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A sensitive gas chromatographic/mass spectrometric method for the resolution and quantification of ethosuximide enantiomers in biological fluids

Lino Sghendo[a], Janet Mifsud[a,*], Roger Ellul-Micallef[a], Joe Portelli[a], Jeff S. Millership[b]

[a]*Department of Clinical Pharmacology and Therapeutics, The University of Malta, Msida, MSD 06, Malta*
[b]*School of Pharmacy, Medical Biology Centre, The Queen's University of Belfast, Belfast BT7 1NN, UK*

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Abstract

A modified specific, sensitive and reproducible chiral gas chromatographic (GC) method for the resolution and quantification of ethosuximide enantiomers in urine and plasma was developed. The samples were extracted by liquid–liquid extraction, using diethylether and the enantiomers were separated and quantified on a chiral gas chromatographic column (25QC2/CYDEX-β 0.25). The method involved the use of GC/MS instrumentation for the acquisition of data in the electron impact selective-ion monitoring mode, collecting ions characteristic of both ethosuximide and α,α-dimethyl-β-methylsuccinimide, the internal standard and of mass-to-charge ratio (m/z) exactly equal to 55 and 70 units. The limit of quantitation of the method was 2.5 μg/ml for both urine and plasma with both enantiomers. The method proved to be linear, precise and reproducible in the 5–300 μg/ml concentration range for urine samples and in the 10–250 μg/ml concentration range for plasma samples. Future research work envisaged the application of this method in pharmacokinetic and pharmacodynamic studies.

Keywords: Enantiomer separation; Ethosuximide

1. Introduction

Ethosuximide ($C_7H_{11}NO_2$; {*R*,*S*}-2-ethyl-2-methyl-succinimide) is a white crystalline material which is readily soluble in water and less soluble in organic solvents [1]. It possesses one chiral centre (Fig. 1) and the drug present in pharmaceutical preparations is available as the racemic mixture [2]. Ethosuximide

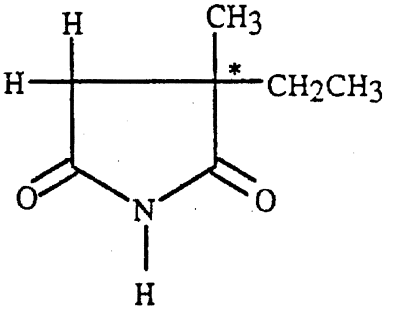

Fig. 1. Chemical structure of ethosuximide [*denotes a chiral centre].

*Corresponding author. Tel.: +356-32-902-910; fax: +356-32-0281.

E-mail address: janmif@cis.um.edu.mt (J. Mifsud).

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is an anti-epileptic drug and is indicated as first line treatment for absence seizures [3]. The internal standard most commonly used for gas chromatographic analysis of ethosuximide in biological fluids is α,α-dimethyl-β-methylsuccinimide.

For the optimisation of therapy with drugs, it is considered highly desirable to obtain knowledge of the pharmacokinetic aspects of drug action and on plasma concentration–pharmacological response relationship through pharmacodynamic modelling [4]. This knowledge may be extended to the study of the varying pharmacokinetic/pharmacodynamic properties of the individual enantiomers of chiral drugs, such as antiepileptic agents [5].

Antiepileptic drugs possess narrow therapeutic ranges and therefore, knowledge on the properties of the individual enantiomers is of great importance [6]. The pharmacodynamic profiles of a chiral drug, administered either as a racemate or as a single enantiomer, may be quite different [7]. Administration of a chiral racemic drug may lead to a complex agonist/antagonist interaction between the individual enantiomers [8]. Knowledge of the effect of chirality may help in the understanding of the idiosyncratic toxicity and efficacy of antiepileptic drugs [9].

To date, only a few studies were carried out regarding the separation and measurement of ethosuximide enantiomers in biological fluids. König successfully separated ethosuximide enantiomers using XE-60-L-valine-*R*-α-phenylethylamide and octakis 6-*O*-methyl-2,3-di-*O*-pentyl-γ-cyclodextrin as the chiral selectors in gas chromatography [10,11].

