Rapid and Simple GLC Determination of Valproic Acid and Ethosuximide in Plasma of Epileptic Patients

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Abstract □ A GLC method for the determination of valproic acid and ethosuximide in plasma was developed. The procedure involved a single solvent extraction of drugs from acidified plasma samples, followed by a GLC injection of the unconcentrated organic phase. This rapid, sensitive, specific, and reproducible method has been used for 2 years for the routine determination of plasma levels of valproic acid and ethosuximide in epileptic patients who receive other antiepileptic drugs simultaneously.

Keyphrases □ Valproic acid—GLC determination in plasma of epileptic patients □ Ethosuximide—GLC determination in plasma of epileptic patients □ Antiepileptic agents—GLC determination of valproic acid and ethosuximide in plasma □ GLC—determination of valproic acid and ethosuximide in human plasma

The use of valproic acid in the treatment of primary generalized epilepsy (particularly petit mal epilepsy) has been reported (1–3). Optimal plasma concentrations of valproic acid are between 40–50 and 100 μg/ml of plasma (1), and the importance of monitoring valproic acid in epileptic patients was discussed (4, 5).

Ethosuximide was introduced 25 years ago in the clinical therapy of petit mal epilepsy (6). There have been several studies (7–9) on the therapeutic use of ethosuximide in epilepsy and on the importance of monitoring plasma ethosuximide concentrations.

Numerous GLC methods are available for the determination of valproic acid (10–18) and ethosuximide concentrations (17, 19–21) in human plasma. However, almost all of these methods require evaporation to concentrate the drugs. This step is crucial because these drugs (particularly valproic acid) are very volatile. A microextractive technique without evaporation of the organic phase is sometimes used to avoid irregular evaporation or decreased sensitivity due to volatility. However, these techniques require a high level of precision due to the minute volumes used.

This report describes a GLC method without evaporation, utilizing 1 ml of plasma. This method has been used in this laboratory for more than 2 years for routine analyses.

EXPERIMENTAL

Reagents—Stock solutions of valproic acid[1] and ethosuximide[2] were prepared by dissolving the drugs in water. Caproic acid[3] (marker for valproic acid) and α,α-dimethyl-β-methyl succinimide[4] (marker for ethosuximide) were dissolved in chloroform[3]. Stock solutions were stored at 4°.

Apparatus—The gas chromatograph[5] was equipped with a flame-ionization detector and a recorder–integrator[6]. The glass column, 1 m long × 3-mm i.d., was packed with 10% diethylene glycol–succinate–phosphate, on 80–100-mesh Supelcoport[7]. The following flow rates were used: hydrogen, 15 ml/min; air, 250 ml/min; and carrier gas (nitrogen), 40 ml/min. The temperature of the column was 145° for valproic acid and 190° for ethosuximide, and the injector temperature was 225° for both drugs.

Extraction Procedure—To 1 ml of plasma containing unknown concentrations of the drugs was added 0.5 ml of 1 *N* HCl and 0.5 ml of chloroform containing 100 μg/ml of caproic acid or 100 μg/ml of α,α-dimethyl-β-methyl succinimide. After being shaken gently for 15 min and centrifuged at 2500 rpm for 10 min, the aqueous phase was discarded and 1–2 μl of the organic phase was injected into the chromatograph.

Plasma samples from patients taking both drugs were extracted into chloroform containing both markers. Calibration curves were prepared by adding exact volumes (5, 10, 20, 40, 100, and 200 μl) of a standard so-

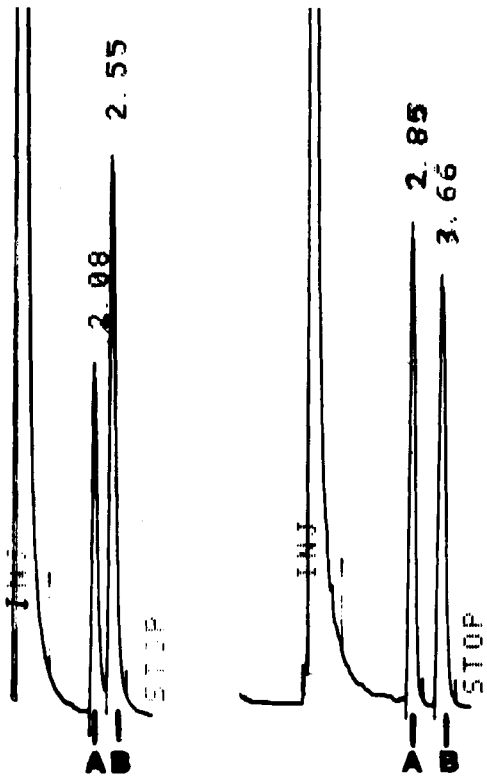

Figure 1—*Response obtained with extract from plasma of a patient being treated with phenobarbital, carbamazepine, and valproic acid (A, caproic acid; and B, valproic acid) (left) and with phenytoin, nitrazepam, and ethosuximide (A, α,α-dimethyl-β-methyl succinimide; and B, ethosuximide) (right).*

