Ion-Chromatographic Determination of Valproic Acid

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A new direct chromatographic assay method of valproic acid (2-propylpentanoic acid) using a carbonic acid solution as the eluent and conductivity detection was developed and applied to its determination in Depakene tablets and human plasma. The analytical recovery and the coefficients of variation of the method obtained by the use of drug-free plasma samples spiked with various amounts of valproic acid were ranged from 96.5 to 104% and from 3.2% at 12.4μg cm^{-3} to 0.8% at 124μg cm^{-3}, respectively. No interference from the plasma constituents or from other anticonvulsants was observed. The procedure is simple, fast, specific, and precise. The plasma levels of valproic acid obtained from patients receiving sodium valproate together with other anticonvulsants are indicated.

Valproic acid is the free-acid form of the drug marketed under the names of Valerin, Depakene and Sodium Valproate; it is clinically used as an anticonvulsant in the treatment of epilepsy.

Recently, several methods have been described in the literature for the analysis of valproic acid in plasma. These include gas chromatography (GC),[1—7] high-performance liquid chromatograpny (HPLC),[8—13] and enzyme immunoassay (EMIT).[14—17] Although a number of methods have been reported, certain problems still remain such as the assay being time-consuming, expensive, nonspecific, and/or insensitive. Three major characteristics of valproic acid which have restricted the development of an analytical method are its poor ultraviolet absorbance, its high volatility as a free acid, and its relatively high adsorptive power onto the GC column packings.[1,5] Because of this poor ultraviolet absorption, a direct sensitive assay of valproic acid by HPLC is difficult. In order to overcome this problem, derivatization to a chromophoric species, such as phenacyl,[10,13] 4-bromophenacyl[12] and 2-naphthylcarbonylmethyl esters,[11] has been necessary.

On the other hand, the GC methods are usable for the direct analysis of carboxylic acids. However, these methods are mostly limited to short-chain fatty acids. In order to improve the separation characteristics and the detection sensitivity of valproic acid, many efforts have been made.[2,5,7] More recently, Rege et al.[6] have reported that, for the GC method, the derivatization of valproic acid to the phenacyl ester was effective in its determination in plasma, although the method required extraction and evaporation steps.

The EMIT method is simple and fast, but requires some expensive reagents. However, a critical comparision of the GC and EMIT methods has been reported recently showing that, although the two methods produced no statistical differences in control samples, there was a highly significant proportional error of 26% when the same patient sample was split and analyzed by both methods.[17] The higher EMIT values for patients' samples than those with GC were probably due to the cross-reactivity of some unsaturated metabolites of valproic acid, which may interfere with the enzyme immunoassay.

In this paper, we describe a novel method for the analysis of valproic acid in drug and human plasma. Having noticed that valproic acid is a fatty acid and has the pK_a of 2.5,[18] we examined its determination by the ion-exclusion technique using a carbonic acid solution as the eluent and conductivity detection.[19] The procedure is simple, fast, specific, and precise.

Experimental

Materials. The sodium valproate was obtained from Kyowa Hakko (Tokyo, Japan). The depakene tablets were from commercial sources. All the other chemicals were of an analytical grade. A stock solution of sodium valproate was prepared by dissolving the salt in distilled and deionized water. Plasma samples were stored at −20 °C.

Chromatographic System. Analyses were performed using a Dionex M-10 Ion Chromatograph equipped with a conductivity detector. The details of the modification of the system, except for a guard and a separator column, have been described in a previous paper.[19] The guard column was made by packing the Aminex Q-150S resin, which is a high-capacity cation exchanger, into a glass column(3 mm i.d.×50 mm). On the other hand, the separator column was made by repacking the Dionex ICE separator resin[19] as a slurry into a glass column(6 mm i.d.×65 mm). The column conditioning was performed as follows. At the flow rate of 0.7 cm^3 min^{-1}, both columns were first rinsed with deionized water for 60 min, then with a 0.5 mol dm^{-3} sulfuric acid solution for 40 min, and again with deionized water for 40 min, and then finally they were equilibrated with an eluent solution.

Procedures. Drug Sample: A tablet of Depakene (200 mg as sodium valproate) was dissolved in deionized water in a volumetric flask(500 cm^3). 5 cm^3 of the solution was diluted to 100 cm^3 with deionized water. This diluted solution was injected onto the chromatograph through the filter(0.42 μm).

