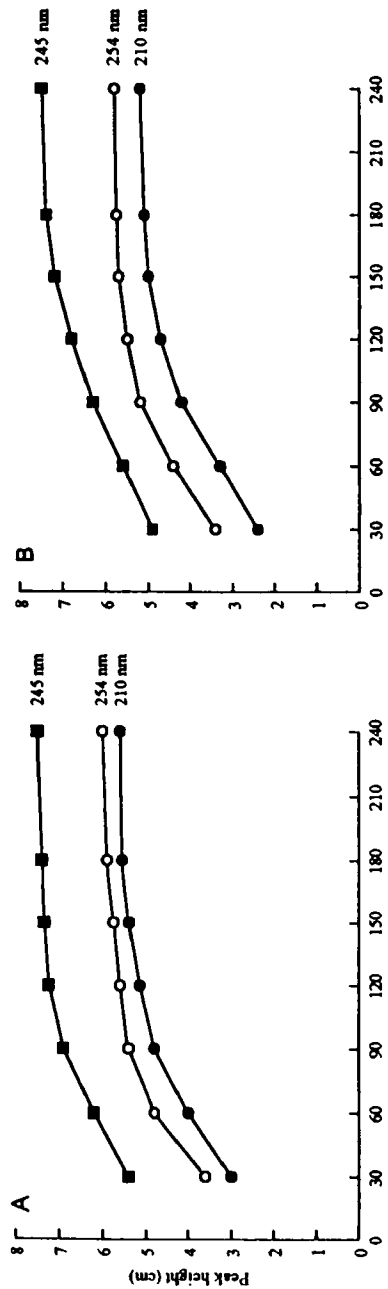

Figure 1. Time course of the derivatization of (A) valproic acid (1.73 µmol) and (B) sodium caproate, the internal standard (1.45 µmol) with phenacyl bromide (3.20 µmol) - triethylamine (4.94 µmol) in acetone at 50°C. For comparative purposes, curves obtained at 210, 245 and 254 nm are shown for each reacting compound.

Table 2

Results of the Assay of Valproic Acid in Commercial Dosage Forms by Proposed HPLC Methd and USP 23 GC Method[a,b]

	Valproic Acid Found, as % of Declared					
	HPLC			GC		
Lot No.	Run 1	Run 2	Mean	Run 1	Run 2	Mean
			Capsules, 250 mg/Capsule			
1	104.5	103.8	104.2	104.8	103.8	104.3
			Syrup, 250 mg/5mL[c]			
1	103.8	103.1	103.5	100.2	100.5	102.9
2	102.3	103.2	102.3	101.8	99.7	100.8
3	103.7	103.8	103.8	100.5	100.5	102.8
			Tablets, 250 mg/Tablet[d]			
1	99.3	100.7	100.0	100.5	100.8	100.7
			Tablets, 500 mg/Tablet[d]			
1	99.7	100.3	100.0	101.1	100.8	100.9

[a] USP 23 requirement: not less than 90.0 percent and not more than 110.0 percent the labeled amount of $C_8H_{16}O_2$.

[b] USP 23 does not list the tablets.

[c] Contains sodium valproate in an amount equivalent to 250 mg/5mL of valproic acid.

[d] Contains divalproex sodium, a 1:1 coordination of valproic acid with sodium valproate.

a verification of accuracy, commercial dosage forms whose drug content had been previously determined by the GC method for valproic acid in USP 23,[32] were spiked with valproic acid (capsules, tablets), or the equivalent in sodium valproate (syrup), in an amount equal to one-half the declared amount, diluted as described for the dosage form preparations, and put through the proposed derivatization method. As presented in Table 1, the mean recovery of valproic acid from a tablet or capsule matrix was, in both instances, 101.1%, whereas that of sodium valproate from a syrup matrix was 102.6% (n = 2).

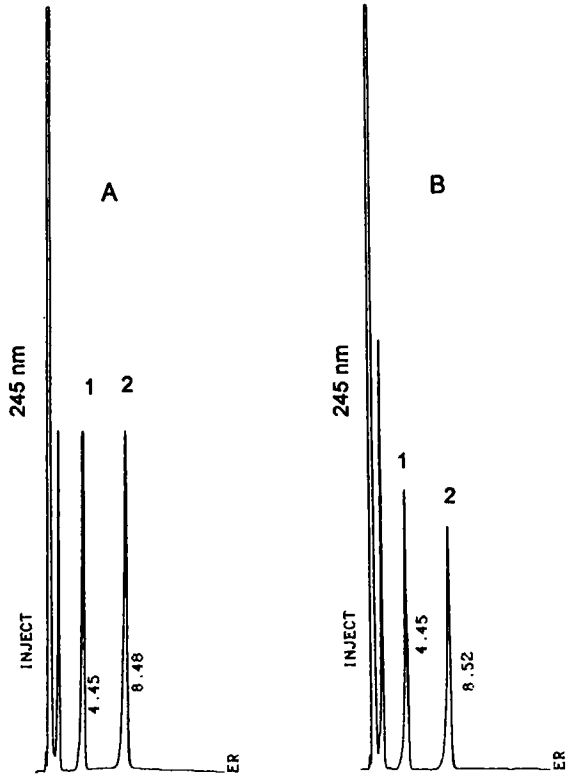

Figure 2. Typical high performance liquid chromatograms of 1, phenacyl caproate, the internal standard, and 2, phenacyl valproate in (A) a standard preparation and (B) a capsule preparation. Flow rate was 2 mL/min.

Table 2 summarizes the results of the assay of commercial dosage forms, comprising capsules, tablets, and syrups, by the proposed method, and they represent the means of duplicate analyses. Typical chromatograms of a standard preparation and a dosage form preparation are shown in Figures 2A and 2B, respectively, with phenacyl caproate eluting ahead of phenacyl valproate. A run was completed in about 10 min. Assay values by the HPLC method were compared with those obtained by the compendial GC assay method for valproic acid tablets and capsules.[28] Intermethod differences were about 0.1 % of labeled for capsules, ≤0.9% of labeled for tablets, and 0.6-1.5% of labeled for syrups. All samples were found to meet the official requirements for labeled drug content. No interferences were noted from excipients or other inert ingredients present in the formulations tested.

In summary, the conversion of valproic acid, sodium valproate or divalproex sodium to phenacyl ester derivatives served as the basis for a specific, accurate, and reproducible means of analyzing these antiepileptic agents in commercial pharmaceutical products by HPLC with spectrophotometric detection. In addition to its simplicity and rapidity, this method was equally applicable to the quantitative analysis of liquid and solid dosage forms, and yielded results that were in close agreement with those obtained by the GC method for valproic acid in USP 23.

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