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Gas chromatography–mass spectrometry assay method for the therapeutic drug monitoring of the antiepileptic drug tiagabine ☆

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Abstract

A gas chromatography–mass spectrometry assay method suitable for the therapeutic drug monitoring of the antiepileptic drug tiagabine is described. Tiagabine and its desmethylated analogue used as internal standard were first extracted from serum by liquid–liquid extraction using an ethyl ether–isobutanol 98:2 mixture. Tiagabine and the internal standard were then methylated in the organic phase in presence of methanol by means of a safe and stable diazomethane derivative. After evaporation, the reconstituted extracts were chromatographed on a crosslinked phenyl methyl siloxane capillary column and detected by mass fragmentometry at $m/z = 156$. No other antiepileptic drug possibly administrated in polytherapy and no metabolite were found to interfere in the assay. The limit of quantification was 5 ng/ml. The precision and the accuracy were found to be suitable for the therapeutic drug monitoring of tiagabine.

Keywords: Tiagabine; Therapeutic drug monitoring; GC–MS; Human serum; Antiepileptic drugs

1. Introduction

Tiagabine (TGA), a potent and specific γ-aminobutyric acid-uptake inhibitor, is a new antiepileptic drug (AED) with a novel mechanism of action [1,2]. TGA is a chiral compound resulting from the development of derivatives of nipecotic acid [3]. Its enantiomeric purity is greater than 99% $R(-)$ [2]. Because through levels of unchanged drug in human plasma following oral doses are in the ng/ml level, sensitive analytical techniques are required for therapeutic drug monitoring of TGA. A recent review on the determination of AEDs in biological fluids [4] revealed that only one high-performance liquid chromatography (HPLC) method has been reported for the determination of TGA in human plasma [5]. The procedure, which involved the solid-phase extraction (SPE) of 1-ml plasma samples on C_8 disposable extraction cartridges followed by separation on a regular C_{18} reversed-phase column and coulometric electrochemical detection was reported to give

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satisfactory results with a quantification limit of 8 ng/ml. However, large negative and positive baseline drifts were obtained under the experimental detection conditions.

In this paper, we described a gas chromatography–mass spectrometry (GC–MS) assay method developed for the therapeutic drug monitoring of TGA as an alternative to the existing HPLC method.

2. Experimental

2.1. Reagents and materials

TGA, (*R*)-*N*-(4,4-di(3-methyl-2-thienyl)but-3-enyl)nipecotic acid (Fig. 1) and its monomethyl analogue used as internal standard (IS, Fig. 1) were kindly supplied by the Swiss Center for Epilepsy (Zürich, Switzerland). (Trimethylsilyl)diazomethane 2 M solution in hexane was from Aldrich (Buchs, Switzerland). Borate buffer pH 7.5 was prepared adjusting 0.11 M borate buffer pH 8 Titrisol solution from Merck (Darmstadt, Germany) to pH 7.5 with 1 M HCl Titrisol solution from Merck. All other chemicals were of analytical grade. Water was obtained by means of a Milli-Q Plus device from Millipore (Le Mont, Switzerland). Pooled human drug-free serum was obtained from Irvine (Santa Ana, CA, USA). Serum samples from patients under different AED therapy as well as drug-free human serum samples from different blood donors were collected by venipuncture using Vacutainer tubes (Becton Dickinson, Basel, Switzerland).

2.2. Standard solutions and calibration

Stock solutions (100 μg/ml) and working solutions (4 μg/ml) of both TGA and IS were prepared in methanol–water (90:10, v/v). Spiked samples 0, 9.11, 45.55, 91.10, 227.8 and 455.5 ng/ml TGA free base were used as standard samples. Those were obtained by mixing suitable volumes of either stock or working solutions with pooled serum and further successive dilutions in serum. Control samples 18.22, 182.2 and 364.4 ng/ml were prepared as the standard samples. These were used for both the validation process and the quality control of each run. The calibration curves were obtained by linear regression of the area ratio of TGA–Me to internal standard and the nominal theoretical concentrations expressed in ng/ml TGA free base. No response weighting was applied and the curves were not forced through origin.

