

Transdermal Reverse Iontophoresis of Valproate: A Noninvasive Method for Therapeutic Drug Monitoring

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Purpose. The objectives of this work were (a) to explore the potential of transdermal reverse iontophoresis for therapeutic drug monitoring and (b) to develop an "internal standard" calibration procedure so as to render the technique completely noninvasive.

Methods. A series of *in vitro* iontophoresis experiments was performed in which the subdermal concentration of sodium valproate was varied from 21 μM to 1 mM. Glutamic acid was also introduced into the subdermal donor at a fixed concentration to act as an "internal standard" for the calibration method.

Results. Both valproate and glutamate anions were recovered, as expected, at the anodal receptor chamber. The iontophoretic extraction flux of valproate was linearly correlated with the subdermal concentration. Glutamate flux was constant. It follows that the ratio of extracted fluxes (valproate/glutamate) was directly dependent upon (a) the subdermal valproate concentration and (b) the subdermal concentration ratio (valproate/glutamate), offering a means, thereby, to a completely noninvasive methodology.

Conclusions. This work demonstrates the potential of reverse iontophoresis for noninvasive therapeutic monitoring. The simultaneous quantification of the analyte of interest and of an "internal standard" renders the withdrawal of a blood sample unnecessary.

KEY WORDS: iontophoresis; reverse iontophoresis; therapeutic drug monitoring; valproate; transdermal extraction.

INTRODUCTION

In general, pharmacokinetic studies and, in particular, therapeutic drug monitoring (TDM) are primarily based on blood sampling. Less invasive sampling compartments, such as urine and/or saliva, are restricted to selected drugs (1–3) and are not easily accessed in poorly cooperative populations (e.g., neonatal, pediatric, and geriatric patients). The invasive nature of venipuncture, with the associated discomfort and risks of infection, limit the frequency with which TDM and pharmacokinetic studies in "sensitive" subjects are performed (1–4). This constitutes a serious drawback for patients who are insufficiently monitored or for whom inadequate pharmacokinetic data are available (1,4–6). Thus, the development of noninvasive techniques that provide information about the plasma concentrations and kinetics of both endogenous and drug substances is of clear interest (1,3–5).

The skin offers an extensive and accessible surface, and its potential as a portal for drug sampling has been considered (7,8). This earlier research addressed the passive transdermal efflux of substances into collection patches. Other devices,

so-called "sweat patches" (developed for doping and for control of substances of abuse) operate on a similar principle (9,10) but also enable drug excretion in the sweat to provide an additional mechanism of transport. However, because passive transport across the skin is so slow, these approaches require long accumulation times before the drug can be detected in the patch, and they are clearly limited for pharmacokinetic tracking.

Transdermal iontophoresis refers to the transfer of charged and polar substances through the skin under the influence of an electric field (11). Iontophoresis is a symmetric process that transports ions across the skin in both directions. In the outward direction, endogenous ions move toward the electrode of opposite sign in accord with their concentration and electrical mobility (the electromigration mechanism). Thus, the anode extracts the ubiquitous chloride and any other anion whose physicochemical properties and concentration result in a reasonable transport number (11). At the cathode, sodium and other cations are extracted. Uncharged solutes are also transported into the cathodal chamber by convective solvent flow (the electroosmosis mechanism) (12). Concentration-dependent "reverse iontophoretic" extraction was first described in 1989 for glucose, theophylline, and clonidine (13), and the potential for noninvasive glucose monitoring was then quickly demonstrated *in vitro* (14) and then *in vivo* (15,16). The approval of the Glucowatch Biographer® by the FDA at the beginning of 2001 has validated reverse iontophoresis as a sampling technique. The Glucowatch Biographer® provides frequent, minimally invasive, and automatic glucose readings in diabetics every 20 min for 12 h. Nevertheless, the device requires a single-point calibration with a blood sample obtained via a conventional fingerstick. Lately, reverse iontophoresis has been further exploited for the monitoring of phenylalanine (17), theophylline, and caffeine (18).

