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Short communication

Simple and rapid liquid chromatographic–turbo ion spray mass spectrometric determination of topiramate in human plasma

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Abstract

We present a simple and fast method for the determination of the novel antiepileptic drug topiramate in human plasma by high-performance liquid chromatography coupled with turbo ion spray mass spectrometry. Plasma sample pre-treatment was based on simple deproteinization by acetonitrile. Liquid chromatographic analysis was carried out on a reversed-phase column (C_{18}, 125×4 mm I.D., 5 μm) using acetonitrile–ammonium acetate buffer, pH 6.3 as the mobile phase, at a flow-rate of 0.8 ml/min. Retention time for topiramate was 2.1 min. The detector was a single quadrupole mass spectrometer coupled to a turbo ion spray ion source and a heated nebulizer probe, operating in the positive ion mode. Ion source temperature was off; voltage was +5800 V; nebulizer and curtain gas flow-rates were 6 and 10 ml/min, respectively. Calibration curves for topiramate were linear over the range 1 to 20 μg/ml. Absolute recovery ranged between 92 and 95%. Intra- and inter-assay precision was <4%. The present procedure, omitting extraction and drying steps, is faster and simpler than the previously reported analytical methods for topiramate and was demonstrated to possess adequate sensitivity for routine therapeutic drug monitoring in plasma from patients with epilepsy.

Keywords: Topiramate

1. Introduction

Topiramate (2,3:4,5-bis-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate) (TPM) is a new antiepileptic drug (AED) approved as add-on therapy in patients with partial epilepsy, with or without secondary generalized seizures [1]. TPM is rapidly and well absorbed from the gastrointestinal tract, with time to peak plasma drug concentration of 2–4 h [2]. The oral bioavailability is high (>80%) and the plasma elimination half-life is around 20 h in healthy volunteers. More than 60% of a dose of topiramate is eliminated unchanged by the renal route, and by different metabolic pathways for most of the remaining absorbed fraction [3]. In patients treated with AED inducers of cytochrome P450 metabolism, such as carbamazepine, phenobarbital and phenytoin, metabolic elimination possibly becomes the major determinant of topiramate disposition, and elimination of unchanged drug into urine is reduced to only about 30% [2].

Different controlled clinical trials have demonstrated TPM efficacy in the management of drug-resistant seizures [1]. More recent studies have also shown a high incidence of adverse effects, mostly related to the central nervous system [4,5].

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Due to the pharmacokinetic characteristics of TPM and the pharmacological profile, monitoring drug plasma concentrations may prove to be useful [6]. The quantitative determination of TPM is complicated by the fact that the molecule has no ultraviolet absorption or fluorescence, hampering the use of the more readily accessible techniques, such as high-pressure liquid chromatography (HPLC) with spectrophotometric or fluorescent detection. The reported analytical methods for TPM in human plasma or serum were mostly carried out by capillary gas chromatography (GC) coupled to flame ionization (FID) [7] or nitrogen phosphorus detection (NPD) [8–11] and, more recently, by HPLC coupled to electrospray (ESP) mass spectrometry (MS) [12,13]. The early GC techniques with FID [7] or NPD [8] lacked specificity and the necessary sensitivity to perform therapeutic monitoring when low daily dosages of TPM are administered, unless complicated and time-consuming multiple extraction steps and concentration procedures are used. Some of the subsequently reported procedures exhibited improved sensitivity [9,11], but were generally laborious due to sample pre-treatment by either liquid–liquid extraction [10–12] or solid-phase extraction [9].

Here we describe a sensitive and selective HPLC–turbo ion spray (TSP) MS method for the measurement of TPM plasma concentrations which is faster and simpler than previous methods and suitable for application in a routine AED therapeutic drug monitoring (TDM) setting.

2. Experimental

2.1. Reagents and standards

Topiramate was kindly provided by Cilag (Schaffhausen, Switzerland). Methanol, acetonitrile, both gradient grade, acetic acid and ammonium hydroxide were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a MilliQ apparatus (Millipore, Bedford, MA, USA). The ammonium acetate buffer employed for the preparation of the mobile phase was composed of a solution of 10 m*M* acetic acid adjusted to pH 6.3 with 6% ammonium hydroxide. This solution was filtered through a 0.22 μm membrane filter (GS type, Millipore). Stock (1 mg/ml) and standard solutions of TPM were prepared in methanol and stored at 4°C.

