[Chem. Pharm. Bull. 36(12)5012—5015(1988)]

Solvent Flush Method for Determination of Valproic Acid in Plasma by Gas Chromatography

YUKINAO KOHDA,*[,a] MASAMI MATSUI,[b] KOUJIROU YAMAMOTO,[a] KAZUYO NISHIHARA[a] and YUKIYA SAITOH[a]

Hospital Pharmacy, Faculty of Medicine, University of Tokyo,[a] Hongo, Bunkyo-ku, Tokyo 113, Japan and Tokyo Research & Applications Laboratory, Shimadzu Corporation,[b] Shibasaki, Chofu-shi, Tokyo 182, Japan

(Received May 6, 1988)

A simple method for the determination of valproic acid (VPA) by gas chromatography, which can be applied to the direct analysis of VPA in plasma without extraction, was developed. The method, the solvent flush method, requires only 1 μl of the plasma. The plasma sample was sandwiched by phosphoric acid phases, and directly injected into the gas chromatograph. The detection limit concentration of VPA using 1 μl of plasma was *ca.* 5 μg/ml.

This method made it possible to determine VPA rapidly and easily, since a few microliters of blood can easily be collected into a capillary tube and only a brief centrifugation is then required before the assay. Thus, physical damage to samll animals, such as rats, caused by frequent blood collection is minimal.

Keywords——valproic acid; solvent flush method; direct injection; gas chromatography

Valproic acid (VPA) is an effective drug in many forms of epilepsy, especially petit mal absences and generalized seizures. Many gas chromatographic methods have been published for the determination of plasma VPA concentration. In most of the published assays, no derivation is used, and the usual procedure entails only one extractive step,[1] sometimes followed by derivation.[2] However, the extraction of the acidified plasma sample with an organic solvent is time-consuming and complicated. A simple and rapid procedure is required for the routine determination of VPA. From this viewpoint, direct methods without extraction for the determination of VPA were developed.[3]

In this paper we have developed a faster and simpler method, the solvent flush method, which requires only a very small amount of sample and does not require extraction, deproteinization or injection of acid between sample injections. A 1 μl volume of the sample sandwiched by 1 μl of diluted phosphoric acid phases is directly injected into the gas chromatograph.

Experimental

Materials——Sodium valproate (VPA·Na) was supplied by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan) and was used as a standard substance. Sodium octanoate was purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan) and was of reagent grade. The reagents were used without further purification. The internal standard solution, 0.1 M sodium hydroxide containing 2 mg/ml of sodium octanoate, was prepared monthly and stored at 4 C in the dark. The rat plasma and human serum standards were prepared by adding VPA·Na to drug-free rat plasma and human serum, respectively, to give concentrations of 5 to 200 μg/ml as VPA. The standards were stored at −20 °C in the dark until analyzed.

Apparatus and Gas Liquid Chromatography (GLC) Conditions——A Shimadzu GC-7A gas chromatograph, equipped with a flame ionization detector (Kyoto, Japan), was used. The column was a 1.5 m × 3 mm i.d. coiled glass tube packed with 10% FAL-M on Shimalite TPA 60—80 mesh (Wako Pure Chemical Ind., Ltd., Osaka, Japan). The

glass insert was a 0.15 m × 3 mm i.d. straight glass tube packed with quartz wool soaked with 1.7 M phosphoric acid, and then dried with a nitrogen gas flow. The column attached with the glass insert was conditioned at 180 °C overnight with a nitrogen flow of 30 ml/min. GLC conditions were: column oven temperature, 180 °C; injection port temperature, 230 °C; detector temperature, 230 °C; nitrogen carrier gas flow rate, 50 ml/min; hydrogen flow rate, 50 ml/min; air flow rate, 500 ml/min.

Assay Procedures——The absolute calibration (AC) method was used. One microliter of 0.34 M phosphoric acid, 0.2 μl of air, 1 μl of the plasma, 0.2 μl of air, and 1 μl of 0.34 M phosphoric acid were continuously sucked up a microliter syringe of full scale 10 μl. The plasma sandwiched by the phosphoric acid phases was injected into the quartz wool in the glass insert attached to the gas chromatograph. The internal standard (IS) method was used as a control method, *i.e.*, 2 μl of internal standard solution was added to 20 μl of plasma in a disposable glass tube and the mixture was analyzed by treating it in the same way as in the AC method after vortex-mixing for 5 s.