The initial goal of this project was to assess the feasibility of "reverse iontophoresis" for therapeutic drug monitoring via the skin. To do so requires identification of analytes of suitable physicochemical, pharmacokinetic, and clinical characteristics, and the frequently monitored sodium valproate (19) was selected as an appropriate model. The second objective was to render the existing technique truly noninvasive, such that calibration via a blood sample would no longer be required. For this, a procedure based on the use of an "internal standard" was developed and evaluated.

MATERIALS AND METHODS

Materials

Full-thickness skin was carefully excised from pig ear obtained at the local slaughterhouse (Société d'Exploitation d'Abbatage, Annecy, France) and stored at -20°C for a maximum of 2 months until use. The number of skin donors per experiment was four to six.

N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), L-glutamic acid, and 2-propylpentanoic acid, sodium salt (sodium valproate) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). Sodium chloride was from Fluka (Buchs, Switzerland). [4,5-³H]valproic acid (55 Ci/mmol) was purchased from Moravex Biochemicals

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(Isotopchim Chimie Fine, Peyruis, France). [$^{14}\text{C}(\text{U})$]-L-Glutamic acid (282 mCi/mmol) was from NENTM Life Sciences Products, Inc. (Paris, France). All other chemicals were analytic grade. Deionized water (resistivity $\geq 18 \text{ M}\Omega \text{ cm}$) was used to prepare all solutions.

Iontophoresis Experiments

Electrode and Subdermal Solutions

Anodal and cathodal solutions consisted of 1 cm^3 of a 18 mM HEPES pH 7.4 buffer to which 75 and 133 mM NaCl had been respectively added. Two series of subdermal solutions ($\sim 6 \text{ cm}^3$) were assayed. In the high-concentration-range series, sodium valproate was added to a pH 7.4 buffer consisting of 133 mM NaCl and 18 mM HEPES. The valproate concentrations were 209, 349, 699 μM and 1.05 mM (30, 50, 100, and 150 mg/cm^3 of valproate), which span the usual therapeutic range of total (bound + free) valproate plasma levels. These donor solutions were "spiked" with the radioactive drug at $\sim 0.3 \mu\text{Ci}/\text{cm}^3$. In the low-concentration-range and internal standard series, the valproate concentrations were reduced to 10% of the above-cited values. The four levels (20.9 to 104.5 μM) tested corresponded, therefore, to the plasma concentrations of free valproate usually observed in therapeutic practice. A fixed concentration of 60 μM glutamic acid as an "internal standard" (see below) was also added to all subdermal drug/buffer solutions. These donor solutions were "spiked" with both [^3H]valproate ($\sim 0.4 \mu\text{Ci}/\text{cm}^3$) and [^{14}C]glutamate ($\sim 0.11 \mu\text{Ci}/\text{cm}^3$).

Iontophoretic Cells and Power Supply

Experiments were performed *in vitro* in vertical diffusion cells (Laboratory Glass Apparatus, Berkeley, CA) (20). The area of skin exposed in each electrode chamber was 0.78 cm^2 . Ag/AgCl electrodes were prepared in the usual manner (21), and a constant current was passed between the electrodes from a custom-built power supply (Professional Design and Development Services, Berkeley, CA) interfaced to a personal computer running Labview software (National Instruments Inc., Austin, TX). High-concentration-range experiments lasted 5 h, during which a constant direct current of 0.4 mA was passed. Low-concentration-range experiments comprised an initial iontophoretic period of 5 h at 0.4 mA followed by a second period of 19 h at 0.1 mA. Note that these two periods allow delivery of approximately the same total charge (7.2 and 6.8 C, respectively). The entire content of the anodal chamber was removed for assay at 1, 2, 3, 4, 5, and 24 h of current application and replaced by fresh buffer. The cathodal chamber was sampled at 5 and 24 h only.

