Sodium valproate is known as a useful anticonvulsant drug in primary generalized epilepsy [1]. The determination of the anticonvulsant in serum is of importance for the correct treatment of epileptic patients, especially in establishing the pharmacotherapy. Optimal therapeutic serum concentrations are known to be ca. 60 μg/ml.

Several gas chromatographic (GC) procedures have been described [2–9], each with its own advantages and limitations. A disadvantage, common to all GC procedures, is the treatment of the sample prior to chromatography. Depending on the specific procedure, solvent extraction, derivatisation and evaporation have to be used.

Isotachophoresis [10] is an electrophoretic method that can provide both qualitative and quantitative results on ionic solutes in a relatively short analysis time. The method requires no sample pretreatment and only minute amounts of sample are necessary for an accurate determination. Since valproate is an ionic solute and its therapeutic concentration level is just below the millimolar range, it is possible to determine it directly by analytical isotachophoresis.

MATERIALS AND METHODS

All chemicals were of analytical grade or additionally purified by conven-
tional methods. Sodium valproate was obtained from Labaz (Maassluis, The Netherlands). Test and reference sera were obtained from a hospital pharmacy (Apotheek Haagse Ziekenhuizen, Den Haag, The Netherlands). In addition to valproic acid the test sera contained phenobarbital, phenytoin, ethosuximide, primidone, clonazepam, carbamazepine and 10,11-epoxycarbamazepine.

Serum samples were taken from venous blood; after clotting and centrifugation the sera were stored at -20°C until use.

Gas chromatography

For the GC determinations a Packard-Becker 421 chromatograph was used. Separations were performed in a glass column, packed with 5% FFAP on Chromosorb W HP (Free Fatty Acid Phase, Chrompack, Middelburg, The Netherlands). The injection temperature was 160°C, the oven temperature was kept isothermal at 150°C. Nitrogen was the carrier gas, and flame ionization detection at 175°C was used. Cyclohexanecarboxonic acid was used as the internal standard.

Isoelectric focusing

For the isoelectric focusing separations the coupled column system developed by Everaerts et al. [11] was used. The inner diameter of the preseparation compartment was 0.8 mm. At a leading ion concentration of 0.01 M an electrical driving current of 377 μA was used. The valproate zone was trapped in the analytical column, which had an inner diameter of 0.2 mm. The electrical driving current in the analytical column was 10 μA. The electrolyte systems and other operational conditions are given in Table I.

The constant electrical driving current was taken from a modified Brandenburg power supply (Thornton Heath, Great Britain). The voltages varied between 1 and 15 kV. Serum samples were injected directly, using a microliter syringe. Separated zones were detected by measuring the electrical conductance as well as the UV absorption at 254 nm.

**TABLE I**

<table>
<thead>
<tr>
<th>ELECTROLYTE SYSTEMS AND OPERATIONAL CONDITIONS</th>
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<tbody>
<tr>
<td>Anion</td>
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<tr>
<td>Chloride</td>
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<td>Concentration (M)</td>
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<td>Counter constituent</td>
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<td>pH</td>
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<tr>
<td>Additive</td>
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<td>Current density (A/cm²)</td>
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*MES = 2-(N-morpholino)ethanesulfonic acid.
**EACA = ε-aminocaproic acid.
***Tris = tris(hydroxymethyl)aminomethane.
†HEC = hydroxyethylcellulose.
RESULTS AND DISCUSSION

One of the major advantages of isotachophoresis is that ionic solutes can often be analyzed without sample pretreatment. Therapeutic levels of valproate in serum, however, differ from the physiological chloride concentration by at least two orders of magnitude. Due to this unfavourable sampling ratio, the electrolyte system will have a rather low separation efficiency [12]. Hence, for a reliable determination, a large column volume must be available, resulting in a large analysis time. Most of these problems can be solved using a coupled column system [11]. This system not only allows the use of a high sample load, but also the use of different electrolytes (see Table 1). For the determination of valproate the concentration of the leading ion in the preseparation compartment was 0.01 M. At a high driving current serum samples were separated in approximately 6 min. The swamping amount of chloride was allowed to pass the bifurcation with the analytical column. The valproate zone was trapped in the analytical column, which contained the leading ion at a concentration of 0.005 M. Fig. 1 shows a representative result when 3 µl of a patient serum were injected. The total analysis time was less than 15 min. Since trapping was started 2 sec before the valproate zone reached the bifurcation point, some other solutes were also analyzed. The valproate zone, however, can easily be localized in both the UV trace (Fig. 1c) and the conductimetric trace (Fig. 1a). From the separation in Fig. 1 it follows that uric acid could have been used as the terminating ion, instead of morpholino-ethanesulfonic acid. As a result a

![Diagram](image)

**Fig. 1.** Isotachophoretic separation of a patient serum. UV = UV absorption at 254 nm; \(R\) = increasing resistance; \(t\) = increasing time. Injected volume: 3.0 µl of serum. a, Conductimetric trace; b, differentiated conductimetric trace; c, UV trace.
lower end-voltage would have been obtained allowing further optimization of the analysis time. For the quantitative analyses, the characteristics of the calibration line, i.e. zone length versus amount of valproic acid, were determined. The calibration points were measured with standard valproate solutions in water and in serum. Additionally several test sera, containing various other drugs, were analyzed (Fig. 2). A good linear relation was found with a correlation coefficient of 0.99914 \((n = 26)\). The response was found to be 6.12 ng/sec and the mean error per point was 3.8 ng in the detection range 50–500 ng. Re-

![Fig. 2. Calibration graph for isotachophoretic valproic acid determinations.](image)

Fig. 2. Calibration graph for isotachophoretic valproic acid determinations. (●) Standard solutions in water; (○) standard solutions in serum; (●) test sera.

![Fig. 3. Comparison of gas chromatographic and isotachophoretic results.](image)

Fig. 3. Comparison of gas chromatographic and isotachophoretic results. Abscissa: μg/ml valproic acid by gas chromatography. Ordinate: μg/ml valproic acid by isotachophoresis. (●). Patient sera, (●) test sera.
producibility was better than 2% and day-to-day variations were small. The additional other drugs did not interfere.

For the GC procedure 50 µl of the internal standard solution (500 µg/ml cyclohexanecarbonic acid in water) were mixed with 200 µl of serum. After the addition of 500 µl of carbon tetrachloride and 50 µl of 10% perchloric acid solution in water, the sample was mixed with a vortex-type mixer. The sample was then centrifuged, the upper layer was removed by suction and the interfacing layer of protein was lifted with a Pasteur pipette. A 5-µl sample of the organic layer was applied to the tip of the moving needle. After the evaporation of the organic solvent the sample was directly injected.

The GC procedure also yielded a good calibration curve, with a correlation coefficient of 0.99969. In the detection range of 2—20 ng the mean error per point was 0.1 ng. Reproducibility and day-to-day variations were better than 5%.

Using these calibration data, valproic acid concentration levels were determined in the sera of twenty patients. The isotachophoretic results were compared with the GC results. As can be seen from Fig. 3 there is a good correlation. The experimental slope deviates by only 1% from the ideal value of unity. A positive intercept of 4.9 ng of valproic acid, however, is present. The group mean was 62.1 µg/ml for the isotachophoretic determinations and 57.8 µg/ml for the GC determinations. The origin of the systematic deviation is still under investigation. The results of four different test sera (calf serum) were in good agreement with the expected values for both methods.

ACKNOWLEDGEMENTS

The authors are indebted to the analytical staff of the Kempenhaege Epileptic Institute (Heeze, The Netherlands) for the accurate GC determinations.

REFERENCES