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VALPROIC ACID ANALYSIS IN SALIVA AND SERUM USING SELECTED ION MONITORING (ELECTRON IONIZATION) OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES

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SUMMARY

A highly sensitive ion monitoring method for the determination of valproic acid in saliva and in serum has been developed based on the gas chromatographic—mass spectrometric analysis of the *tert.*-butyldimethylsilyl derivatives. Extraction methods are simple and the techniques for derivatization are rapid and convenient. Selected ion monitoring was carried out using electron ionization conditions and a common ion m/z 201 ($M^+ - 57$) present in valproic acid and the internal standard octanoic acid. The lower limit of sensitivity that has acceptable precision for assay purposes is 0.1 mg/l based on a 200-μl sample size. The ion monitoring method (derivatized) was compared to a gas chromatographic method (underivatized) for serum valproate assays and found to be essentially identical.

The assay methodology was used in a kinetic study of valproic acid in two normal subjects. Saliva levels of drug were found to give reasonably good correlations with serum total and with serum free concentrations of drug in both individuals.

INTRODUCTION

Many methods have been described for the analysis of valproic acid, the bulk of these being by gas—liquid chromatography. The increasing popularity of therapeutic drug monitoring particularly with the anticonvulsant drugs has led to assays for valproate that are rapid and relatively easy to perform. More recent methods [1—3] generally involve solvent extraction of small volumes of acidified plasma or serum samples and direct injection without derivatization onto columns of highly polar acidified liquid phases. Sioufi et al. [1] have referenced a number of the methods described for valproate analysis since 1977. These methods are adequate for measuring therapeutic levels (50 mg/l and higher) of valproic acid and have lower limits of sensitivity in

the range of 5—20 mg/l with a few down to 1 mg/l [1,4]. Derivatization methods for valproic acid using phenacyl esters [5,6] have the potential to provide even greater sensitivity when using electron-capture detection [6]. Selected ion monitoring methods using gas chromatography—mass spectrometry (GC—MS) have recently been reported for valproic acid analysis. Chemical ionization (CI) of the free acid on a short column using paramethadione as internal standard provides specific and rapid analysis of patient samples [7]. Another CI method using the direct insertion probe with [$^{13}C_2$]valproic acid as internal standard claims sensitivity down to 0.2 mg/l [8]. Analysis of the methyl esters of [$^2H_4$]valproic acid and [$^2H_{14}$]valproic acid (internal standard) under electron ionization (EI) conditions gave good sensitivity with the calibration curve for [$^2H_4$]valproic acid in plasma extending down to 0.1 mg/l [9].

Our interest in developing a selective and sensitive assay for valproic acid stemmed from reports that the levels of valproic acid in saliva show poor correlation to either total plasma concentrations [10] or to the unbound fraction found in serum [11]. The low pK_a of valproic acid [12] may account in part for the variability observed in the ratio of saliva concentration to the free fraction in plasma. The poor correlation could also result from a lack of precision of the assays for the low levels (0.5—5.0 mg/l) of valproic acid found in saliva. We report here on a sensitive ion monitoring assay for valproic acid based on the formation of the *tert.*-butyldimethylsilyl (t-BDMS) esters. The method is rapid and would appear convenient for routine clinical use. Initial tests of the assay were made by performing a single-dose pharmacokinetic study of valproic acid in two normal volunteers. Serum levels of drug were determined by a gas chromatographic method and saliva levels by selected ion monitoring. A reasonably good correlation of the serum total to saliva concentrations and the serum free to saliva concentrations were found. Variability in the data, however, suggests special protocols for saliva sampling would be necessary in order to use saliva in a reliable manner for predicting serum total or serum free concentrations of valproic acid.