Analytic Procedures

Samples from the electrode chambers were mixed with 5 cm^3 of scintillation cocktail (Ultima Gold XR, Packard Instrument S.A., Rungis, France), and the extracted amounts of valproate and glutamate were determined by liquid scintillation counting (Beckman LS 6500, Beckman Instruments France, S.A., Gagny, France). The disintegrations per minute were converted to molar flux by an appropriate mathematical transformation. The skin was digested in 5 cm^3 of Soluene 350 (Ultima Gold XR, Packard Instrument S.A., Rungis, France),

10 cm^3 of Hionic Fluor (Ultima Gold XR, Packard Instrument S.A., Rungis, France) was then added to the digested tissue, and the radioactivity present in the skin was quantified. The total radioactivity recovered from the experiment (samples + skin + donor) allowed mass balance to be performed.

The "Internal Standard" Hypothesis

In this context, an "internal standard" is defined as an endogenous substance typically found in the body at a concentration that may be considered constant. Such a steady level should result in an invariant iontophoretic extraction flux. In contrast, the iontophoretic flux (J) of the analyte of interest, in the presence of competitor co-ions, will vary as a function of its concentration in the subdermal fluids (11,22,23).

In the experiments reported here, we have used glutamate as an internal standard, having fixed its concentration (C_{glu}) at a value of 60 μM . We acknowledge that, *in vivo*, it is unreasonable to expect the concentration of glutamate to be constant. However, *in vitro*, this artificial condition can be imposed to test the hypothesis now proposed. The therapeutic range of valproate can thus be expressed as a function of the minimum and maximum subdermal ratios of concentrations $C_{\text{min}}/C_{\text{glu}}$ and $C_{\text{max}}/C_{\text{glu}}$, and the iontophoretic flux of glutamate should be constant ($J_{\text{glutamate}}$), whereas valproate fluxes should vary proportionately to the subdermal concentration. In other words, the therapeutic range can now be defined in terms of the iontophoretic flux ratios ($J_{\text{valproate-min}}/J_{\text{glutamate}}$) and ($J_{\text{valproate-max}}/J_{\text{glutamate}}$).

The internal standard hypothesis can be formulated as:

$$\frac{J_{\text{Valproate}}}{J_{\text{Glutamate}}} = K \cdot \frac{[\text{Valproate}]}{[\text{Glutamate}]} \quad (1)$$

where K is a constant. Because the internal standard concentration is fixed, it follows that

$$\frac{J_{\text{Valproate}}}{J_{\text{Glutamate}}} = K^{\#} \cdot [\text{Valproate}] \quad (2)$$

where $K^{\#} = K/[\text{glutamate}]$. A key objective of this work, therefore, was to test the validity of Eqs. (1) and (2) *in vitro*.

Statistics

Valproate fluxes exhibited unequal variance (heteroscedasticity). The square root transformation, commonly used when the group variances are proportional to the means, was used to transform the fluxes (24). Consequently, the internal standard hypothesis can be reformulated as:

$$\frac{\sqrt{J_{\text{Valproate}}}}{\sqrt{J_{\text{Glutamate}}}} = k \cdot \sqrt{\frac{[\text{Valproate}]}{[\text{Glutamate}]}} \quad (3)$$

and

$$\frac{\sqrt{J_{\text{Valproate}}}}{\sqrt{J_{\text{Glutamate}}}} = k^{\#} \cdot [\text{Valproate}] \quad (4)$$

where k and $k^{\#}$ are constants. SigmaStatTM for Windows V.2.03 and Graph Pad Prism V.3.02 were used for analyzing the data. Linear regression procedures were always followed

by the corresponding ANOVAs. All the regressions reported in this work were significant ($p < 0.001$). Glutamic acid fluxes were compared by ANOVA.

