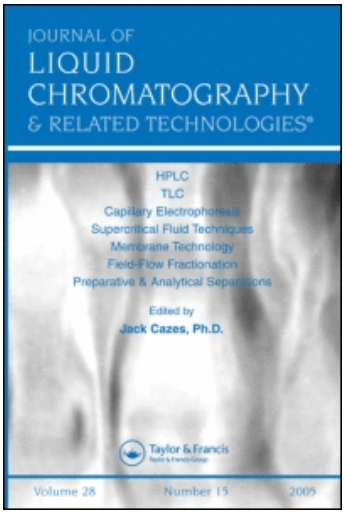

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N. Wad[a]
[a] Swiss Epilepsy Center, Zurich, Switzerland

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SEPARATION OF THE ENANTIOMERS OF PHENETURIDE IN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

N. Wad

Swiss Epilepsy Center
Bleulerstrasse 60
8008 Zurich, Switzerland

ABSTRACT

Total pheneturide (PTE) was isolated from serum by preparative high-performance liquid chromatography (HPLC). A reversed-phase column was used. The total PTE collected was separated into its two enantiomers using a chiral stationary phase. It was found that (+)-PTE was present in serum at a concentration 15 times higher than (-)-PTE. The interest of this result lies in the fact that the two enantiomers have a different pharmacological action. In guinea pigs (+)-PTE showed psychostimulation whereas (-)-PTE showed sedation. The question arises as to whether the racemic (equimolecular) mixture is the best therapeutic combination of the enantiomers or whether another concentration ratio or one of the enantiomers would give a better antiepileptic efficacy versus toxicity ratio.

INTRODUCTION

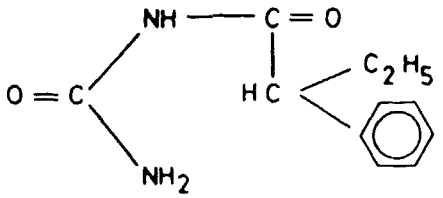
Some antiepileptic drugs (AEDs) determinable in serum are optically active such as ethosuximide, mephenytoin, methsuximide and pheneturide (PTE). This is also the case for some of the meta-

bolites of AEDs, such as desmethyl-mephenytoin, desmethyl-methsuximide and 5-(4-hydroxyphenyl)-5-phenylhydantoin and 2-phenylbutyric acid (metabolite from PTE). The determination of the enantiomeric composition of urinary phenolic metabolites of phenytoin has already been described (1). Recently, many companies introduced HPLC columns packed with chiral complex agents with the capability of separating optically active isomers. In this study a chiral stationary phase from J.T. Baker was selected which has been developed by Pirkle et al (2). The resolution of the individual enantiomers is of importance because they may have different pharmacological properties. Racemic PTE (Fig. 1) was introduced as an AED by Gold-Aubert in 1952 (3) and has been shown to be an effective drug (4), in particular against complex partial seizures (5-7). Gold-Aubert also studied the pharmacological action of the enantiomers. In guinea pigs (+)-PTE showed psychostimulation whereas (-)-PTE showed sedation (8). The concentration of total PTE in serum can be measured by means of gas chromatography (9), thin-layer chromatography (10) or HPLC (11). The last technique (11) has been implemented in our laboratory since 1983 for the routine determination of total PTE in serum. During 1986 the number of total PTE concentrations performed was 303, which represented 1.1% of the AEDs measured that year.

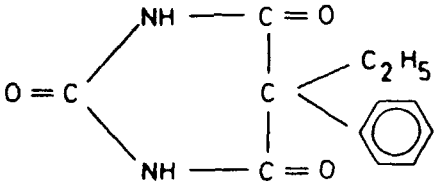
METHODS

Apparatus

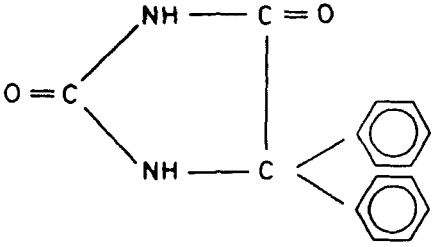
HPLC: Hewlett-Packard 1084B equipped with a variable wavelength detector. Column: LiChrosorb RP-8, 200 x 4.6 mm, 10 µm (Hewlett-Packard). Mobile phase: Gradient elution with 16 - 23% acetonitrile in 2.5 mM KH_2PO_4 for 6 min, remaining isocratic for the rest of the run. Flow: 1.6 ml/min. Temperature: 35 °C. Detection wavelength: 204 nm.