Plasma Sample: A plasma sample was mixed with an equivolume of a 0.1 mol dm^{-3} sodium hydroxide solution and then with eight volumes of acetonitrile by means of a Voltex-type mixer. After centrifugation for 5 min at 3000 rpm, the supernatant was diluted with an equivolume of deionized water. An aliquot of the solution was then injected onto the

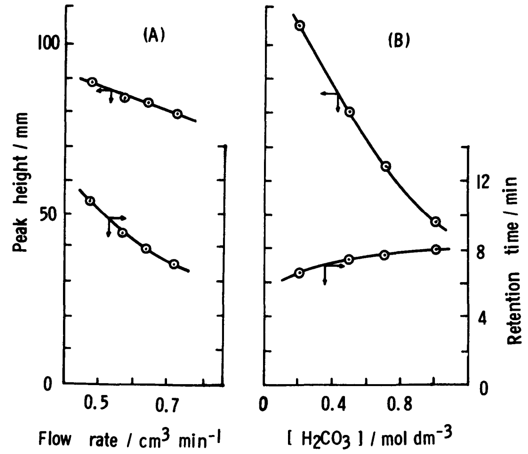

Fig. 1. Variations in the retention time and in the peak height of valproic acid with the flow rate(A) and with the concentration of carbonic acid in the eluent. All chromatographic conditions are the same as shown in Table 1 except for the flow rate(A) and the concentration of carbonic acid in eluent(B). Valproic acid: 10.0 μg cm^{-3}.

chromatograph.

Results and Discussion

1. Elution Behavior of Valproic Acid. At first, as a separator, we tried to use a Dionex ICE separator column(9 mm i.d.×200 mm), which has been used for the separation of low-molecular-weight organic acids.[19,20] However, in this case, using a 0.5 mmol dm^{-3} H_2CO_3 eluent at a flow rate of 0.7 cm^3 min^{-1}, valproic acid was eluted as a broad peak at about 60 min. After considerable exploratory experiments directed at faster elution and a more sensitive detection of valproic acid, we found that the small column described above was fit for our purposes. By the use of this small column, the elution characteristics of valproic acid were investigated. Figure 1 shows the variations in the retention time and in the peak height of valproic acid with the flow rate(A) and with the concentration of carbonic acid in the eluent(B). With the increase in the concentration of carbonic acid in the eluent, — that is, with the decrease in the pH of the eluent[19] accompanied by an increase in the background conductivity, the variation in the retention time is not very much, but the decrease in the peak height, that is, the detector response, is quite large. On the other hand, the increase in the flow rate does not appreciably affect the peak height, but makes the retention time decrease sharply. In other words, the use of a low concentration of carbonic acid in the eluent is effective for the more sensitive detection of, and the higher flow rate is effective for the faster elution of, valproic acid. Thus, the chromatographic conditions were set as shown in Table 1.

2. Valproic Acid in Drugs. The calibration curve constructed by the use of the peak height of a standard solution was linear from 0.2 to 25 μg cm^{-3}. The detection limit of the assay was 50 ng cm^{-3} of valproic acid in the sample solution. Figure 2 shows a typical chromatogram obtained from the analysis of valproic acid in a Depakene® sample solution.

Table 1. Chromatographic Conditions

Eluent	:	0.5 mmol dm^{-3} H_2CO_3
Guard column	:	3 mm i.d.×50 mm Aminex Q-150S resin column
Separator	:	6 mm i.d.×65 mm Dionex ICE separator resin column
Flow rate	:	0.7 cm^{-3} min^{-1}
Sample loop	:	50 mm^3
Meter setting	:	1 μS

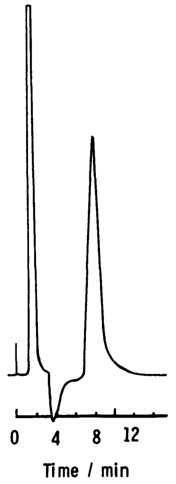

Fig. 2. Chromatogram of valproic acid in Depakene® tablet sample solution.
All chromatographic conditions are the same as shown in Table 1.

The precision and accuracy of the method were examined by analyzing valproic acid in Depakene® tablets. Against the calculated value (17.35 μg cm^{-3}) from the appended content table, the assay for three sample solutions prepared by following the procedure indicated 17.37—17.44 μg cm^{-3}. Furthermore three Depakene® sample solutions spiked with valproic acid (5 μg cm^{-3}) were also analyzed. Recoveries ranging from 99.4% to 100.2% was obtained. It was thus proven that the method was satisfactorily precise and accurate.

3. Valproic Acid in Plasma. In analyzing biological samples by the chromatographic method, it is always a great problem how to carry out deproteinization while retaining an adequate recovery of the material of interest. In order to ascertain the optimal conditions for deproteinization, a study using a drug-free plasma sample spiked with valproic acid (40 μg) was performed.

Optimization of Deproteinization Conditions. Afer considerable exploratory experiments by the use of such solutions as methanol, acetonitrile, hydrochloric

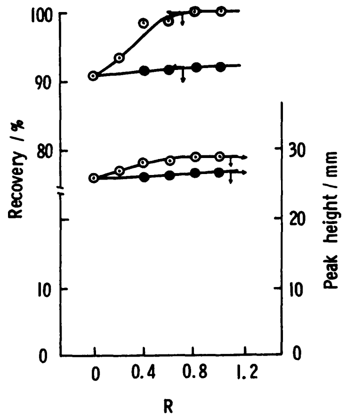

Fig. 3. Effect of acid and base on valproic acid recovery in deproteinization step.
All chromatographic conditions are the same as shown in Table 1. R: the volume ratio denoted in 0.1 mol dm^{-3} hydrochloric acid (●) and/or sodium hydroxide solution (○) to plasma.