RESULTS AND DISCUSSION

Iontophoretic Extraction of Valproate

Valproic acid is a small molecule (MW = 144), negatively charged at physiologic pH ($pK_a = 4.6$). It is transported primarily by electromigration toward the anode (positive electrode). Figure 1A shows, at different sampling times, the anodal extraction fluxes of valproate for the high range of subdermal concentrations. The fluxes increased with time and had not achieved a steady value at 5 h. The factors that determine the attainment of "steady-state" iontophoretic fluxes have not been completely identified, although they have been related, for example, to the depletion of the ion reservoir in the membrane (23).

Importantly, valproate fluxes increased as a function of the subdermal drug concentration, and linear regressions between these parameters (Table I) were significant at all sampling periods; however, acceptable correlation coefficients were obtained only after the third hour of iontophoresis. These findings are consistent with previous observations (22,23). When the current is initiated, all ions present in the system compete to transport the charge. The transport number of a specific ion increases with its concentration and its electrical mobility. In our case, most of the charge is probably transported by chloride coming from beneath the skin and by sodium ions "delivered" from the anodal chamber. Valproate competes with chloride and other co-ions to carry the charge, and, under these circumstances, its transport number (i.e., its iontophoretic flux) is directly proportional to its concentration.

The subdermal levels of valproate assayed in the first series of experiments corresponded to typical total valproate plasma concentrations. However, because valproate binds strongly and saturably to albumin in this therapeutic range (19), only the free fraction of the drug is available for distribution. Microdialysis (25) has shown a good correlation be-

Table I. Regressions of the Data Shown in Fig. 1 according to the Equation $(\text{Flux})^{1/2} = k^* \cdot (\text{Concentration}) + \text{Intercept}$, where "Flux" Is the Reverse Iontophoretic Extraction Rate of Valproate ($\text{pmole} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$) and "Concentration" is the Subdermal Level of the Drug (μM).

Concentration range (μM)	Time (h)	$k^*{}^a$	Intercept ^b	r
209–1050	1	0.018	11.8	0.52
	2	0.027	23.6	0.67
	3	0.034	27.5	0.77
	4	0.038	29.6	0.87
	5	0.043	29.6	0.89
21–104.5	1	0.070	3.0	0.74
	2	0.116	5.4	0.86
	3	0.135	6.8	0.90
	4	0.151	7.6	0.92
	5	0.157	8.1	0.93
	24	0.100	5.0	0.96

^a Units are $(\text{flux})^{1/2}/(\text{concentration})$.

^b Units are $(\text{flux})^{1/2}$.

tween the valproate concentrations present in the subcutaneous extracellular fluids, the subdural cerebrospinal fluid, and the unbound plasma level. It was also shown that valproate readily distributes to the subcutaneous extracellular fluid and that the ratio of the subcutaneous extracellular concentration to the free plasma level was close to unity.

It may be reasonably expected (26) that only free valproate will be reverse iontophoretically extracted from the subcutaneous extracellular fluids through the skin. A valproate-tissue protein complex will be too large to be efficiently transported. Thus, iontophoresis should provide a noninvasive method for monitoring unbound drug concentrations.

Consequently, a second series of experiments was undertaken in which the subdermal concentration of valproate was reduced by an order of magnitude. Two protocols were considered: the first involved a relatively short sampling period at a high current (5 h at 0.4 mA); the second lasted for 19 h but used a lower current (0.1 mA). As mentioned before, these two approaches delivered approximately the same total