Villen et al. [12] reported the determination of the enantiomer ratios of ethosuximide in plasma samples using a fused-silica gas chromatographic column coated with Chirasil-Val III®, although separation was not baseline. Baseline separation and quantification of ethosuximide enantiomers in plasma and urine were later obtained by Mifsud and the extraction procedure devised was applied in pharmacokinetic studies in rats [13].

The aim of this study was the development of a modified specific, sensitive and reproducible chiral gas chromatographic method for the resolution and quantification of ethosuximide enantiomers in urine and plasma for future application in pharmacokinetic and pharmacodynamic studies.

2. Materials and methods

2.1. Chemicals and solutions

Chloroform, diethylether, hydrochloric acid and anhydrous sodium sulphate were all of Analar® grade (BDH, Poole, UK). *Rac*-ethosuximide was donated by Parke Davis (Ann Arbor, MI, USA) while the internal standard, α,α-dimethyl-β-methylsuccinimide and dimethoxypropane were purchased from Sigma Company (Poole, Dorset, UK).

Calibration standards for *rac*-ethosuximide were prepared at 10 different concentrations from stock solutions in 25 ml glass volumetric flasks. In urine, calibration standards were prepared at 5, 10, 20, 40, 50, 100, 150, 200, 250 and 300 μg/ml. In plasma, calibration standards were prepared at 10, 20, 30, 40, 50, 80, 100, 150, 200 and 250 μg/ml.

2.2. Equipment

The GC/MS system used in this study consisted of a quadrupole instrument with a direct capillary column interface and an electron-ionization type ion source (Shimadzu GCMS-QP5050, Kyoto, Japan). The instrument was equipped with a turbomolecular pump (Turbotronik NT 151/361, Shimadzu, Kyoto, Japan), backed up by a rotary mechanical pump (Edwards, Sussex, UK). The peaks were recorded and measured with CLASS-5000 software (Shimadzu, Kyoto, Japan) on a Funai computer. The instrument operated on both a scan mode and on a selective-ion monitoring mode. The gas chromatograph (Shimadzu, GC-17A, Kyoto, Japan) was equipped with a chiral fused-silica capillary column –25 QC2/CYDEX-β, 0.25 μm×0.22 m (I.D.)×25 m (SGE, Kiln Farm, Milton Keynes, UK), operating at a temperature range of 30 to 270 °C.

2.3. Chromatographic conditions

A 1 μl extracted urine or plasma sample was introduced in a split mode into the injection port maintained at 250 °C. Urine samples were analysed at a split ratio of 1:20, while plasma samples were

analysed at a split ratio of 1:4. The column head pressure was maintained at 15 p.s.i. using helium as carrier gas, at a purity of 99.9999%. The transfer line temperature was held at 250 °C and the oven was temperature programmed at 100 °C for 1 min and then ramped at 5 °C per min to 250 °C. A final hold time of 5 min was set.

The mass spectrometer was operated in the electron ionization/scanning mode for the preliminary qualitative work, collecting data between 30 and 450 mass units (m.u.), at 2 scans/s. For quantitative analysis, the mass spectrometer was operated in the electron ionization/selective-ion monitoring mode, collecting ions characteristic of both ethosuximide and the internal standard and of mass-to-charge ratio (m/z) exactly equal to 55 and 70 units.

2.4. Urine extraction

The urine samples were extracted using a modification of previously published procedures [10,12,13]. The urine sample (0.5 ml) was mixed with 0.1 ml 3 *M* HCl in a 58×17 mm foil-face lined, bakelite screw-capped, glass vial (BDH, Poole, UK). The internal standard (0.5 ml, 100 μg/ml) was then added. After mixing, 4 ml of diethylether were added and the contents vigorously shaken for 30 s on a vortex mixer and mixed gently for 15 min on a rotary shaker. The contents were then centrifuged at 2850 rev./min for 5 min and the ether layer was removed. The remaining aqueous layer was then extracted two more times with diethylether.