MATERIALS AND METHODS

Reagents

Valproic acid (di-*n*-propylacetic acid) was obtained from K and K Fine Chemicals, ICN Pharmaceuticals (Plainview, NY, U.S.A.). Purity of valproic acid was checked by programmed GC—MS and high-performance liquid chromatography (HPLC). Octanoic acid was obtained from Nutritional Biochemicals Corporation (Cleveland, OH, U.S.A.) and cyclohexanecarboxylic acid from Aldrich (Milwaukee, WI, U.S.A.). Stock solutions of internal standard for GC analyses were prepared in 1 *N* hydrochloric acid to contain either 33 μg/ml octanoic acid or 21.45 μg/ml of cyclohexanecarboxylic acid. The stock solution of internal standard for saliva analyses using GC—MS contained 5.0 mg/l of octanoic acid in 1 *N* hydrochloric acid. Purchase of *tert.*-butyldimethylchlorosilane—imidazole (t-BDMCS) reagent was from Applied Science Division, Milton Roy (State College, PA, U.S.A.). Solvents were distilled-in-glass grade obtained from Caledone (Georgetown, Canada).

Instrumentation

Gas chromatography was performed on a Hewlett-Packard 5830A gas chromatograph with flame ionization detector. The column was 1.8 m × 2 mm I.D. coiled glass packed with 10% SP-216-PS on 100—120 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). GC conditions were injector temperature, 200°C; detector, 300°C; oven, 135°C when cyclohexanecarboxylic acid was the internal standard or 150°C when octanoic acid was used. Carrier gas (helium) flow-rate was 40 ml/min. Retention times for valproic and octanoic acid were 3.4 and 5.6 min respectively at 150°C.

A Varian MAT 111 gas chromatograph—mass spectrometer in which the gas chromatograph was replaced with a Hewlett-Packard 5700 gas chromatograph was used for the ion monitoring assays. The mass spectrometer is interfaced to a Varian 620L computer and has been modified to allow for selected ion monitoring. Columns were glass (1.8 m × 2 mm I.D.) packed with 3% Dexsil 300 on 100—120 mesh Supelcoport and carrier gas (helium) at a flow-rate of 25 ml/min. A column packed with 3% OV-17 on 80—100 mesh Chromosorb W HP has also been used. Column temperature was 135°C; separator and inlet line temperatures were 250°C; injector temperature 250°C. The mass spectrometer was operated with an electron ionization voltage of 80 eV and a source temperature of 285°C. Trap current was 300 μA. A single ion window (*m/z* 201) was set up for monitoring by initially centering a window on *m/z* 205 of PFA-225 US (Pierce, Rockford, IL, U.S.A.). The retention times for the t-BDMS derivatives of valproic and octanoic acid on the Dexsil column were 1.8 min and 3.0 min, respectively.

Drug study

Two adult male volunteers who had fasted overnight were each administered a single 600-mg oral dose of valproic acid given in 200 ml of water with the pH adjusted to 7—8. The drug was taken on an empty stomach and food was not permitted until 3 h after dosing. Blood samples were taken during the first 4 h using an indwelling catheter (heparin locked) and thereafter by venipuncture. Saliva samples were taken coincident to blood sample collections via expectoration into clean coded scintillation vials. If food or drink had been taken, volunteers were asked to rinse the mouth thoroughly with water at least 10 min prior to sampling. Saliva production was aided by rolling a solid piece of PTFE about in the mouth like a marble. Approximately 5 ml of saliva was collected. The pH of the saliva was measured immediately following collection. Saliva and recovered serum samples were stored frozen (—20°C) until time for analysis.

Extraction and sample processing

To serum samples (200 μl) in a 1.0-ml conical shaped reaction vial fitted with a PTFE-lined cap are added 200 μl of internal standard solution and 200 μl of extraction solvent (10% ethyl acetate in *n*-hexane). The mixture is vortex mixed for 60 sec, then centrifuged for 15—20 min at 1000 *g* to ensure complete separation of the phases. An aliquot (2 μl) of the upper organic phase is injected into the gas chromatograph. For GC—MS analysis only 50 μl of serum are required. After the centrifugation step, 150 μl of the top organic

layer are transferred to a second vial and 5—7 μl of t-BDMCS reagent is added. The mixture is vortex mixed for 30 sec, centrifuged for 5 min, and 1—5 μl injected into the GC—MS system. Saliva (200 μl) samples, following centrifugation, are treated exactly as serum samples except that the strength of the internal standard, octanoic acid, is reduced to correspond to the levels of valproic acid found in saliva.