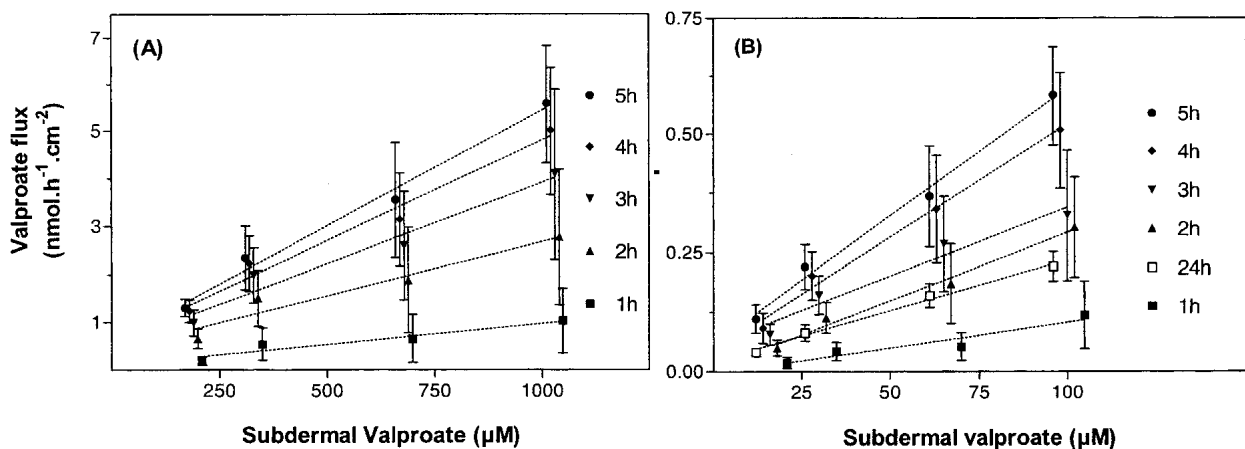


Fig. 1. Reverse iontophoretic extraction fluxes of valproate as a function of time and subdermal concentration (A) in the range 209–1050 μM , and (B) in the range 21–104.5 μM . Each data point represents the mean \pm standard deviation ($n = 6$). For clarity, the data points on the x-axis have been displaced slightly. Lines of linear regression are drawn through the data. Table I contains the regression statistics based on the square root of the flux values.

charge; however, under steady-state conditions, the extraction flux of valproate was expected to be four times higher at 0.4 mA than at 0.1 mA.

Figure 1B shows the valproate extraction fluxes measured, as a function of concentration, at different sampling times. As for the higher-concentration series, the extraction flux is proportional to the subdermal concentration (Table I), and the correlation coefficient is greater than 0.85 from the second hour of sampling. It follows, therefore, that iontophoresis is also efficient for monitoring valproate in this low concentration range.

Figure 2 demonstrates that valproate fluxes during the first sampling protocol of 5 h at 0.4 mA were not four-fold higher than those measured during the second phase (19 h at 0.1 mA). For example, the fluxes measured at 3 and at 5 h were only, on average, 1.8 and 2.7 times, respectively, those determined at 24 h. In fact, the iontophoretic extraction fluxes of valproate increased progressively with time toward a steady value, which had not yet been attained at 5 h. However, the decent correlations observed beyond the second hour suggest that valproate monitoring could nevertheless be performed at the shorter sampling times. It should also be noted that the longer time sampling protocol would also result in a satisfactory outcome with respect to reverse iontophoretic monitoring.

Finally, a pooled linear regression, including all the data obtained at the high and low concentration ranges, was performed. The correlations between the subdermal concentrations and the fluxes at different times (1–5 h) were all significant, with $r > 0.86$ from the second hour of sampling. This is a remarkable result given the wide range of concentration considered (20 μM to 1 mM). It also means that reverse iontophoresis would continue to sample valproate linearly even if the free concentration suddenly increased as a result of saturation of protein binding.

Although these results show that reverse iontophoresis is able to sample valproate in a concentration-dependent manner, an important analytic effort would be necessary in order to quantify the small extracted amounts in any practical device. It should be emphasized, however, that the approach

