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DETERMINATION OF SULTHIAME, TETRAHYDRO-2-*p*-SULPHAMOYL-PHENYL-2H-1,2-THIAZINE-1,1-DIOXIDE, IN PLASMA AT THERAPEUTIC CONCENTRATIONS, USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A specific and sensitive liquid chromatographic method for the determination of sulthiame in small (0.5 ml) plasma samples is described. After adding an internal standard, a direct extract of the sample is examined by reversed-phase liquid chromatography with ultraviolet spectrophotometric detection. The method is rapid, simple and capable of determining plasma levels after therapeutic ingestion of sulthiame.

INTRODUCTION

Sulthiame has been used in Europe since the early 1960's, usually in combination with other anticonvulsants and mainly for the treatment of psychomotor and grand mal seizures. Lerman and Nussbaum[1] however, also found the drug to be effective when used alone in cases of myoclonic seizures. Varying degrees of side effects have been reported: while Lerman and Nussbaum[1] found the occurrence to be infrequent and usually mild, other authors (Green and Kupferberg[2], Callaghan *et al.*[3]) encountered a high incidence of toxic effects, which were often severe. Hyperventilation was the most frequent manifestation, with paraesthesia, headaches, weight loss, ataxia, hallucinations, agitation and personality changes also occurring.

There are few reports which include sulthiame blood concentrations mainly because of the difficulty previously with analysing this compound in body fluids. Olesen[4] described a method, where the drug was measured by UV spectrophotometry after an initial separation by thin-layer chromatography, but this was time-consuming, tedious and, in our hands, gave variable results.

Several gas–liquid chromatographic (GLC) methods have been reported. Simons and Levy[5] advocated chromatography of the underivatised drug on an OV-17 column at 295° after purifying by back extraction. Unfortunately they recommended the use of an internal standard which is not commercially available. In addition, sensitivity was poor since the peaks eluted on the down slope of the solvent front and

the column absorbed some drug irreversibly. Friel *et al.*[6] found direct chromatography of sulthiame to be unsatisfactory so resorted to methylation with tetramethylammonium hydroxide. However, the limit of sensitivity for this method was only 5 mg/l and each injection took 20 min to elute. On-column methylation was also employed by Hacket and Dusci[7], but unfortunately they found it necessary to condition the column by injecting a concentrated solution of sulthiame and thioridazine (internal standard) both at the start of every batch of analyses and after every twelfth injection in order to achieve good reproducibility. In addition they required a large (2 ml) sample and lengthy purification in order to obtain a detection limit of 2 mg/l. Patsalos *et al.*[8] described a method for the determination of sulthiame in plasma, which involves a single direct extraction and subsequent concentration, but no details of reproducibility or limits of sensitivity were reported and the methylated drug chromatographed as two peaks. Flachs *et al.*[9] alluded to an enzymatic method but did not give any details.

A high-performance liquid chromatographic (HPLC) method was first reported by Riedel *et al.*[10] who separated several anticonvulsant drugs. However, their attempts left much room for improvement and the present procedure describes a rapid direct extraction of sulthiame from plasma with subsequent separation by reversed-phase liquid chromatography, and spectrophotometric detection. This method is now part of a routine service currently dealing with about 6000 anticonvulsant assay requests per year.

METHOD

Principle

Sulthiame is extracted directly from buffered plasma, to which an internal standard has been added, into dichloroethane. After concentration and reconstitution in a small amount of solvent, the extract is injected onto a liquid chromatograph and measured against a range of plasma standards, which are carried through the procedure simultaneously.

Liquid chromatography

An Applied Chromatography Systems constant flow reciprocating pump was used throughout, in conjunction with a Pye LC3 UV detector and a Bryans 2800 recorder. The column was a 10 cm × 5.5 mm I.D. stainless-steel tube which was packed with LiChrosorb RP-8 (5 μm) (HPLC Technology, Wilmslow, Great Britain). The running solvent was a mixture of 0.5 *M* acetate buffer (pH 5.8) and acetonitrile in the ratio 70:30. The system was kept at ambient temperature, the wavelength setting on the UV detector was 248 nm, and the sensitivity was set at 0.16. The pressure was 70 bar achieving a flow-rate of 1 ml/min. A septum injection technique was used throughout.

Reagents

Dichloroethane and acetonitrile (HPLC S grade) were obtained from Rathburn Chemicals (Walkerburn, Great Britain). A phosphate buffer (pH 4.5) comprising 0.5 *M* sodium dihydrogen orthophosphate in distilled water was used. The internal standard consisted of 5-ethyl-5-*p*-tolylbarbituric acid (Sigma, St. Louis, Mo., U.S.A.)