PHENETURIDE



PHENOBARBITAL



PHENYTOIN

Fig. 1. Chemical formular of pheneturide, phenobarbital and phenytoin showing their structural relationship

Hewlett-Packard 1090 equipped with a photo diode-array detector. Column: Bakerbond Chiral Phase, (R)-N-3,5-dinitrobenzoyl-phenylglycine (DNBPG) bound covalently to 5 µm aminopropyl silica, 250 x 4.6 mm (J.T. Baker). Mobile phase: 90% n-Hexane and 10% 2-propanol. Flow: 1.5 ml/min. Temperature: 35 °C. Detection wavelength: 204 nm.

Shaker: Gerhardt.

Evaporator: Büchi, Rotavapor-R.

Reagents and standards

(+)-PTE and (-)-PTE and racemic PTE were kindly supplied by Professor Gold-Aubert, Sapos S.A., Genève, Switzerland. Racemic 2-phenyl-butyric acid was obtained from Aldrich. The standards were dissolved in ethanol. Acetonitrile, ethanol, n-hexane and 2-propanol were LiChrosolv reagents from Merck.

Procedure

The concentration of total PTE in serum was measured by a previously described HPLC method (11). In short, this method is as follows: 500 µl serum was extracted with ethyl acetate at pH 3.9 and the dried extract is dissolved in 70% ethanol in water and an aliquot is injected into the Hewlett-Packard 1084B liquid chromatograph equipped with a RP-8 column. A chromatographic separation is shown in Fig.2. This method (11) was also used for preparative isolation of total PTE from 500 µl serum samples prior to the enantiomer analysis. The collected total PTE, dissolved in the mobile phase of acetonitrile and water, was evaporated to dryness in the Rotavapor. The residue was dissolved in 200 µl ethanol and 25 µl was injected into the liquid chromatograph equipped with the chiral column. Separation of racemic PTE standard and isolated total PTE from pooled patient serum into its enantiomers are shown in Fig. 3. The racemic PTE standard was given to drug-free serum and analysed as a patient serum.

RESULTS AND DISCUSSION

The day-to-day coefficient of variation for the determination of total PTE in serum was 3.5% (n=30) with a recovery of 90% and the linearity is guaranteed to at least 350 µmol/l (11). The within-day precision for the determination of the enantiomers was examined in pooled patient serum. The results are shown in Table 1. The results are well within the accepted limits of deviation (Table 1), although no internal standard was used.