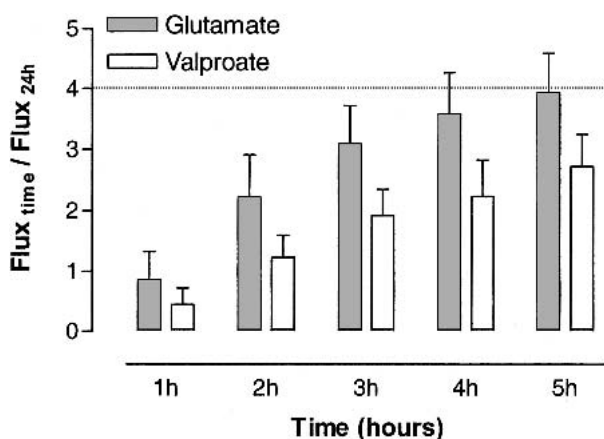


Fig. 2. Reverse iontophoretic extraction fluxes of valproate and glutamate as a function of time relative to their values at 24 h (mean + standard deviation; $n = 6$). At steady state, these ratios should achieve, under the experimental protocol used, a value of 4 (see text for details).

described has not been optimized, and higher extraction fluxes should be achievable. Furthermore, a reverse iontophoresis device for drug monitoring need not sample as frequently as that for glucose monitoring; longer sampling times (on the order of hours, for example) and “off-line” analysis and quantification would be acceptable and simpler (and less expensive) to design.

The Internal Standard Calibration

The second objective of this work was to develop a calibration procedure that would obviate the need for a blood sample. Why is such a calibration necessary? In a blood sample, the concentration of analyte therein is identical to that in the compartment sampled (i.e., the blood). On the other hand, when an iontophoretic extraction is performed, the concentration of the analyte in the “sample” is diluted with respect to the compartment from which it originated. This has two basic causes, which lie in electromigration and electroosmotic flux.

Electromigration

Iontophoretic fluxes caused by electromigration depend on the transport numbers of the ions that carry the current. The transport number of each ion is determined by its concentration and mobility (11,23,27). For the analyte of interest, the amount recovered in the collection chamber (Q moles) during a sampling period of duration T_s is given by:

$$Q = J_{\text{analyte}} \cdot T_s = \frac{t_{\text{analyte}} \cdot I \cdot T_s}{F \cdot z_{\text{analyte}}} \quad (5)$$

where J_{analyte} is the electromigration flux of the analyte, t_{analyte} and z_{analyte} are the transport number and valence, respectively, of the analyte, F is Faraday’s constant, and I the applied current. The only unknown is the transport number. However, it must be noted that a transport number is not a “dilution factor”; i.e., it is not a simple linear function of the analyte concentration (as would result from the simple dilution of a drop of blood in the receptor solution). Rather, the transport number determines the efficiency of extraction for a specific ion and, as a result, the degree with which it will concentrate in the receptor chamber. The transport numbers of the ions present in the system are defined by a complex function of their mobilities and concentrations (23,27). Furthermore, the sum of the transport numbers must equal 1, as the total charge passed between the electrodes must equal that transported by the ions through the skin. At present, there is no known algorithm to link a given ion transport number (or flux) to its concentration in the donor solution. To establish that relationship, the “donor” concentration of the ion has to be known. Although this presents no problem in an *in vitro* experiment like those described in this paper, the only approach *in vivo* to calibrate the iontophoretic extraction efficiency is via a blood sample.

Electroosmotic Flux

The electroosmotic flux across the skin during iontophoresis is the product of the convective solvent flow and the concentration of the analyte of interest (12,28). Because there is no *a priori* procedure to estimate solvent flow at any par-

ticular skin site, a blood sample is, once again, essential to calibrate the reverse iontophoretic approach.

In this study, a pair of anions was used to address the calibration problem: valproate was the analyte of interest, glutamate (MW = 147; $pK_a = 4.25$) the internal standard, which was added to the subdermal buffer at a fixed concentration of 60 μM . The latter was selected purely for analytic convenience; the issue of "practical" internal standards is discussed later. Given the physicochemical characteristics of these two anions, their iontophoretic transport is exclusively electromigrative.