The total ether layers were then transferred to a 100×16 mm rubber-stoppered evacuated glass tube (Vacutainer®, Becton Dickinson, USA) and dried with anhydrous sodium sulphate overnight, after mixing on a rotary mixer for 5 min. Next day, the sodium sulphate was removed by filtering through a small plug of cotton wool placed in a Pasteur pipette. The sample was later evaporated under a stream of air at room temperature, for approximately 15 min, to around 250 μl. Some dimethoxypropane (200 μl) was added to the sample at the final stages of evaporation, to ensure thorough removal of water. The sample was shaken on the vortex mixer prior to injecting onto the GC column.

2.5. Plasma extraction

The extraction procedure used was a modification of the one employed by Galan-Valiente and his colleagues [14]. The internal standard solution (50 μl, 100 μg/ml) was added to 50 μl of the thawed plasma sample in a 100×16 mm rubber-stoppered evacuated glass tube (Vacutainer, Becton Dickinson, USA) together with 15 μl 3 *M* HCl and mixed. Diethylether (1 ml) was then added and the sample vortexed for 6 min. The sample was later centrifuged at 2850 rev./min for 5 min.

The upper ether layer was then transferred to a clean dry tube, excess ammonium sulphate was added using a spatula and the sample was vortex mixed for 15 min and centrifuged. The ether layer was subsequently evaporated down to approximately 50 μl under a stream of air. Some dimethoxypropane (40 μl) was added at the final stage of the evaporation step in order to ensure that the sample was thoroughly dried. The dry ether layer obtained was injected into the GC apparatus for analysis.

2.6. Peak area measurement and statistical analysis

Concentrations of the drug in the standard samples were calculated using peak area ratios. Peak area data was generated from the computer software controlling the instrument. The ratios obtained were used to construct calibration graphs. Linear regression and correlation coefficient were estimated for all curves using Sigma Plot® software. Intra-day and inter-day coefficients of variation were estimated from injections of extracted standard samples of *rac*-ethosuximide at 20 and 150 μg/ml.

Intra-day variation in analysis was determined from six consecutive injections of extracted urine samples and nine consecutive injections of extracted plasma samples. Inter-day variation in peak area measurement was determined by injecting an extracted urine sample on 6 separate days and an extracted plasma sample on 9 separate days. Recovery was estimated by adding exact quantities (18 and 120 μg/ml) of *rac*-ethosuximide to blank urine or blank plasma and extracting as above. Statistical

analysis was carried out on the data using Microsoft Excel® Software.

The precision of the analytical system was determined by the repetitive injection of the same extracted urine ($n=6$) or plasma ($n=9$) samples, spiked with *rac*-ethosuximide at 18 and 120 μg/ml, respectively and according to Eqs. (1) and (2). In these equations, the coefficient of variation represented the level of precision of the system, s represented the standard deviation of the group of values while $\bar{x}$ referred to the mean of the values. The precision of the extraction method was similarly determined but this time, repetitive extractions of the same spiked solutions (18 and 120 μg/ml, respectively) were analysed.

The accuracy of the technique was determined by the repetitive analysis of spiked urine ($n=6$) or plasma ($n=9$) samples (18 and 120 μg/ml of *rac*-ethosuximide, respectively) and according to Eq. (3). In this equation, $\bar{x}$ represented the mean of the group of values while μ referred to the true (spiked) value. The limit of detection (LOD) of the analytical method was taken to be the lowest concentration of enantiomer that could be detected but necessarily not quantitated, under the above experimental conditions. The limit of quantitation (LOQ) of the technique was equal to 3.3 times the limit of detection (Eq. (4)). Microsoft Excel® Software was again used for statistical analysis.

$$\text{Coefficient of variation (\%)} = s/\bar{x} \cdot 100\% \quad (1)$$

$$\text{Precision (\%)} = 100\% - \text{variation\%} \quad (2)$$

$$\text{Accuracy (\%)} = \bar{x}/\mu \cdot 100\% \quad (3)$$

$$\text{Limit of quantitation} = 3.3\text{LOD} \quad (4)$$

3. Results and discussion

3.1. Fragmentation data

The mass spectra of ethosuximide enantiomers were identical (Fig. 2A). The major peaks were detected at m/z 113, m/z 70 and m/z 55. A small molecular ion peak was detected at m/z 141. This was in relatively low abundance, as is frequently