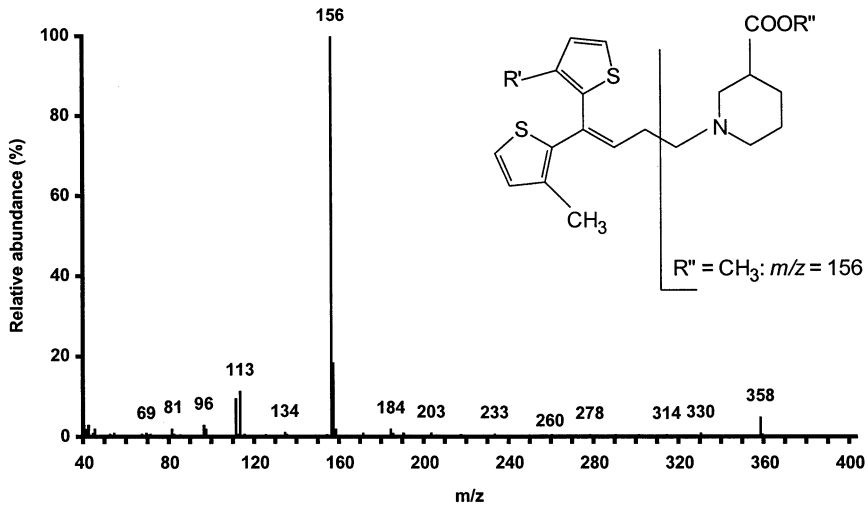

Fig. 1. Chemical structures of TGA derivatives and electron impact (70eV) mass spectrum of TGA–Me. IS: $R' = R'' = H$; TGA: $R' = CH_3$, $R'' = H$; IS methyl ester: $R' = H$, $R'' = CH_3$; TGA–Me: $R' = R'' = CH_3$.

2.3. Sample preparation

Aliquots of 500 μl of both spiked and unknown serum samples were mixed in a 12-ml glass tube with 25 μl of internal standard working solution (= 100 ng). 500 μl of borate buffer pH 7.5 were then added to the mixture and the tubes were briefly vortexed. A 7-ml volume of a mixture ethyl ether–isobutanol (98:2, v/v) were added and the tubes were shaken for 20 min on a reciprocating shaker at 300 rpm. The tubes were then centrifuged ($1500 \times g$ for 10 min at 4°C) and frozen at −20°C. The organic phase was quantitatively transferred to another tube. After the addition of 2 ml of methanol and 100 μl of trimethylsilyldiazomethane, the tubes were left at room temperature for 1 h. The solvents were evaporated to dryness under nitrogen by means of a Zymark Turbovap LV evaporator (Brechbühler SA, Grand-Lancy, Switzerland). The extracts reconstituted in 100 μl of methanol were ready for injection.

2.4. Instrumentation

GC–MS analysis was performed on a HP-5MS column (30 m × 0.25 mm I.D., crosslinked 5% phenyl methyl siloxane, 0.25 μm film thickness) using a Model 6890 gas chromatograph coupled to a Model 5973 mass selective detector (all from Hewlett Packard, Meyrin, Switzerland). The initial oven temperature of the gas chromatograph was 100°C. The oven temperature was increased at a rate of 20°C/min to reach a final temperature at 300°C. The carrier gas was helium. The column head pressure was 97 kPa with an average linear velocity of 43 cm/s. The solvent delay was 7 min and the injector port temperature was 300°C. The injections were performed in splitless mode using unrestricted liners pre-filled with deactivated glass wool. The mass spectrometer was operated in selective ion monitoring mode using electron impact ionization. The ion being monitored was m/z 156 (corresponding fragment see Fig. 1). The ionization energy was 70 eV and the electron multiplier was typically set to 1.5 kV. The temperature of the transfer line and the source were 290 and 250°C, respectively.

2.5. Method validation

The method was validated according to guidelines of [6]. The analyses carried out to study the inter-day precision and accuracy were performed by different operators using different pipetting devices on different days. The levels of possible concomitant AEDs assayed during the specificity investigations were all within their therapeutic range.

3. Results

3.1. Mass spectra and chromatography

The mass spectrum of TGA–Me is shown in Fig. 1. As expected, the fragment due to the cleavage of the nipecotic acid moiety ($m/z = 156$) was the base peak (100% relative abundance) of both TGA–Me and IS methyl ester because IS differs from TGA only by a desmethylated thienyl group. Typical chromatograms are shown in Fig. 2. The retention times were found to be 11.581 ± 0.003 ($n = 24$) and 11.879 ± 0.003 ($n = 24$) for IS and TGA derivatives, respectively.

3.2. Validation data

No endogenous compound interfering with either TGA or IS was detected in the serum samples collected from six different blood donors. The specificity of the assay against possible AEDs concomitantly administrated in polytherapy is given in Table 1. No interference was observed with 16 AEDs and their metabolites. However, GC–MS and HPLC investigations showed that TGA was found to be present at less than 0.5% in the available IS sample. For this reason, the amount of IS added was limited to 100 ng per sample.