To validate the internal standard calibration procedure requires three conditions to be met: (a) the extraction flux of the internal standard (glutamate) must remain constant as the analyte concentration fluctuates; (b) the extraction flux of the analyte (valproate) must vary with its subdermal concentration; and (c) the ratio of the iontophoretic fluxes (analyte/internal standard) must be proportional to their subdermal concentration ratio and, given that the internal standard concentration is constant, to the analyte concentration.

Figure 3 illustrates that the first criterion is satisfied. Glutamate extraction is unchanged as the valproate subdermal concentration is increased from 21 to 104.5 μM . These data further demonstrate that glutamate extraction had attained a steady rate by 5 h. Equally (as shown in Fig. 2), the glutamate flux at this time equals the theoretically predicted value of fourfold greater than that observed after the second, low-current protocol, at 24 h postinitiation of iontophoresis. The exact reason why glutamate reaches a steady extraction rate more quickly than valproate remains unknown but may be related to the greater lipophilicity of the drug relative to the amino acid [the calculated log (octanol-water partition coefficients) of the unionized valproic and glutamic acids are 2.9 and -3.8, respectively (<http://esc.syrres.com/interkow>)].

With respect to the second condition, it has already been shown that valproate extraction is proportional to its subdermal concentration (Fig. 3). The valproate fluxes at 5 h and the deduced transport number of the analyte increase linearly with concentration. At the same time, glutamate flux and transport number remain constant.

Figure 4 shows that the final condition is supported by the results from the very first hour of sampling; correlation coefficients for the linear dependence of the iontophoretic

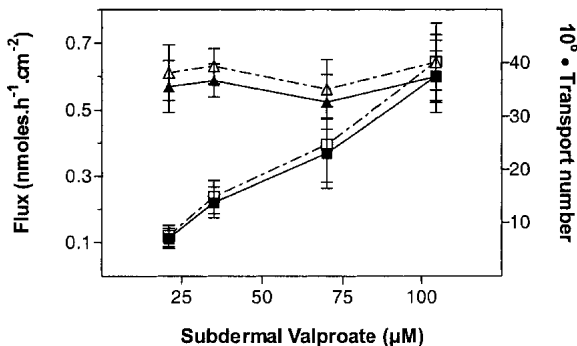


Fig. 3. Extraction fluxes after 5 h of iontophoresis of valproate and glutamate (filled squares and filled triangles, respectively) and their corresponding transport numbers (open squares and open triangles, respectively) as a function of the subdermal valproate concentration (range 21–104.5 μM). Each data point is the mean \pm standard deviation ($n = 6$).

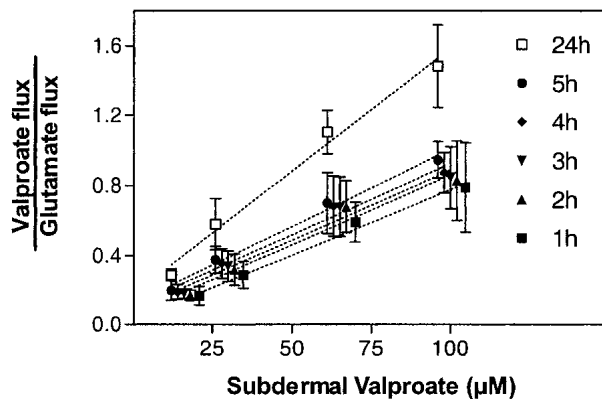


Fig. 4. Ratio of the reverse iontophoretic extraction fluxes of valproate and glutamate as a function of time and subdermal valproate concentration (range 21–104.5 μM). Each data point represents the mean \pm standard deviation ($n = 6$). For clarity, the data points on the x-axis have been displaced slightly. Lines of linear regression are drawn through the data. Table II contains the regression statistics following a square root transformation of the data.

extraction ratio (valproate/glutamate) on the subdermal valproate concentration are greater than 0.9 for all the time points (Table II). It would appear, therefore, that the internal standard calibration approach may correct for some of the variability associated with the extraction process before "steady state" is achieved. The latter contention is supported by Fig. 5, which plots the derived values of k^* (Table I) and k [defined in Eq.(3)] as a function of time. Although the values of k^* , the proportionality constant between valproate extraction flux and valproate concentration, increase progressively with time, k (which relates the ratio of extraction fluxes to the subdermal concentration ratio) is constant from the first hour of sampling.