[1] Sigma-Tau, Rome, Italy.
[2] Parke-Davis, Milan, Italy.
[3] Carlo Erba, Milan, Italy.
[4] Aldrich-Europe, Beerse, Belgium.
[5] Model Fractovap 2350, Carlo Erba, Milan, Italy.
[6] Model HP 3380A, Hewlett-Packard, Avondale, Pa.
[7] Catalog Number 1-1999, Supelco Inc., Bellefonte, PA 16823.

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Table I—Reproducibility of Valproic Acid and Ethosuximide Analyses in Human Plasma Samples

Amount Added, μg/ml	Valproic Acid			Ethosuximide		
	Amount Found, μg/ml[a]	*SD*	*CV*, %	Amount Found, μg/ml[a]	*SD*	*CV*, %
10	9.9	0.43	4.3	10.0	0.42	4.2
30	29.9	1.27	4.2	30.2	1.29	4.3
60	60.3	2.04	3.4	60.3	2.10	3.5
100	100.1	3.00	3.0	100.2	3.41	3.4

[a] Mean of 10 determinations.

lution of valproic acid or ethosuximide (1 mg/ml) to 1 ml of drug-free plasma. These plasma samples were treated as already described for samples containing unknown concentrations.

RESULTS AND DISCUSSION

Typical chromatograms of extracts from plasma of two patients undergoing multiple antiepileptic drug therapy, one receiving valproic acid and the other receiving ethosuximide, are shown in Fig. 1. There was no interference from endogenous plasma compounds or from other antiepileptic drugs.

Calibration curves from plasma extract showed a linear correlation between concentration and the respective reading: $y = 0.044x - 0.039$, $r = 0.995$, for valproic acid; and $y = 0.023x - 0.034$, $r = 0.992$, for ethosuximide. To calculate these curves, a least-squares linear regression method was used. The minimal concentration detectable for both drugs was ~1–2 μg/ml of plasma.

Analytical recoveries of substances were established as follows. Various amounts (5, 10, 20, 40, 100, and 200 μg) of valproic acid or ethosuximide were dissolved in 1 ml of drug-free plasma. After acidification, plasma samples were extracted into 0.5 ml of pure chloroform. Then 0.2 ml of a solution containing the marker (100 μl/ml) in chloroform was added to 0.2 ml of the organic phase.

A series of external standards was prepared by adding 0.5 ml of the marker solution (100 μg/ml) to 0.5 ml of chloroform containing various amounts (5, 10, 20, 40, 100, and 200 μg) of valproic acid or ethosuximide.

Analytical recoveries were calculated by comparing peak area ratios of the extracted standards to the ratios of the external standards. Expressed as concentrations in micrograms per milliliter, they were as follows (theoretical values of 5, 10, 20, 40, 100, and 200): 5.0, 10.1, 20.3, 40.7, 102.3, and 202.8 for valproic acid and 4.4, 8.9, 18.0, 35.6, 88.8, and 176.4 for ethosuximide.

Reproducibility was determined by performing 10 replicate analyses of four control samples containing 10, 30, 60, and 100 μg/ml of both drugs on different days over 4 months. The results are shown in Table I.

The total time required to analyze 22 plasma samples (plasma samples from 16 patients and six calibrators) was ~1.6 hr.

This method has been utilized for 2 years, analyzing ~1600 plasma samples from patients being treated with valproic acid and ~250 plasma samples from patients being treated with ethosuximide. Only ~30 samples contained both drugs.

If these drugs appear together in the same plasma, they may be extracted simultaneously, as described, and injected onto the column at two different temperatures. At the highest temperature (190°), valproic acid and its marker elute in front of the solvent; at the lowest temperature (145°), ethosuximide and its marker have a retention time of 20 and 16 min, respectively.

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