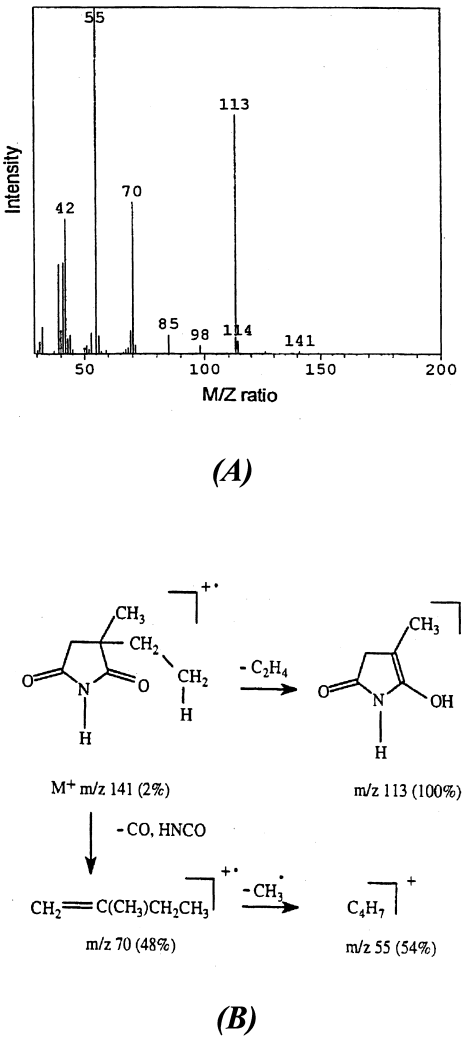

Fig. 2. (A) Mass spectrum of rac-ethosuximide. The mass spectra of ethosuximide enantiomers are identical and the major peaks are detected at m/z 113, m/z 70 and m/z 55. A small molecular ion peak is detected at m/z 141. (B) Fragmentation pattern for ethosuximide. The peak at m/z 113 occurs due to a McLafferty rearrangement in the molecule. The ethosuximide ring structure may also lose neutral molecules and radicals to produce peaks at m/z 70 and m/z 55, respectively.

observed for succinimide derivatives. The peak at m/z 113 occurred due to a McLafferty rearrangement in the molecule. The ethosuximide ring structure also broke down to produce ring opening fragments, with the loss of neutral molecules, CO and HNCO and resulting in a radical ion–methyl propenyl alkene ion (m/z 70). The ion formed subsequently lost a methyl

radical, probably the terminal methyl group of the ethyl moieties (Fig. 2B).

3.2. Calibration analysis

The resolution of ethosuximide enantiomers by the chiral gas chromatographic column was baseline (Fig. 3A). (*S*)-ethosuximide, the first enantiomer eluting from the column [13] had a retention time of 14.00 min, while (*R*)-ethosuximide had a retention time of 14.30 min. α,α-Dimethyl-β-methylsuccinimide, the internal standard, had a retention time of 13.60 min. The column retained its ability to separate the enantiomers throughout all the study. The detector response was somewhat higher at *m*/*z* 55 than at *m*/*z* 70 (Fig. 3B) and ethosuximide peaks were larger, compared to the internal standard peak, at *m*/*z* 55. However, at *m*/*z* 70, the baseline was somewhat more stable. The peak shape was symmetrical and adequate for peak area measurements.