It is also interesting to note that the value of k at 5 h (~ 0.75) at the end of the first sampling protocol increases to ~ 0.95 following the 19-h period of the second sampling protocol (Fig. 5). This confirms that the ratio of extraction fluxes directly reflects their relative concentration in the subdermal compartment and that their extraction efficiencies are quite similar (a conclusion consistent with the facts that their subdermal levels are of the same order of magnitude and that their transport numbers are alike, as confirmed by calculation—see Fig. 3). The concept of the internal standard hypothesis is well demonstrated by the valproate–glutamate couple, therefore. If glutamate was indeed present systematically at a fixed concentration of 60 μM , one could envisage

Table II. Regressions of the Data Shown in Fig. 5 according to the Equation $(\text{Flux Ratio})^{1/2} = k^{\#} \cdot (\text{Concentration}) + \text{Intercept}$, where "Flux Ratio" = $J_{\text{valproate}}/J_{\text{glutamate}}$ and "Concentration" Is the Subdermal Level of the Drug (μM)

Time (h)	$10^3 \cdot k^{\#a}$	Intercept	r
1	5.84	0.31	0.89
2	6.12	0.33	0.90
3	6.10	0.34	0.91
4	6.11	0.36	0.93
5	6.45	0.35	0.93
24	7.69	0.44	0.95

^a Units are $(\text{Concentration})^{-1}$

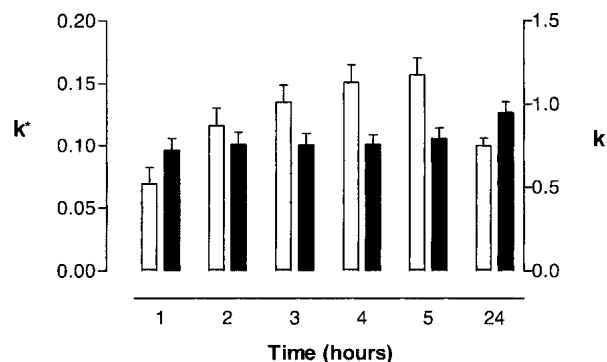


Fig. 5. Values of k^* (open bars) as shown in Table I, and k (filled bars) as defined in Eq. (3), as a function of time of iontophoresis. The mean values are shown together with the 95% confidence interval.

the development of a completely noninvasive reverse iontophoretic procedure for monitoring valproate in the therapeutic concentration range without the need for a blood sample to be withdrawn.

However, this encouraging conclusion also leads to a series of questions requiring further work. First, as glutamate concentrations are not constant under normal conditions, what might be a more appropriate, alternative internal standard for a drug such as valproate? Would an electrolyte ion, such as chloride, be practical given that, in reality, it is likely to be the major charge carrier from beneath the skin toward the anode? The mobility and concentration of Cl^- , it must be remembered, will be much greater than those for a drug like valproate, so might we expect k under such circumstances to approach an ideal value of unity? Second, do the principles established here for a pair of anions hold similarly for cations? What would be the internal standard for a cationic drug; an electrolyte like Na^+ or K^+ , perhaps? Again, is the better choice the major charge carrier (Na^+) or one that is responsible for much smaller fraction of the current flow (e.g., K^+)? Third, what about neutral analytes, such as glucose, that are transported by electroosmosis and not electromigration? Is a similarly uncharged internal standard required, or might a cation also be useful (given that convective solvent flow, under normal conditions, goes in the anode-to-cathode direction)? Finally, to what extent is k really constant? In a typical population of patients, what range of variability in k would be acceptable, or would one need to pinpoint this parameter for each individual? These and other important questions form the basis of ongoing research.

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