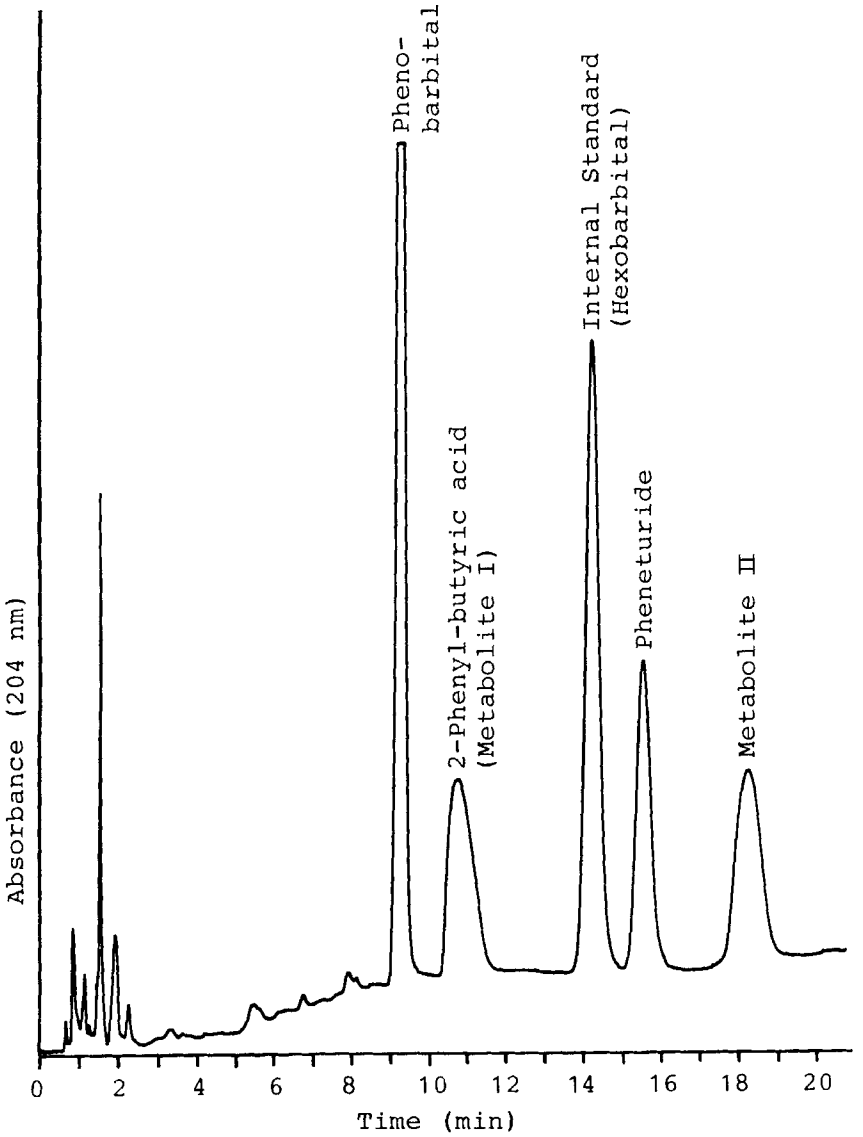


Fig. 2. Chromatographic separation of a serum extract from a patient receiving racemic pheneturide and phenobarbital. The concentration of 2-phenyl-butyric acid is 173 µmol/l, total pheneturide 72 µmol/l and phenobarbital 160 µmol/l. LC: Hewlett-Packard 1084B. Column: LiChrosorb RP-8, 200 x 4.6 mm, 10 µm (Hewlett-Packard). Mobile phase: Gradient elution with 16-23% acetonitrile in 2.5 mM KH_2PO_4 for 6 min, remaining isocratic for the rest of the run. Flow: 1.6 ml/min. Temperature: 35 °C.