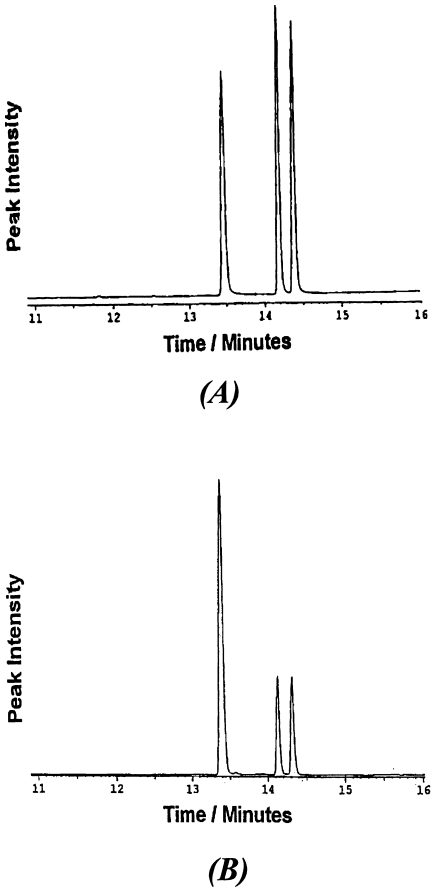

Fig. 3. Chromatogram for urine samples spiked with 100 μg/ml *rac*-ethosuximide at (A) *m*/*z* 55 and (B) *m*/*z* 70. α,α-dimethyl-β-methylsuccinimide (13.60 min), (*S*)-ethosuximide (14.00 min) and (*R*)-ethosuximide (14.30 min). A CYDEX-β GC column was used with the following conditions: (1) Carrier gas: Helium: 15 p.s.i.; Injector temperature: 250 °C; Detector temperature: 250 °C. The oven was set at 100 °C for 1 min and then ramped at 5 °C per min up to 230 °C for the duration of the run. The split/splitless injector was set at a split mode (1:20) with an injection size of 1 μl.

The calibration graphs constructed at both *m*/*z* 55 and *m*/*z* 70, for both urine and plasma samples were linear and the curves nearly passed through the origin. Linear regressions and correlation coefficients were acceptable for both (*R*)- and (*S*)-enantiomers. At *m*/*z* 55, r^2 for urine samples was 0.999, while for plasma samples it was 0.997 (Fig. 4A). At *m*/*z* 70, r^2 for urine samples was 0.994 while for plasma samples it was 0.991 (Fig. 4B). The calibration graphs obtained were described by the following equations:

1. $y = 0.013x + 0.03$ (*S*)-;
   $y = 0.013x + 0.03$ (*R*)- (urine, *m*/*z* 55)

2. $y = 0.005x + 0.01$ (*S*)-;
   $y = 0.005x + 0.008$ (*R*)- (urine, *m*/*z* 70)

3. $y = 0.014x + 0.01$ (*S*)-;
   $y = 0.014x + 0.013$ (*R*)- (plasma, *m*/*z* 55)

4. $y = 0.005x + 0.006$ (*S*)-;
   $y = 0.005x + 0.006$ (*R*)- (plasma, *m*/*z* 70)

where *y* was the peak area ratio while *x* was the enantiomer concentration. Regression analysis confirmed that the levels of ethosuximide enantiomers in the calibration standards were within the linear range of the instrument.

3.3. Intra-day/inter-day variation

In urine samples, the intra-day coefficient of variation ranged between 0.97 and 3.89%, while the inter-day coefficient was in the limits of 1.41 and 6.17% (Table 1). In plasma samples, intra-day