Standard curves are prepared from the addition of valproic acid to drug-free serum to provide concentrations of 20, 40, 60, 80, 100, 120, and 160 mg/l. Valproic acid stock solutions in methanol are prepared such that 0.1 ml of stock solution is made up to the 5-ml mark with serum in a volumetric flask to give the required concentration. These standards can be stored frozen and used repeatedly over a period of 4—7 weeks and still provide reproducible results. Extraction recoveries of these prepared standards were measured using a standard curve of valproic acid prepared in 10% ethyl acetate in hexane and containing a constant concentration of octanoic acid. Standard curves for saliva are prepared similarly but with concentrations of valproic acid being 0.1, 0.25, 0.5, 1.0, 1.5, 3.0, and 6.0 mg/l. The calibration curves are prepared in the usual manner by a plot of the area ratio of the valproic acid peak to that of the internal standard versus the concentration of valproic acid. A standard curve is run prior to each batch of saliva samples analyzed and should have an r^2 value of at least 0.995 before samples are analyzed. Serum samples are usually read twice by GC and saliva samples 2 to 3 times by GC—MS.

The free fraction of valproic acid in serum was determined by equilibrium dialysis. The plexiglass dialysis blocks contained five 2-ml cells separated into 1-ml compartments by cellulose dialysis membranes with a molecular weight cut-off of 10,000—12,000 (Sigma). The membranes were boiled for 1 h in distilled water and soaked in isotonic buffer solution pH 7.4 for approximately 1 h prior to use. Serum (0.5 ml) is placed into one half of the cell with 0.5 ml of isotonic phosphate buffer pH 7.4 placed into the other side of the cell. The cells are rotated in a water bath at 37°C for 4 h. Equilibration was determined to be reached within 2 h. The serum side of the cell (200 μl) is analyzed in the same manner as for total serum concentrations. The buffer side (200 μl) is analyzed by GC with the concentrations determined from standard curves (1—10 mg/l) prepared from buffer solutions to which valproic acid had been added. Recoveries of the dialysis procedure were determined by summing the concentrations of the serum and buffer sides of the cell and comparing to the total concentrations measured prior to dialysis. Percent recoveries were 90—95%.

RESULTS AND DISCUSSION

The extraction procedure used here for serum and saliva samples is very similar to other single extraction methods reported in the literature [1] and in particular to that of Peyton et al. [13]. The extraction recoveries of valproic acid from serum samples were found to be 100—105% (reported 100% [13]) over the range of concentrations used for the standard curve. Recoveries from spiked saliva samples (1 mg/l and 6 mg/l) were 100%. In our hands the best results for direct injection of the underivatized acids were obtained on the

SP-216-PS (polyester phase modified by phosphoric acid) column packing as described previously by others [1,14] for valproic acid. The method described here, using cyclohexanecarboxylic acid as internal standard is essentially the same as that used at a local hospital. Of 20 patient serum samples sent to us for comparison of results the values we obtained ranged from 85 to 103% of the reported values with a mean of 95%. Since octanoic acid is the internal standard used in the selected ion monitoring assay it is also now routinely used for GC analyses. Typical standard curves using this internal standard over the 20—160 mg/l range give mean slopes of the linear responses of 0.04111 ± 0.00063 (n = 7, over a period of 8 days) with a coefficient of variation of ±1.53%.