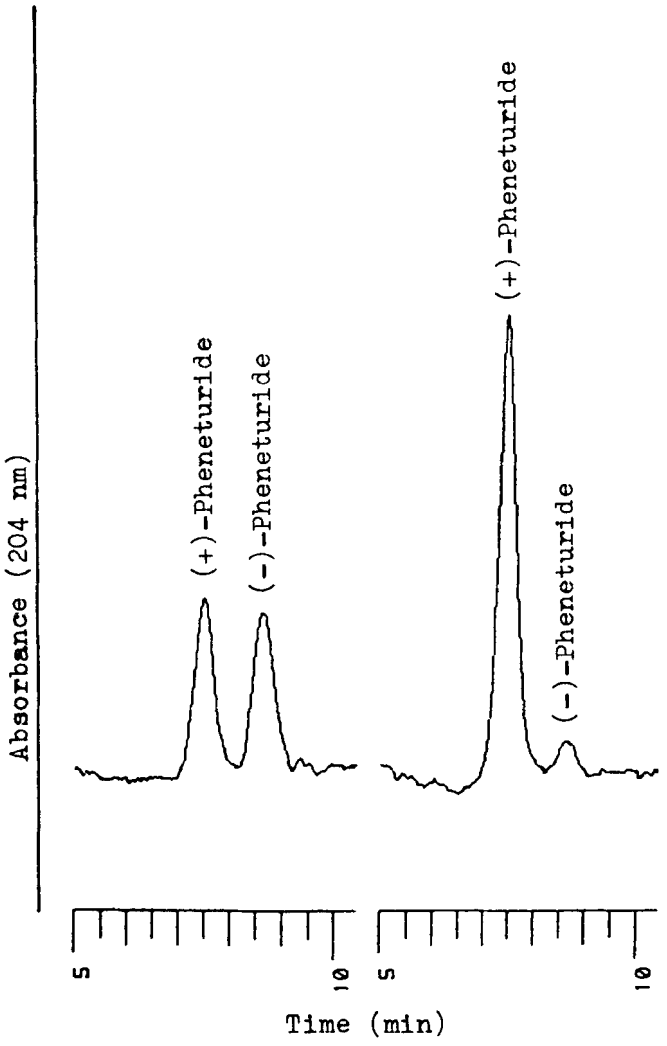


Fig. 3. Left: Chromatographic separation of racemic pheneturide (PTE) standard into its enantiomers. The standard solution was given to drug-free serum and treated as a patient serum. The concentration of (+)-PTE and (-)-PTE are 28.7 µmol/l. LC: Hewlett-Packard 1090. Column: Bakerbond Chiral Phase, DNBPG (Covalent), 250 x 4.6 mm, 5 µm. Mobile phase: 90% n-Hexane and 10% 2-propanol. Flow: 1.5 ml/min. Temperature: 35 °C.

Right: Chromatogram of (+)-PTE and (-)-PTE from pooled serum used in the calculation of the within-day variation of the method. The concentration of (+)-PTE is 75.5 µmol/l and of (-)-PTE 6.1 µmol/l.

TABLE 1

Precision for the determination of the enantiomers of pheneturide (n=12).

	Range ± S.D. (μmol/l)	C.V. (%)
(+)-Pheneturide	71.3 ± 3.0	4.2
(-)-Pheneturide	5.2 ± 0.4	7.1

The prior isolation of the racemate of PTE was done to rule out interactions from comedications and from the metabolites of PTE by the determinations of the enantiomers of PTE. The concentrations of total PTE, (+)-PTE and (-)-PTE were measured in serum from 10 patients with steady-state serum concentrations of PTE who received racemic PTE either as a monotherapy or in combination with other AEDs. The blood samples used in the study were obtained in the morning prior to the first dose of the day. The concentrations of total PTE were between 53 and 122 μmol/l (mean 80 μmol/l) with a (+)-PTE fraction between 85 and 97% (mean 94%) of total PTE. In other words, the concentration of the psychostimulant (+)-PTE in serum is around 15 times higher than that of the sedative (-)-PTE. The present report would indicate that (+)-PTE and (-)-PTE are not metabolised by the same enzyme system or that the enzyme affinity is much higher for (-)-PTE, or that (-)-PTE is converted to (+)-PTE in vivo. Based on these findings it should be investigated whether the racemic PTE is the best therapeutic combination of the enantiomers or

whether anotner concentration ratio or one of the enantiomers would give a better antiepileptic efficacy versus toxicity ratio. The issue of whether the comedication had an influence on the distribution of the fraction of (+)-PTE and (-)-PTE could not be settled with this small number of patients. Freezing and thawing of serum samples did not change the fractions of (+)-PTE and (-)-PTE. Racemic PTE is not available for clinical use in the United States and therefore has a smaller market than the other AEDs, which could influence investigations on its antiepileptic and toxic effects. Patients taking racemic PTE have two metabilites from PTE in serum as shown in Fig.2 (11). One of these was identified by means of chromatographic and mass spectrometric (MS) data to be 2-phenyl-butyric acid, which is also optically active. The concentration of this metabilite in serum is in excess of the parent compound. This metabolite has been identified in human urine and is, together with 2-(4-hydroxyphenyl)--butyroylurea the main metabolite of racemic PTE (12). Our second metabolite has a MS spectrum which has not yet been clarified and contrary to what was found in urin (12) no 4-hydroxylation of the phenyl ring could be demonstrated in serum. To our knowledge, the toxic and antiepileptic effects of these two metabolites in serum have not been investigated.

ACKNOWLEDGMENTS

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