0.5 g/l in ethanol. A 0.5 *M* acetate buffer (pH 5.8) was prepared by adding 50 ml 1 *M* NaOH to 53.8 ml 1 *M* CH_3COOH and diluting to 1 l with distilled water.

Extraction procedure

Plasma (500 μl), phosphate buffer (250 μl) and 5-ethyl-5-*p*-tolylbarbituric acid (50 μl) were added to a 10-ml conical tube with a ground glass stopper. Eppendorf pipettes were used to dispense the plasma and buffer, the internal standard being added by means of a repeating Hamilton syringe. A 7-ml volume of dichloroethane was dispensed into the tube which was stoppered and then whirlimixed for 1 min. After standing for a few minutes, to allow the phases to separate, the plasma layer was discarded and the organic phase passed through a Whatman No. 1 filter paper into a clean conical tube. The solvent was evaporated to dryness using a stream of air and the residue reconstituted in 50 μl of the acetate buffer–acetonitrile solvent mixture with vigorous whirlimixing to ensure complete dissolution. A 5-μl volume of the extract was then injected onto the liquid chromatograph.

Measurement

Standard plasma solutions of sulthiame are carried through the extraction procedure with each batch of samples and these are prepared as follows. Multiples of 50 μl of a stock solution of sulthiame (0.25 g/l in ethanol) are dispensed using a repeating Hamilton syringe and diluted to 10 ml with plasma as described in Table I, giving a range of standards containing 1.25–10.0 mg/l. It was found convenient to prepare these calibration standards using reconstituted Biotrol freeze dried blank plasma (Laboratoires Biotrol, Paris, France).

TABLE I

THE COMPOSITION OF STANDARD SOLUTIONS OF SULTHIAME OBTAINED BY DILUTING AN ETHANOLIC STOCK SOLUTION (0.25 g/l) TO 10 ml WITH PLASMA

Volume of stock solution (μl)	*Concentration of sulthiame (mg/l)*
50	1.25
100	2.50
200	5.00
400	10.00

A 5-μl volume of an extract from each standard is injected onto the column and the peak heights measured from an extrapolated baseline. A calibration graph is prepared by plotting the ratio of the peak height of drug to the peak height of internal standard against sulthiame concentration (Fig. 1). The sulthiame concentration in a sample is calculated by direct comparison of the peak height ratio of the sample with the standard calibration graph.

Reproducibility

Two plasma samples were each analysed 20 times to obtain the reproducibility of the method. The results are shown in Table II.

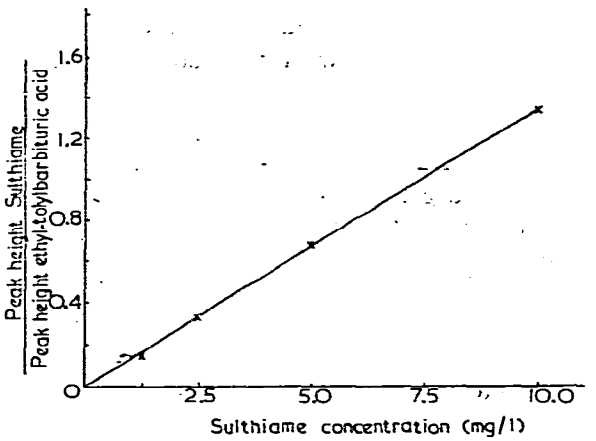

Fig. 1. Standard calibration graph relating the ratio of the peak heights of sulthiame and 5-ethyl-5-*p*-tolylbarbituric acid to the concentration of sulthiame in the extract.

TABLE II

REPRODUCIBILITY ($n = 20$) FOR ANALYSES OF SERUM POOLS

Sample No.	*Mean concentration (mg/l)*	*Standard deviation (mg/l)*	*Coefficient of variation (%)*
1	3.2	0.089	2.8
2	9.3	0.44	4.7

RESULTS AND DISCUSSION

Under the conditions described, both drug and internal standard give sharp, symmetrical peaks with baseline separation, (Fig. 2). No interfering peaks in the region of either sulthiame or the internal standard have been encountered from the constituents of normal human plasma. Other anticonvulsant drugs are extracted by this method, eluted from the column and are detected when present at therapeutic concentrations in plasma; however, they are all well separated from sulthiame. A trace obtained from a mixture of several anticonvulsants and their metabolites, together with sulthiame, is shown in Fig. 3. A problem may arise if a patient is prescribed sulthiame and carbamazepine or beclamide concurrently, in that these two drugs have almost the same retention time as tolylbarbituric acid (Table III). However, in these cases, which in our experience are not common, 10-methoxycarbamazepine (Ciba-Geigy, Basle, Switzerland) which is included in the mixture illustrated in Fig. 3, may be used as a suitable alternative internal standard. This is not the compound of choice for routine use since it has a longer retention time than tolylbarbituric acid and is not freely available. Despite this disadvantage it is currently being used in the development of a method for the determination of carbamazepine and its epoxide metabolite in plasma by liquid chromatography[11]. Fig. 4 illustrates a trace obtained from a plasma sample from a patient who was prescribed sulthiame, phenobarbitone and phenytoin concurrently.