The choice of the *tert.*-butyldimethylsilyl derivative of valproic acid for the selected ion monitoring assay was based primarily on the increased sensitivity observed with the instrumentation available. Not having chemical ionization capabilities we found this derivatization technique to be the most suitable under electron ionization conditions. Phillipou et al. [15] reported successful application of this derivatization technique to the detection of picogram quantities of fatty acids using mass fragmentography. The t-BDMS derivatives of organic acids were also reported by De Jong et al. [16] to be superior to trimethylsilyl derivatives for GC—MS analysis of organic acid profiles. As shown in Fig. 1 the mass spectrum of this ester derivative of valproic acid gives the typical $M^+ - 57$ fragment at m/z 201 corresponding to the loss of the *tert.*-butyl radical. Our attempts to use an SP-216-PS column and monitor the m/z 102 ion of underivatized valproic acid resulted in a high

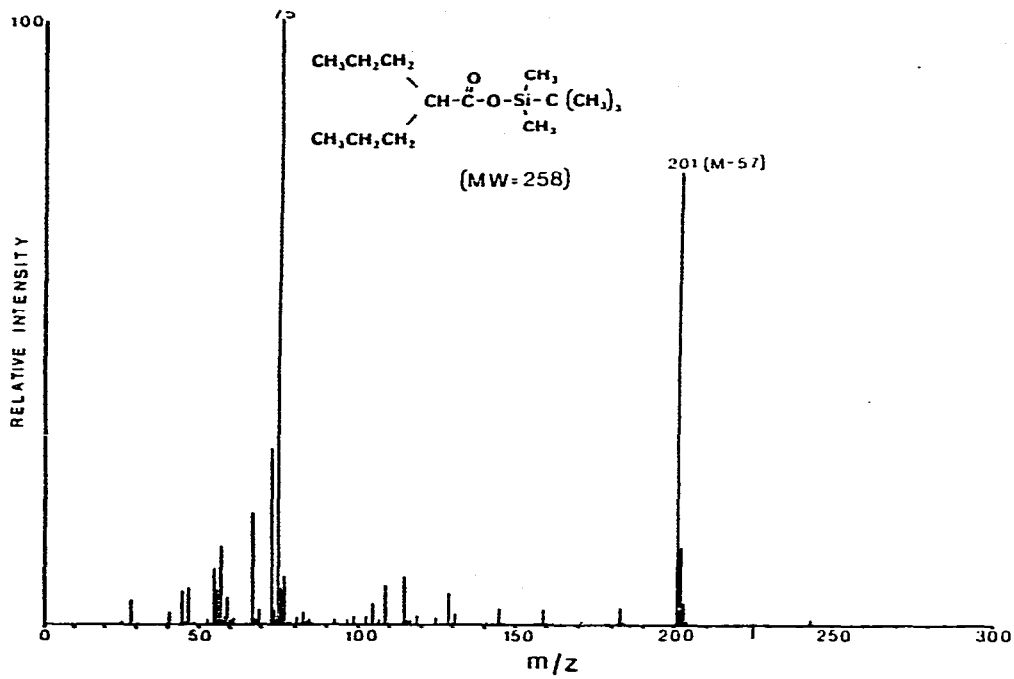

Fig. 1. Mass spectrum of the t-BDMS derivative of valproic acid obtained by GC—MS on a 3% OV-17 column.

background which was in part due to excessive column bleed. The sensitivity obtained was similar if not less than that obtained by GC with flame ionization detection. Monitoring *m/z* 87 the base peak of the methyl esters of valproic and octanoic acid produced by diazomethane esterification as described by Von Unruh et al. [9] did not produce narrow peaks when chromatographed on polar columns such as Silar 10C, nor did the sensitivity observed with the methyl esters compare with that achieved with t-BDMS esters. It was also felt that the higher mass ion at *m/z* 201 would prove more selective than monitoring the lower mass ion *m/z* 87.

Another advantage of using the t-BDMS esters for the ion monitoring analysis of valproic acid is the simplicity of the method. The derivatization procedure is a routine extension of the extraction process and samples suitable for placement in an automatic sampler are easily prepared. No evaporation step is necessary to concentrate the sample. The amount of reagent added into the organic phase containing the extracted drug and internal standard is approximately 100-fold in excess of that required to derivatize the concentrations to be found in saliva. Ester formation is rapid since longer mixing times or heating at 60°C for 10 min in a sealed container, the usual recommended method for this derivatization, did not result in peaks with greater intensity. One precaution that was necessary to observe included ensuring that none of the lower aqueous phase is transferred to the vial in which the reagent is to be added. Stability of the derivatives was not rigorously tested since derivatized samples were always run the same day on which they were prepared.