The overall extraction recoveries and the yield of the derivatization step were not determined because of the lack of both reference TGA and IS derivatives. The relative standard deviation of the IS peak area after liquid–liquid extraction and GC–MS analysis was found to be 7.6% ($n = 24$).

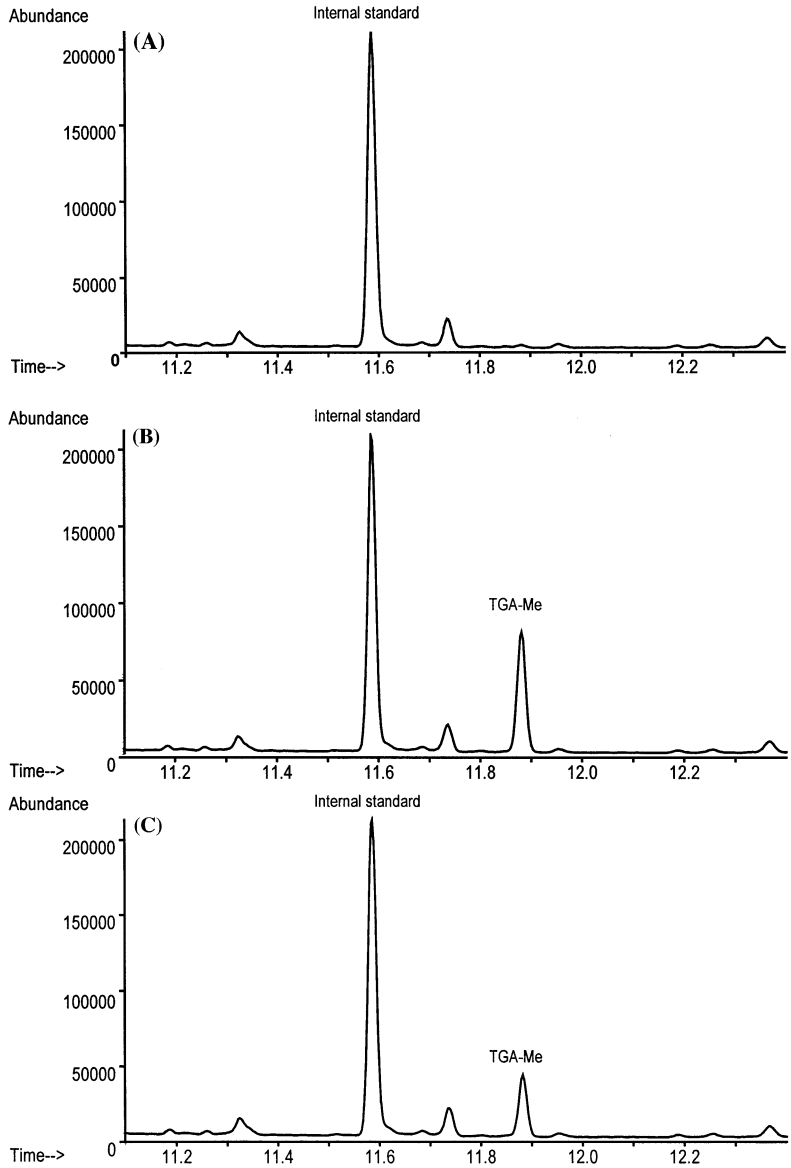

Fig. 2. Typical chromatograms obtained under the described experimental conditions (time in min). Selected ion monitoring $m/z = 156$. (A) blank serum, (B) spiked serum sample 45.55 ng/ml TGA, C: patient sample under TGA therapy.

The overall mean of the slopes within the range 0–455.5 ng/ml was 235.5 ± 7.7 (RSD = 3.3%, $n = 6$). A mean intercept value of -1.7 ± 0.9 ($n = 6$) was calculated. The coefficient of correlation were found to be > 0.9997 ($n = 6$).

The intra-day and inter-day precision and accuracy data are summarized in Table 2. The repeatability of the assay was found to be $< 3.7\%$ ($n = 6$) at 18.22, 182.2 and 364.4 ng/ml, respectively. The reproductibility of the assay at the same concentration levels was found to be within 3.0–7.5% ($n = 6$) The intra-day and inter-day accuracy defined as the mean deviation from the theoretical nominal concentration of the spikes was within $+1.2$ and $+5.6\%$ ($n = 6$) and within 7.3 and 9.0% ($n = 6$), respectively.