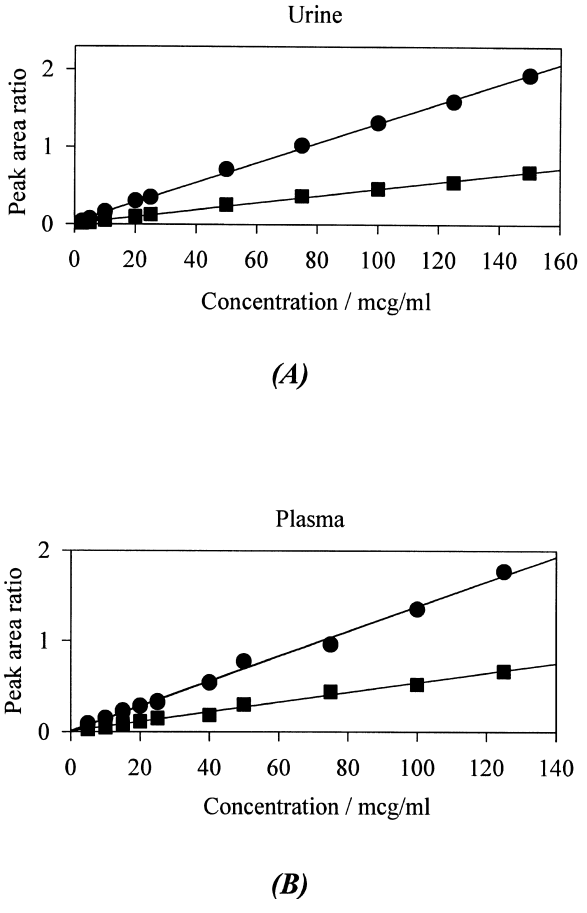

Fig. 4. Typical calibration graphs for (a) urine samples and (b) plasma samples at m/z 55 (●) and m/z 70 (■). (Statistical analysis: Data points = 10; Standard Deviation: Slope = 0.03–0.04, Intercept = 0.001–0.004; Standard Error of the Mean: Slope = 0.01, Intercept = 0.0002–0.001).

variation ranged from 1.81 to 8.24%, while inter-day variation was never lower than 2.09% and never higher than 9.42% (Table 2). Overall, the intra-day and inter-day coefficients of variation were low and less than 10%. Intra-day variation for urine samples at m/z 55 and m/z 70 were similar, although variation at 20 μg/ml of *rac*-ethosuximide was slightly higher than at 150 μg/ml. Inter-day variation for urine samples was higher than intra-day variation and more pronounced at m/z 70 than at m/z 55. In inter-day analysis, variation at 20 μg/ml was again higher than at 150 μg/ml.

Intra-day variation of plasma samples at m/z 55 and m/z 70 was nearly always higher than variation in urine. A similar trend occurred in this case, with higher variation at m/z 70 than at m/z 55 and higher at 20 μg/ml of *rac*-ethosuximide than at 150 μg/ml. Inter-day coefficients of variation were the highest in this study, although still relatively low (<10%). In fact, inter-day variation at 150 μg/ml was in the same range as variation at the same concentration in urine (≈2%). Only the coefficients at 20 μg/ml varied to a considerable extent from urine to plasma and from intra-day to inter-day analysis.

3.4. Recovery

In urine samples, recovery was high and in the range of 93.79 to 103.57%, while the coefficient of variation ranged between 0.59 and 3.83%. In plasma samples, recovery was again high and ranging from 95.30 to 100.42%, while the coefficient of variation was never lower than 0.52% and never higher than 4.58% (Table 3). The results suggested that the recovery rates of ethosuximide enantiomers from biological fluids were very high and within the 93–104% range. The coefficients of variation, although low, were usually higher at m/z 70 and at 18 μg/ml of *rac*-ethosuximide in both plasma and urine. The only exception occurred in plasma at m/z 55 and for the (*S*)-enantiomer, where variation was higher at 120 μg/ml. The highest variation in recovery data was obtained in plasma, at 18 μg/ml of *rac*-ethosuximide and m/z 70, where variation exceeded 4.5%. All in all, the extraction procedure used in the study seemed reliable and reproducible.

3.5. Other validation parameters

In urine samples, precision was high and in the range of 95.40 to 99.43%, while accuracy ranged between 94.00 and 103.57%. In plasma samples, precision was again high and in the limits of 95.71 to 99.52%, while accuracy was higher than 95.33% but lower than 100.44% (Table 4). The limit of detection and the limit of quantitation of the analytical technique were similar for both urine and plasma samples, at both m/z 55 and m/z 70 and were equal to 0.83 and 2.5 μg/ml of the enantiomers, respectively. These results suggested that both precision and accuracy of the analytical method were very high