A typical ion chromatogram is shown in Fig. 2 from a saliva sample extract containing 1.0 mg/l of valproic acid. Blank saliva extracts do not show interfering peaks. At the lower limits of sensitivity (0.1 mg) an occasional background peak appears near valproic acid that can only be attributed to the reagent. Table I describes the precision data obtained using this method. Peaks below 0.1 mg/l are detected but the precision at this level is poor.

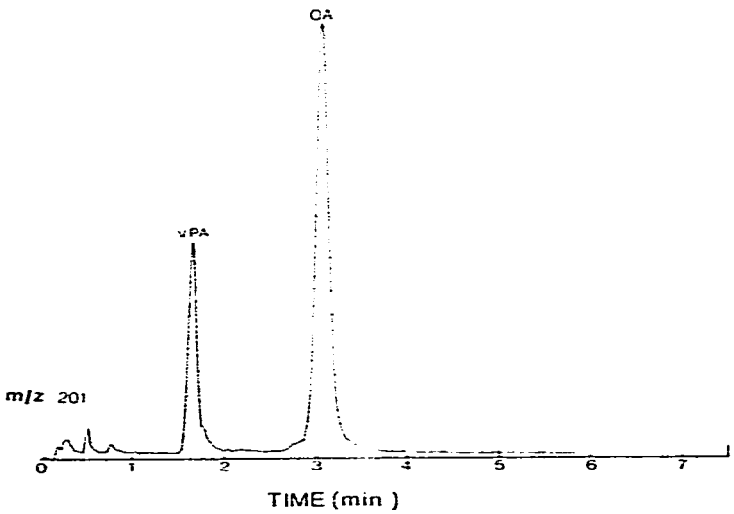

Fig. 2. Selected ion monitoring (*m/z* 201) of t-BDMS derivative of valproic acid from saliva (1.0 mg/l) on a 3% Dexsil column. OA = octanoic acid (internal standard).

TABLE I

CALIBRATION CURVE DATA OF THE t-BDMS DERIVATIVE OF VALPROIC ACID IN SALIVA

Saliva concentration (mg/l)	Peak area Ratio mean* (±R.S.D., %)	Linear regression parameters**
0.10	0.0292 (±8.5)	
0.25	0.0691 (±0.99)	$a^0 = 0.0000331$
0.50	0.1291 (±0.87)	
1.00	0.2405 (±0.87)	$a_1 = 0.240$
1.50	0.3302 (±2.0)	
3.00	0.7192 (±1.6)	$r^2 = 0.9993$
6.00	1.446 (±2.3)	

*$n = 4$.
**r^2 is the coefficient of determination, a_1 is the slope and a^0 is the intercept. Equation for the line is $y = a_1x + a^0$ where y = the peak area ratio mean and x = drug concentration.

The derivatization method using t-BDMCS reagent has been investigated for possible application to measurement by GC with flame ionization detection of valproic acid levels found in serum. A gas chromatogram is shown in Fig. 3. However, interference from a reagent peak that occurs at the same retention time as the internal standard does not permit transferring this method directly for GC use. Since the reagent peak does not appear using ion monitoring, serum samples containing valproic acid over the range of 20—100

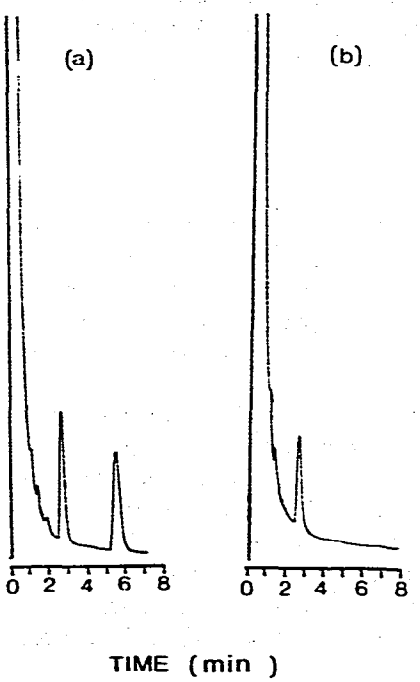

Fig. 3. Gas chromatograms of the t-BDMS derivatives of (a) valproic acid (20 mg/l) and the internal standard, octanoic acid (66 mg/l); (b) control serum extract.