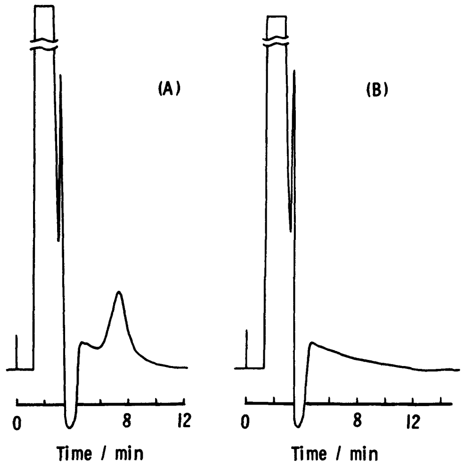

Fig. 4. Chromatograms of valproic acid in human plasma.
All chromatographic conditions are the same as shown in Table 1. (A): plasma from a patient receiving treatment with sodium valproate together with other anticonvulsants. (B): plasma from a patient receiving treatment with some anticonvulsants other than sodium valproate.

Table 2. Analytical Recovery and Precision Data of Valproic Acid in Human Plasma

Sample No.	Added μg cm^{-3}	Found μg cm^{-3} Mean±s.d.	C.V. %	Recovery %
1	12.4	12.6±0.4	3.2	102
2	17.3	16.7±0.5	3.0	96.5
3	24.8	24.3±0.4	1.6	98.0
4	34.6	34.6±0.6	1.7	100
5	52.0	54.1±0.5	0.9	104
6	86.8	87.7±0.8	0.9	101
7	121	120 ±1	0.8	99.2
8	124	126 ±1	0.8	102

acid, and sodium hydroxide and/or SEP-PAK® C_{18} cartridges (Waters Associates, Massachusetts), it was found that the complete recovery of valproic acid could be obtained by the addition of 0.1 mol dm^{-3} of a sodium hydroxide solution with a volume ratio of 0.8 or more(sodium hydroxide solution to plasma) prior to the addition of acetonitrile, as is shown in Fig. 3. In this case, the valproic acid recovery was calculated by comparing the peak height of the valproic acid obtained from the sample solution prepared by spiking with valproic acid before and after the deproteinization treatment.

Calibration Curve. The calibration curve was constructed by the use of a drug-free plasma spiked with valproic acid. The curve using the peak height was linear over the concentration range of 5—200 μg of valproic acid in 1.0 cm^3 of plasma. The detection limit of the plasma assay was 2 μg cm^{-3} of valproic acid.

Analytical Recovery and Precision. The analytical recovery and precision of the method were determined by analyzing plasma samples spiked with various known amounts of valproic acid. The results are shown in Table 2. The recovery of valproic acid was in the range from 96.5% to 104%. The coefficients of variation ranged from 3.2% at 12.4 μg cm^{-3} to 0.8% at 124 μg cm^{-3}. The procedure was found to be satisfactorily reproducible and precise.

Figure 4(A) shows a typical chromatogram obtained from the analysis of valproic acid in a plasma sample containing other anticonvulsants(primidone, ethotoin, clonazepam, and acetazolamide). Figure 4(B) also shows a chromatogram obtained by means of a plasma sample from a patient receiving treatment with primidone, ethotoin, sulthiame, and clonazepam. No interference from the plasma constituents or other anticonvulsants was observed.

Valproic-Acid Levels in Patient Plasma. The assay was performed on plasma from patients receiving sodium valproate together with various anticonvulsants, such as primidone, sulthiame, ethotoin, phenytoin, clonazepam, diazepam, acetazolamide, and carbamazepine. The results are shown in Table 3. These data indicated that the plasma levels of valproic acid were in the range of 24—74 mg dm^{-3} when daily medication was between 10—91 mg kg^{-1}. There were, however, marked variations among different patients in the relationship between drug dose and plasma level.

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Table 3. Plasma Levels of Valproic Acid from Patients Receiving Sodium Valproate

Patient	Weight	Daily medication		Time after administration	Plasma level
	kg	mg	mg kg^{-1}	h	mg dm^{-3}
1	23	2100	91	2.0	68
				15.0	39
2	21	1800	86	2.0	54
				15.0	36
3	22	1500	68	2.0	63
				15.0	51
4	37	2400	65	2.0	71
				15.0	31
5	50	1200	24	2.5	74
6	52	1200	23	4.2	60
7	54	800	15	4.0	24
8	56	800	14	4.0	24
9	57	800	14	3.5	70
10	59	600	10	3.0	34

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