Table 1
Intra-day and inter-day variation for urine samples at m/z 55 and m/z 70

Descriptive statistics ($n=6$)	Urine m/z 55				Urine m/z 70			
	10 μg/ml		75 μg/ml		10 μg/ml		75 μg/ml	
	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-
Variation[a] (%)	3.50	3.89	0.97	2.17	3.78	1.78	1.10	1.29
Std. Dev.[c]	0.39	0.43	0.78	1.76	0.41	0.19	0.84	0.98
Variation[b] (%)	4.60	4.57	1.41	2.87	5.84	6.17	2.06	2.04
Std. Dev.	0.51	0.50	1.10	2.23	0.66	0.70	1.57	1.57

Variation was measured at concentrations of 20 and 150 μg/ml of *rac*-ethosuximide. The symbols (*S*)- and (*R*)- refer to the enantiomers of ethosuximide. (Results: m/z 55: Intra-day variation = 0.97–3.89% [$s=0.39$–1.76], Inter-day variation = 1.41–4.60% [$s=0.50$–2.23]; m/z 70: Intra-day variation = 1.10–3.78% [$s=0.19$–0.98], Inter-day variation = 2.04–6.17% [$s=0.66$–1.57]). The symbol *s* refers to the standard deviation of the values from the mean.

[a] Intra-day coefficient of variation (%).
[b] Inter-day coefficient of variation (%).
[c] Standard deviation.

and acceptable, while the limit of detection and the limit of quantitation were low and adequate for the measurement of low concentrations of ethosuximide enantiomers in biological fluids.

4. Further discussion

A modified extraction procedure and chiral gas chromatographic technique of high specificity and sensitivity, for the resolution and quantification of the enantiomers of ethosuximide in biological fluids is thus reported. The internal standard peak did not co-elute with the enantiomer's peaks or with peaks generated by any other compound co-extracted from the samples. The retention times obtained in this study (14.00 and 14.30 min) were relatively shorter than the times that had been previously reported with other successful chiral gas chromatographic separations for ethosuximide (16.94 and 17.14 min) [13]. On the other hand, calibration analysis, intra-day/

Table 2
Intra-day and inter-day variation for plasma samples at m/z 55 and m/z 70

Descriptive statistics ($n=9$)	Plasma m/z 55				Plasma m/z 70			
	10 μg/ml		75 μg/ml		10 μg/ml		75 μg/ml	
	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-
Variation[a] (%)	4.36	5.98	2.62	1.81	7.26	8.24	1.99	2.02
Std. Dev.[c]	0.45	0.60	1.89	1.32	0.72	0.82	1.59	1.62
Variation[b] (%)	7.46	5.98	3.26	2.44	9.03	9.42	2.09	2.13
Std. Dev.	0.78	0.61	2.34	3.18	0.90	0.95	1.67	1.71

Variation was measured at concentrations of 20 and 150 μg/ml of *rac*-ethosuximide. (Results: m/z 55: Intra-day variation = 1.81–5.98% [$s=0.45$–1.89], Inter-day variation = 2.44–7.46% [$s=0.61$–3.18]; m/z 70: Intra-day variation = 1.99–8.24% [$s=0.72$–1.62], Inter-day variation = 2.09–9.42% [$s=0.90$–1.71]).

[a] Intra-day coefficient of variation (%).
[b] Inter-day coefficient of variation (%).
[c] Standard deviation.

Table 3
Recovery data for urine and plasma samples at *m/z* 55 and *m/z* 70

Descriptive statistics ($n=6$); ($n=9$)	Urine/plasma *m/z* 55				Urine/plasma *m/z* 70			
	9 μg/ml		60 μg/ml		9 μg/ml		60 μg/ml	
	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-
Recovery[a]	95.54	93.79	101.84	102.46	98.80	99.26	103.48	103.57
Variation[a]	1.51	1.81	0.69	0.59	2.53	3.83	1.16	1.16
Recovery[b]	100.42	100.19	98.10	98.74	95.30	96.01	96.04	96.05
Variation[b]	0.90	1.80	1.43	1.01	4.51	4.58	0.73	0.52

Recovery was measured at concentrations of 18 and 120 μg/ml of *rac*-ethosuximide. (Results: Urine samples, *m/z* 55: Recovery = 93.79–102.46% [variation = 0.59–1.81%], *m/z* 70: Recovery = 98.80–103.57% [variation = 1.16–3.83%]; Plasma samples, *m/z* 55: Recovery = 98.10–100.42% [variation = 0.90–1.80%], *m/z* 70: Recovery = 95.30–96.05% [variation = 0.52–4.58%]).