TABLE II

COMPARISON OF SERUM CONCENTRATIONS OF VALPROIC ACID AS MEASURED BY GC AND GC—MS

	GC (mg/l)	GC—MS (mg/l)	
	57	60	
	60	66	
	70	70	
	82	76	
	83	85	
	85	80	
	90	87	
	100	105	
	115	118	
	120	118	
	125	130	
	128	132	
	128	133	
	133	139	
	142	141	
	150	155	
$\bar{X}$	104.25	105.94	$r^2 = 0.9846$

Paired t-test ($n = 16$), t-Stat = —1.6895, $p = 0.1118$.

mg/l give a straight-line calibration curve ($r^2 = 0.9995$) that passes through zero. A comparison of serum sample analyses (underivatized by GC and derivatized by GC—MS) is shown in Table II. The two methods do not give significantly different results and serum samples were therefore run by the more simple GC procedure.

Drug study in two volunteers

The serum valproate versus time curves for the two normal subjects studied are shown in Fig. 4. Absorption of the drug was, as expected, rapid with peak serum concentrations occurring within the first 30 min. The serum elimination—time curves in both subjects appear to be biphasic like those first described by Gugler et al. [10] for valproic acid studies in normal subjects. The start of the true terminal elimination phase occurs between 8 and 24 h following the dose.

Table III summarizes the pharmacokinetic parameters for valproic acid calculated following an assumption of a one-compartment open model. The terminal half-lives of 17.6 h and 11.8 h for subjects A and B respectively are very similar to values reported by Gugler et al. [10] (15.9 ± 2.6 h) and by Perucca et al. [17] (12.7 ± 2 h for both oral and intravenous doses).

In three of their intravenous dose subjects, Perucca et al. [17] were able to collect sufficiently frequent blood samples to manifest an apparent distribution phase in the plasma drug curve. These data were fitted to and were compatible with a two-compartment open model.

In our experiments, the sampling times were frequent enough to also demonstrate a distribution phase in both subjects after 600-mg oral doses of valproic

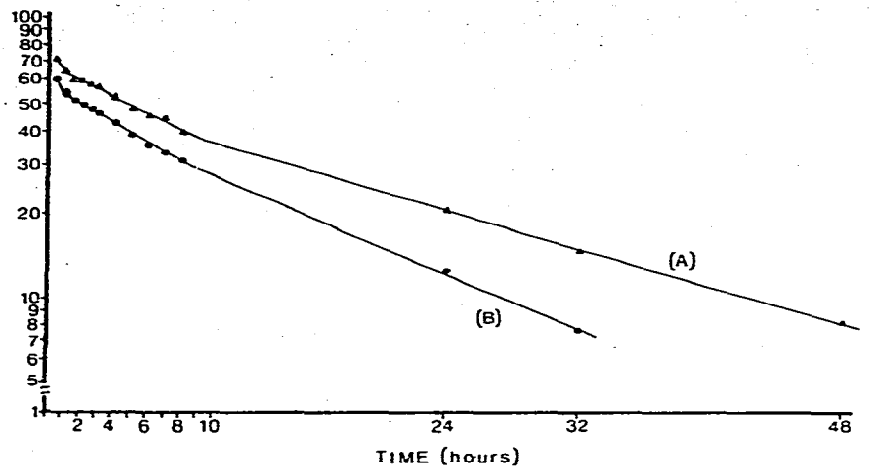

Fig. 4. Semi-logarithmic plot of serum valproate concentrations vs. time following a single 600-mg oral dose of valproate in two subjects, A and B.