A number of plasma samples have been analysed from patients prescribed

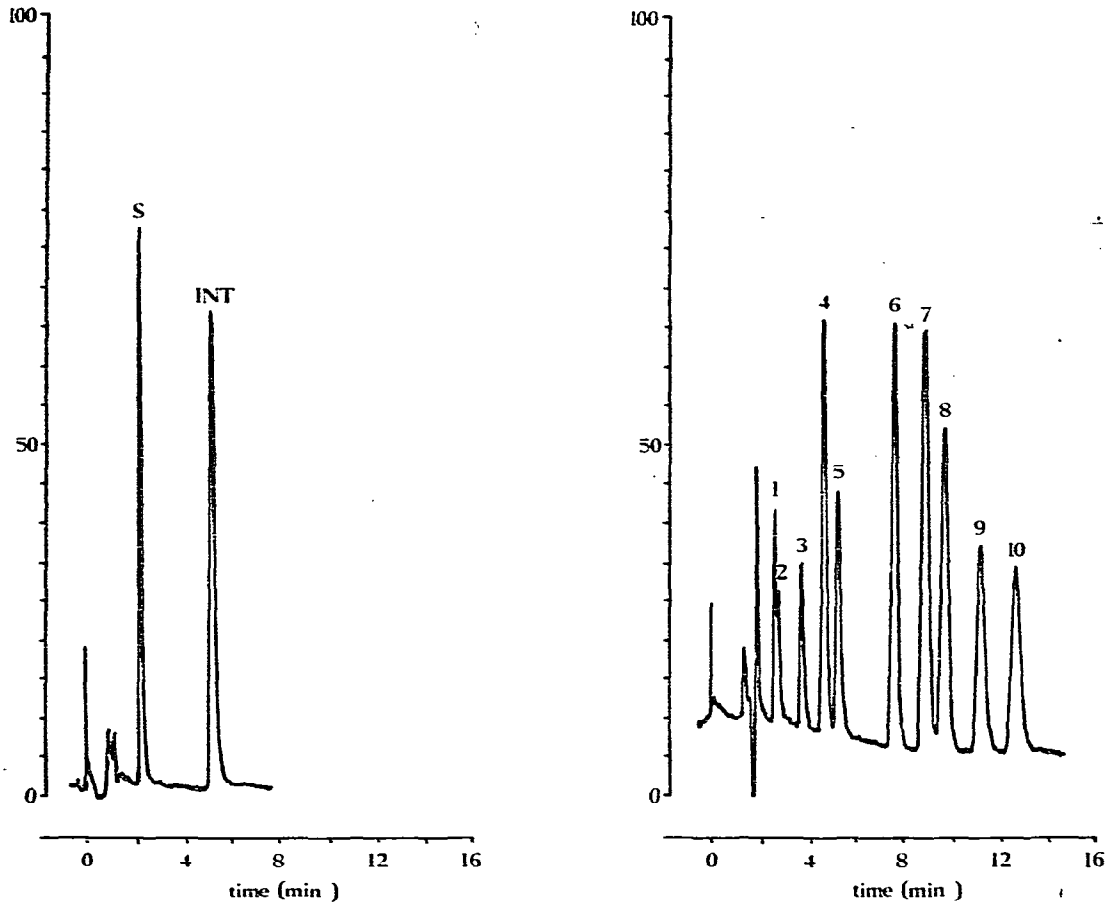

Fig. 2. Separation of sulthiame and 5-ethyl-5-*p*-tolylbarbituric acid under the chromatographic conditions described. S = Sulthiame, INT = internal standard.

Fig. 3. Separation of a mixture of several anticonvulsants with the chromatographic conditions modified to a flow-rate of 0.7 ml/min. Peaks: 1 = primidone; 2 = ethosuximide; 3 = sulthiame; 4 = carbamazepine-10,11-epoxide; 5 = phenobarbitone; 6 = beclamide; 7 = carbamazepine; 8 = pheneturide; 9 = phenytoin; 10 = 10-methoxycarbamazepine.

drugs other than anticonvulsants *e.g.*, antibiotics, antidepressants, but no interferences have arisen due to the presence of these compounds. Also, as Table III shows, acetazolamide which is structurally quite similar and occasionally used in the management of epilepsy, is well separated by the system.