About 50 μl samples of blood were collected from the femoral artery of a male Wistar rat intravenously administered VPA·Na, through a polyethylene cannula into heparinized glass tubes (50 μl, Drummond Scientific Co., U.S.A.) at appropriate time intervals. Plasma samples were obtained by centrifugation at 15000 × *g* for 5 min and immediately analyzed by the AC method. The rest of the plasma was analyzed by the IS method. Patients' serum samples containing VPA were obtained during routine therapeutic antiepileptic drug monitoring and analyzed by the AC method.

The peak areas of VPA and the internal standard, octanoic acid (OA), were integrated by a Shimadzu Chromatopac C-R1A data processor. The calibration curve was prepared by plotting the peak area of VPA in the AC method or the peak area ratio of VPA to OA in the IS method against the known concentrations of VPA.

Results and Discussion

Typical chromatograms are shown in Fig. 1. The retention times for VPA and OA were 5.3 and 7.8 min, respectively. The chromatograms obtained from drug-free rat plasma showed that no interference would occur owing to endogenous substances. Shimalite TPA, which is a support made of macro-crystalline terephthalic acid monomer, is suitable because it retains little water compared with one made of diatomite, when a water-rich sample is injected. However, in continuous analysis, at least 20 min intervals after injection were required to restore the column to its original state. This solvent flush method was adopted to prevent the needle tip of the microliter syringe from plugging caused by denatured plasma proteins produced by heating in the injection port, because only the phosphoric acid remained in the needle tip after injection. Repeated injections of sample caused the plasma proteins to become denatured owing to the high temperature of the quartz wool in the glass insert. Consequently, the peaks of VPA and OA slowly decreased after about 250 injections or more. However, the changes of the retention times and peak area ratio of VPA/OA were negligible. The glass insert with quartz wool soaked in phosphoric acid was renewed after about 200 injections.

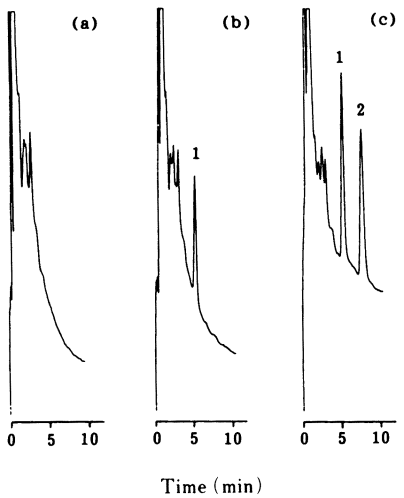

Fig. 1. Typical Chromatograms

(a) Drug-free rat plasma by the absolute calibration (AC) method; (b) plasma obtained from a rat administered VPA·Na by the AC method; (c) plasma obtained from a rat administered VPA·Na by the internal standard method. A 1 μl aliquot of plasma, sandwiched between 0.34 M phosphoric acid phases (1 μl each), was injected. Sensitivity was set at 8×10^{-10} A full scale. Peaks: 1 = valproic acid; 2 = octanoic acid (internal standard).

TABLE I. Reproducibility of Assays for Rat Plasma Spiked with Valproic Acid

Method	Spiked concentration (μg/ml)	Within-run ($n=10$)		Between-run ($n=12$)	
		Mean (μg/ml)	C.V. (%)	Mean (μg/ml)	C.V. (%)
AC[a]	5	4.3	7.4	6.2	7.9
	12.5	12.4	5.9	12.8	7.0
	50	49.5	4.2	52.3	4.7
	150	153.0	2.0	151.5	3.3
IS[b]	5	6.1	6.9	6.8	7.0
	12.5	12.7	6.0	12.2	6.9
	50	49.3	3.5	50.5	4.5
	150	150.8	1.9	149.2	2.0

a) Absolute calibration method. *b*) Internal standard method. C.V., coefficients of variation.

The analytical column has been able to withstand at least 1000 analyses.