TABLE III

KINETIC PARAMETERS AFTER ORAL ADMINISTRATION OF VALPROIC ACID (600 mg) IN TWO NORMAL SUBJECTS

Assumptions: $f = 1$; AUC^{*}_{∞} = (trapezoidal rule to 32 h + $\frac{Cp32}{\beta}$); $t_{1/2\beta}$ = terminal half-life; β = terminal rate constant; AUC = area under the serum concentration curve; Cl = serum drug clearance; V_D = volume of distribution; f = oral availability; Cp32 = serum VPA concentration, 32 h post administration.

Subject code	Weight (kg)	$t_{1/2(1-8)}$ (h)	$t_{1/2\beta}$ (h)	β (h^{-1})	AUC^{*}_{∞} ($\mu g \cdot h/ml$)	Cl (l/h)	Cl (l/h·kg)	$V_{D_{area}}$ (l/kg)
A	63.64	8.3	17.6	0.0394	1432.30	0.41891	0.00658	0.1672
B	97.73	7.4	11.8	0.0587	903.41	0.66415	0.00680	0.1157

acid administered as solutions. The distributive half-life was estimated to be about 7—8 h. During multiple dosing therapy, this distribution phase would tend to disappear at steady state and the plasma drug decay kinetics would then appear to obey a one-compartment open model.

Using a combination nonlinear least squares parameter estimation and fitting routine (AUTOAN) [18] the serum concentration versus time data were found to fit a two-compartment open model with first-order absorption. The derived pharmacokinetic elimination half-lives were estimated to be 14.38 h and 9.09 h for subjects A and B respectively. These values were similar to those determined by Perucca et al. [17] (6.5—10.2 h). The scatter in the data is a result of the small number of subjects studied and the known high degree of intersubject variability observed for valproic acid.

Saliva data

Table IV summarizes the concentrations of valproic acid as measured for serum and saliva samples obtained in the single-dose studies of two subjects.

TABLE IV

SALIVA CONCENTRATIONS OF VALPROIC ACID IN SINGLE-DOSE STUDIES OF TWO SUBJECTS AND THEIR RELATIONSHIPS TO SERUM TOTAL AND SERUM FREE LEVELS

Subject A						Subject B					
Time (h)	Valproic acid concentrations (mg/l)*			Saliva/ total serum ratio	Saliva/ free serum ratio	Time (h)	Valproic acid concentrations (mg/l)*			Saliva/ total serum ratio	Saliva/ free serum ratio
	Total serum	Free serum	Saliva				Total serum	Free serum	Saliva		
1.0	65.8	4.2	0.66	0.010	0.157	1.0	53.2	3.4	0.69	0.013	0.203
1.5	59.6	3.6	0.57	0.010	0.158	1.5	51.7	3.1	0.71	0.014	0.229
2.0	60.5	3.4	0.61	0.010	0.179	2.0	49.0	2.7	0.64	0.013	0.237
2.5	59.2	3.3	0.54	0.009	0.164	2.5	47.6	2.5	0.41	0.009	0.164
3.0	57.1	3.2	0.58	0.010	0.181	3.0	47.0	2.1	0.43	0.009	0.205
4.0	53.7	2.8	0.68	0.013	0.243	4.0	42.6	2.1	0.39	0.009	0.186
5.0	48.4	2.6	0.45	0.009	0.173	5.0	38.7	1.6	0.29	0.007	0.181
6.0	46.1	2.2	0.36	0.008	0.164	6.0	35.4	1.5	0.30	0.008	0.200
7.0	44.6	2.1	0.35	0.008	0.167	7.0	33.5	1.5	0.30	0.009	0.200
8.0	40.2	1.9	0.35	0.009	0.184	8.0	32.0	1.0	0.41	0.013	0.410
Mean				0.0096**	0.177***					0.0104**	0.221***
S.D.				0.0014	0.025					0.0026	0.069
C.V. (%)				14.58	14.12					24.04	31.22

*Each an average of two determinations.