[a] Recovery and coefficient of variation for urine samples (%).

[b] Recovery and coefficient of variation for plasma samples (%).

inter-day variation and recovery data obtained was similar to that observed by other researchers [13].

The extraction procedure utilised diethylether, which with its greater volatility, compared to other solvents previously used, helped to minimise ethosuximide losses at the evaporation stage. This procedure also incorporated the use of dimethoxypropane to aid removal of water, enabling the CYDEX-β column to retain its high efficiency and therefore maintain baseline resolution of the enantiomers. The use of certain previously reported extraction methods led to reduced resolution of the ethosuximide enantiomers possibly resulting from the interaction of moisture from the samples with the stationary phase. The extraction procedure was relatively quick and the samples required no tedious derivatization steps prior to analysis.

5. Theory

5.1. Chiral selectivity

Capillary columns having a chiral stationary phase are used for the separation of optically active isomers or enantiomers, namely species that have the same

Table 4
Precision and accuracy data for urine and plasma samples at *m/z* 55 and *m/z* 70

Descriptive statistics ($n=6$); ($n=9$)	Urine/plasma *m/z* 55				Urine/plasma *m/z* 70			
	9 μg/ml		60 μg/ml		9 μg/ml		60 μg/ml	
	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-
Precision[a]*	98.89	98.23	99.34	99.39	97.48	96.18	98.83	98.81
Precision[a]**	99.15	98.17	98.54	98.98	95.48	95.40	99.23	99.43
Precision[b]*	98.74	98.57	99.48	99.52	97.61	97.07	98.94	98.79
Precision[b]**	99.26	99.11	98.68	98.79	95.71	95.81	99.32	99.44
Accuracy[c]	95.55	94.00	101.83	102.45	98.77	99.22	103.48	103.57
Accuracy[d]	100.44	100.22	98.10	98.75	95.33	96.00	96.04	96.05

Precision and recovery were measured at concentrations of 18 and 120 μg/ml of *rac*-ethosuximide. (Results: Urine samples, *m/z* 55: Precision = 98.17–99.39%, Accuracy = 94.00–102.45%, *m/z* 70: Precision = 95.40–99.43%, Accuracy = 98.77–103.57%; Plasma samples, *m/z* 55: Precision = 98.57–99.52%, Accuracy = 98.10–100.44%, *m/z* 70: Precision = 95.71–99.44%, Accuracy = 95.33–96.05%).

[a] Precision of analytical system* and extraction method** for urine samples (%).

[b] Precision of analytical system* and extraction method** for plasma samples (%).

[c] Accuracy of method for urine samples (%).

[d] Accuracy of method for plasma samples (%).

physical and chemical properties with the exception of the direction in which they rotate plane-polarized light. A chiral stationary phase can recognize differences in the optical activity of the solutes to varying extent. The conformation of cyclodextrins in an aqueous system approximates to a truncated cone, with an internal cavity possessing a hydrophobic surface [15]. One of the mechanisms of separation by β-cyclodextrins is based on the formation of solute-β-cyclodextrin complexes occuring in the barrel-shaped opening of the cyclodextrin [16]. The stability of these inclusion complexes is dependent on the size, intrinsic structural features, and hydrophobic and steric character of the guest molecule [17].

6. Conclusion

A modified specific, sensitive and reproducible chiral GC/MS method is described here for the resolution and quantification of ethosuximide enantiomers in biological fluids. The chiral stationary phase used in the study proved to be efficient in the separation and determination of these enantiomers in urine and plasma. The separation was high and allowed for the repeated analyses of extracted biological samples. In this technique, the analytical column was well protected from decomposition and thus several hundred samples could be injected on the system. In addition, the total analysis time was not long and recovery and reproducibility were excellent. Future research work in this field envisaged the application of this method in pharmacokinetic and pharmacodynamic studies.

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