Recoveries of VPA and OA (50 and 182 ng per 1 μl injection volume) spiked into rat plasma were 98.2 ± 3.1% and 99.8 ± 2.4% (mean ± S.D., $n=10$), respectively, relative to those spiked into water. It seems that negligible adsorption of VPA and OA occurs on the denatured protein residues in the glass insert.

As shown in Table I, the coefficients of variation for within-run precision ranged from 2.0 to 7.4% in the AC method and from 1.9 to 6.9% in the IS method, and those for between-run precision ranged from 3.3 to 7.9% in the AC method and from 2.0 to 7.0% in the IS method. No coefficient of variation for within-run or between-run precision was more than 8%, which is generally acceptable for quantitative analyses.

The calibration curves in the AC method and in the IS method were both linear in the range of 5 to 200 μg/ml in rat plasma, and approximately passed through the origin. The correlation coefficients were more than 0.99. The minimal concentration detectable for VPA was *ca.* 5 μg/ml in 1 μl of plasma. The observed values determined by the AC method were compared with those obtained with the IS method. There was good agreement between the results obtained by both methods ($y=0.967x+0.345$; $x=$IS method, $y=$AC method), and the correlation coefficient was 0.993 ($n=39$).

The AC method was also applied to the measurement of serum levels of VPA in 45 epileptic patients who were receiving VPA·Na with or without other antiepileptics or other drugs. Hemolytic, icteric and lipemic human serum, like normal serum, did not interfere with quantitative analysis. Also there was no interference from other antiepileptic drugs such as phenytoin, phenobarbital, carbamazepine, primidone and ethosuximide. The observed values were compared with those obtained with the conventional extractive method[4] used in our laboratory. There was good agreement between the results obtained by both methods ($y=0.975x+0.241$; $x=$extractive method, $y=$AC method), and the correlation coefficient was 0.988 ($n=45$).

In conclusion, this AC method made it possible for us to carry out the assay immediately after blood collection, because the assay procedure is rapid and easy, requiring no extraction or deproteinization process, and there is no requirement for column maintenance with acid between sample injections. Furthermore, the amount of sample required for the AC method is actually not more than that which is needed for injection, 1 μl. Therefore, this method makes it possible to minimize physical damage to a small animal such as a rat by frequent blood collection during the experiments, and will also be useful for the therapeutic drug monitoring of VPA for pediatric patients, since only a few microliters of blood obtained from a fingertip

or an earlobe by using a hematocrit capillary tube is sufficient.

References

1) S. I. Johannessen, *Arzneim. Forsch.*, **27**, 1083 (1977); D. J. Berry and L. A. Clarke, *J. Chromatogr.*, **156**, 301 (1978); Y. Kohda, K. Nishihara, Y. Saitoh, F. Nakagawa and Z. Tamura, *Igaku No Ayumi*, **115**, 813 (1980); K. A. Odusote and A. L. Sherwin, *Ther. Drug Monitor.*, **3**, 103 (1981); C. Manfredi and L. Zinterhofer, *Clin. Chem.*, **28**, 246 (1982); N. J. Cook and D. A. Jowett, *J. Chromatogr.*, **272**, 181 (1983).
2) A. Hulshoff and H. Roseboom, *Clin. Chim. Acta*, **93**, 9 (1979); S. C. Chan, *Clin. Chem.*, **26**, 1528 (1980); R. Nishioka, S. Kawai and S. Toyoda, *J. Chromatogr.*, **277**, 356 (1983); A. B. Rege, J. J. L. Lertora, L. E. White and W. J. George, *ibid.*, **309**, 397 (1984).
3) C. Jakobs, M. Bojasch and F. Hanefeld, *J. Chromatogr.*, **146**, 494 (1978); B. Pileire, *ibid.*, **162**, 446 (1979); R. Nishioka, M. Takeuchi, S. Kawai, M. Nakamura and K. Kondo, *ibid.*, **342**, 89 (1985).
4) Y. Kohda, "Yakubutsu Ketchunodo Sokutei No Jissai (Practice of Therapeutic Drug Monitoring)," 2nd ed., eds. by Z. Tamura and M. Horioka, Yakugyo-jihosha, Tokyo, 1984, pp. 253—267.