**Correlation between saliva and total serum: (A) $r = 0.87066$, (B) $r = 0.81875$.

***Correlation between saliva and free serum: (A) $r = 0.83701$, (B) $r = 0.85776$.

Total serum and free serum levels obtained by equilibrium dialysis were measured using GC while saliva levels were exclusively measured using GC—MS since the concentrations found in saliva were all below the level of detection using GC. The data reported are considered to be reasonably precise, since serum total and saliva concentrations were repeated on a second occasion by other analysts and the saliva to serum ratios found were essentially unchanged. Only the 1—8 h sample times are included in the table. The 0.5-h saliva levels were exceedingly high and appeared to reflect residual valproic acid retained in the oral cavity. The 24-h saliva levels were below the level of detection even by GC—MS. The mean saliva to total serum concentration ratio was found to be practically identical in both subjects with saliva valproate concentration at 1% of total serum levels. This compares with values found previously in six subjects under steady state conditions where the mean saliva concentrations ranged from 0.7 to 2.4% of plasma concentrations [10]. Linear regression of the saliva versus serum total drug values in the two subjects gave correlations of $r = 0.87066$ and 0.81875 with a combined correlation of the 20 sample times of $r = 0.7877$. These intrasubject correlations of serum total and saliva concentrations are very comparable to results reported for two of six volunteer subjects on a multiple dose regimen [10]. Intersubject variability (saliva/serum total) is reported to be quite large ($r = 0.64$) [12] and our preliminary results of patient samples analyzed using GC—MS indicate similar results.

Comparison of saliva levels of valproic acid with the free levels of the drug found in serum gave a mean of 17.7% (saliva/serum free) in one subject and 22% in the other and correspond closely to the values found for fifteen epileptic patients [11]. While saliva levels of valproic acid are not equivalent to the free serum levels, the intrasubject correlations are reasonably strong (A, $r = 0.8370$ and B, $r = 0.8578$) and are comparable to correlations between serum total and saliva valproate concentrations. This is not surprising since serum free and serum total concentrations are highly correlated (A, $r = 0.9678$; B, $r = 0.9280$). If the serum free fractions for the two subjects are calculated the mean values (1—8 h) are fairly constant (approximately 5.0%) and interestingly both subjects show a small but gradual decline in the free fraction as the total serum levels of drug fall.

A consideration of the intrasubject variation in our study of two volunteers shows that there is, in each case, at least one elevated saliva level which contributes considerably to the variation. These erratic elevated saliva levels are apparently unrelated to saliva pH or to free levels in the serum. Repeating the regression of the data using a program that identifies outliers at the 0.05 level gave strong correlation values for both subjects. Subject A data with the 4-h value excluded gave saliva/serum total, $r = 0.97240$, C.V., 9.03%; saliva/serum free, $r = 0.97177$, C.V., 5.89%. Subject B data with the 8-h sample excluded gave saliva/serum total, $r = 0.89237$, C.V., 24.9%; saliva/serum free $r = 0.94923$, C.V., 11.33%. Spurious and inflated levels for saliva valproate have also been observed in patient studies and occur unrelated to free levels or saliva pH and thus indicate a possible facilitated transport of the drug into saliva. The occurrence of sporadic changes in saliva valproic acid concentration points out the hazard of single point saliva and blood sampling in attempting to draw con-

clusions regarding correlations. This also indicates that if saliva sampling were to be of any value, even for predicting intraindividual serum total or free levels of drug, saliva sampling protocols would need to be more sophisticated than taking a single sample.

In conclusion the assay of valproic acid using selected ion monitoring (GC—MS) of the t-BDMS esters has proved to be a highly sensitive method for precise measurement of valproic acid in either saliva or serum down to levels of 0.1 mg/l. Only 50—200 μl of sample are required and the method of derivatization is convenient and rapid. The use of this technique for valproate saliva assays in a kinetic study in two volunteers as reported here, may in part account for the good correlations of saliva/serum total or saliva/serum free that were observed. This assay method has also been used for saliva analysis in kinetic studies involving pediatric patients and comparable results were found.

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