The procedure described has been shown to be specific for sulthiame and is rapid, requiring only a short extraction and subsequent concentration. It is therefore ideally suited to the analysis of large numbers of samples. Since this method was introduced, we have analysed 37 samples and have obtained plasma sulthiame concentrations ranging from 0.2 to 14.6 mg/l. Sulthiame was the only anticonvulsant drug prescribed to 14% of these patients. These results are similar to those reported by Oleson[4], who found plasma sulthiame concentrations in the range 0.5–12.5 mg/l, Callaghan *et al.*[3] (2.5–18.0 mg/l) and Hackett and Dusci[7] (0.7–14.1 mg/l). Green *et al.*[12],

TABLE III
RELATIVE RETENTION TIMES OF VARIOUS ANTICONVULSANT DRUGS AND THEIR METABOLITES

Drug	*Relative retention time*
Acetazolamide	0.33
Primidone	0.37
Ethosuximide	0.38
Sulthiame	0.53
Carbamazepine-10,4-epoxide	0.64
Phenobarbitone	0.73
5-Ethyl-5-*p*-tolylbarbituric acid	1.00
Beclamide	1.05
Carbamazepine	1.23
Pheneturide	1.36
Phenytoin	1.58
10-Methoxycarbamazepine	1.78

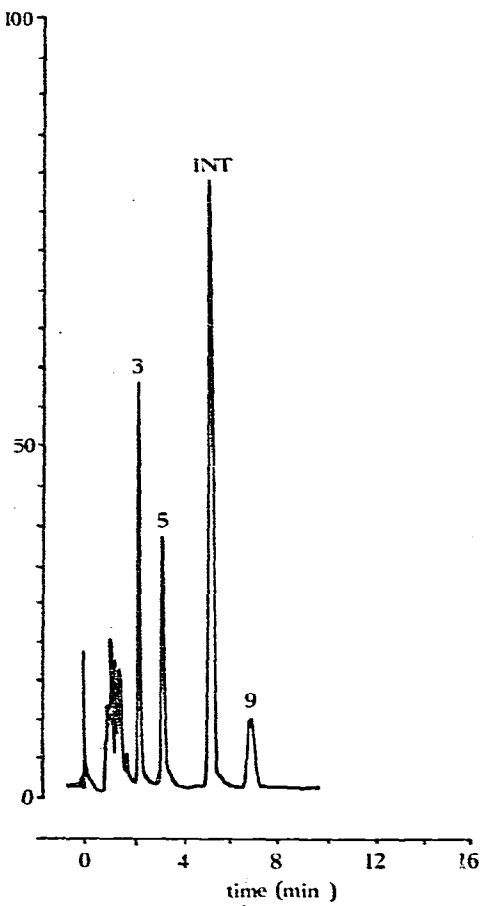

Fig. 4. Liquid chromatogram of a plasma extract from an individual treated with sulthiame in addition to phenobarbitone and phenytoin. Peaks: 3 = sulthiame; 5 = phenobarbitone; INT = internal standard; 9 = phenytoin.

however, reported plasma sulthiame concentrations ranging from 5 to 60 mg/l, although the doses of drug prescribed to their patients were generally higher than those used in the other studies.

In one of the 37 samples no drug was detected so poor compliance was suspected, and for three patients the dose of sulthiame prescribed was not known. Fig. 5 relates the dose of drug prescribed to the plasma sulthiame concentration in the remaining 33 patients. Although, in common with other studies, the plasma levels are scattered for a given dose of drug, there is a general trend towards an increasing plasma concentration with an increase in dose. Unfortunately, since the results for this study were derived from our routine workload, complete details of patients were not always provided and it was not possible to compare a weight related dose to the plasma concentration.

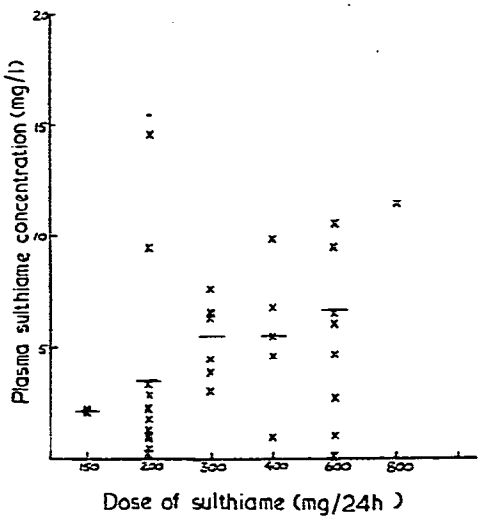

Fig. 5. Scattergram relating dose of drug prescribed to plasma sulthiame concentration in 33 patients.

Now that a simple reliable method is available for sulthiame measurement we are engaged upon an assessment of our patient workload to see whether the common side effects of this anticonvulsant are related to plasma concentration.

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