Table 1
Specificity of the developed method

Antiepileptic drugs and metabolites	Possible interference
Carbamazepine	ND*
Carbamazepine-10,11 epoxide	ND
Carbamazepine-*trans*-diol	ND
Clobazam	ND
Clonazepam	ND
Desmethylclonazepam	ND
Ethosuximide	ND
Felbamate	ND
Gabapentin	ND
Lamotrigine	ND
Oxcarbazepine	ND
Hydroxy-10 carbamazepine	ND
Pheneturide	ND
Phenobarbital	ND
Phenytoin	ND
m-Hydroxyphenyl-5 phenylhydantoin	ND
p-Hydroxyphenyl-5 phenylhydantoin	ND
Primidone	ND
Phenylethylmalondiamide	ND
Remacemide	ND
Desglycinylremacemide	ND
Topiramate	ND
Valproic acid	ND
Vigabatrin	ND

* ND = not detected

As shown in Table 2, the limit of quantification (precision $< 15\%$, $n = 6$; accuracy $\pm 15\%$, $n = 6$) was 5.0 ng/ml with a signal-to-noise 38:1. The limit of detection was found to be in order of 0.4 ng/ml (signal-to-noise 3:1).

TGA and IS derivatives were found to be stable in reconstituted extracted control samples (three levels, $n = 6$ for each level) placed in the injector rack for more than 38 h. The mean deviations found after 38 h were $< 1.6\%$ for all levels. The stability of TGA in frozen plasma and at room temperature has been already reported [4]. Investigations done in serum gave the same results.

4. Discussion

Liquid–liquid extraction from serum samples using ethyl ether [7] was found to be a suitable alternative to the reported solid-phase extraction procedure [4]. Investigations done using an ASPEC sample preparation device showed that the liquid–liquid extraction procedure allowed larger throughputs than SPE for series runs including six calibration levels, three quality controls and several unknown samples. In addition, the SPE procedure required an additional step for the evaporation of the solvent necessary for the elution of TGA from the disposable extraction cartridges used in SPE. Isobutanol, which has been

Table 2
Intra-day and inter-day precision and accuracy, and limit of quantification of the determination of tiagabine in human serum by the present method

Nominal theoretical concentration (ng/ml)	Concentration found (ng/ml)			*n*	Confidence interval of the mean ($P = 95\%$, $n = 6$)	Deviation (%)
	Mean	SD	RSD (%)			
Intra-day precision and accuracy						
18.22	18.44	0.67	3.7	6	18.44 ± 0.71	1.2
182.2	188.9	3.2	1.7	6	188.9 ± 3.3	3.7
364.4	384.8	7.9	2.1	6	384.8 ± 8.3	5.6
Inter-day precision and accuracy						
18.22	19.86	1.48	7.5	6	19.9 ± 1.55	9.0
182.2	195.43	7.08	3.6	6	195.4 ± 7.4	7.3
364.4	396.93	12.10	3.0	6	396.9 ± 12.7	8.9
Limit of quantification						
8.00	7.60	0.11	1.4	6	7.60 ± 0.12	−5.0
5.00	4.75	0.37	7.9	6	4.75 ± 0.39	−5.0
2.00	1.39	0.20	14.5	6	1.39 ± 0.21	−30.4

shown to prevent basic drug adsorption on glassware, was systematically added to ethyl ether [8].

No peak was detected applying total ion current detection when reference TGA was directly injected on the column. As a consequence, derivatization of the carboxylic function of the nipecotic acid moiety was required. Trimethylsilyldiazomethane is a stable and safe substitute for either hazardous diazomethane or corrosive reagents containing methanol and acidic catalysts such as hydrogen chloride or boron trifluoride. Trimethylsilyldiazomethane reacts quickly at room temperature with carboxylic acids in presence of methanol to give methyl esters in yields suitable for analytical gas chromatography [9]. Investigations done by HPLC showed that no TGA remained after trimethysilyldiazomethane treatment under the experimental conditions described. In addition, the derivatization is easy because the evaporation of the organic phase of the extract separated after freezing of the aqueous phase was not required. 3 M hydrogen chloride in methanol was found to be an effective esterification agent but required an additional evaporation step and handling of corrosive volatile reagent.

The limit of quantification of 5 ng/ml was found to be similar to that one obtained by means of the HPLC involving coulometric detection i.e. 8 ng/ml. However, the ratio signal-to-noise at the limit of quantification was 38:1 and no baseline disturbance was observed in the chromatograms. In addition, the necessary sample volume is a half of that one required by the HPLC procedure. The use of an internal standard free of TGA traces would improve the limit of quantification.

The developed method was applied to the therapeutic drug monitoring of tiagabine and was found to be suitable for its intended use.

5. Conclusion

The developed method is suitable for the therapeutic monitoring of tiagabine. It involved a liquid–liquid extraction and the derivatization of TGA by means of a stable and safe esterification reagent easily usable in clinical laboratories. The validation data demonstrate its specificity and its reliability.

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