Plasma standards of 1.0, 2.0, 5.0, 10 and 20 μg/ml were prepared by pipetting suitable amounts of standard TPM solutions into 500 μl aliquots of blank pooled plasma and then treated exactly as the patient specimens.

2.2. Blood sampling and plasma processing

Venous blood samples (5 ml) were drawn from patients at 8 a.m. before the first morning dose of TPM and concomitant AEDs, transferred into heparinized tubes (8 IU heparin/ml blood) and centrifuged at 1500 *g* for 10 min at 4°C. Plasma was immediately separated and 500 μl aliquots were deproteinized by addition of 2 ml acetonitrile, vortexed for 15 s and then centrifuged at 2500 *g* at 4°C for 10 min. Twenty microliters of the clean upper layer was injected directly into the chromatographic system.

2.3. Chromatographic apparatus and conditions

The HPLC system consisted of a Series 200 liquid chromatograph (Perkin-Elmer, Norwalk, CA, USA), a Rheodyne Model 7125 S injection valve (Rheodyne, Cotati, CA, USA) fitted with a 50 μl sample loop and a Purosphere C_{18} (5 μm) endcapped column, 125×4 mm I.D., protected by a C_{18} Purosphere precolumn, both purchased from Merck. The mobile phase was prepared by mixing ammonium acetate buffer with acetonitrile (45:55, v/v) and sparged with helium. The mobile phase flow-rate was set at 0.8 ml/min with post-column split of the eluent flow (65 μl/min to MS).

Quantitative analysis of TPM was carried out using a API 150EX single quadrupole mass spectrometer (Perkin-Elmer Sciex, Foster City, CA, USA), equipped with a turbo ion spray ion source and a heated nebulizer probe operating in the positive ion mode. Nitrogen (99.9% purity) from a Whatman Model 75-72 membrane nitrogen generator (Whatman, Haverhill, MA, USA) was used as nebulizer and curtain gas. The following conditions were used: ion source temperature, off; voltage, +5800 V; nebulizer gas flow-rate, 6.0 ml/min; curtain gas

flow-rate, 10 ml/min; orifice voltage, +40 V; ring voltage, +180 V. The ammonium adduct cation of TPM (*m/z* 357) [14] was recorded at *m/z* 356.9–357.6 (centroid) by a step size of 0.1 amu and a dwell time of 30 ms. Data were acquired and processed by a Power Macintosh 8600/250 series computer (Apple, Cupertino, CA, USA) using LC2 Tune software.

2.4. Method validation

Duplicate standard curves were run on each analysis day ($n = 10$) over 4 months. The TPM peak area was plotted against the drug matched concentration added to the blank plasma. The calibration curve was obtained by the least square method. For assay precision and accuracy assessment, spiked plasma pools were prepared at two concentrations (1 and 10 μg/ml), separated into 500 μl aliquots and stored frozen at −20°C. Three samples from each pool were analyzed with six calibration curves over 4 months. The intra-assay precision was measured by the relative standard deviation (RSD = 100 × SD/mean) of the daily mean of triplicate determinations of each concentration point. The inter-assay precision was evaluated from the RSD of all individually determined values of each concentration point. The accuracy of the method was determined by comparing the means of the calculated concentrations with the nominal concentrations. The absolute recovery of the procedure was calculated as the ratio of the drug peak area from deproteinized blank plasma spiked with TPM (5 μg/ml) to the peak area obtained from the injection of a TPM standard solution at the same concentration. The lower limit of quantitation (LLQ) was defined as the lowest quantifiable concentration with an associated variation of <20%.

2.5. Method specificity

Blank plasma from 10 pools was tested for endogenous interference. Furthermore, a series of plasma samples from patients with epilepsy not taking TPM and treated with commonly prescribed AEDs and benzodiazepines was analyzed to check for drugs which could potentially interfere with TPM determination (Table 1).

Table 1
List of drugs checked for TPM assay interference

AEDs
Carbamazepine
Ethosuximide
Felbamate
Gabapentin
Lamotrigine
Oxcarbazepine
Phenobarbital
Phenytoin
Primidone
Tiagabine
Valproic acid
Vigabatrin
Benzodiazepines
Clobazam
Clonazepam
Diazepam
Lorazepam
Nitrazepam

3. Results and discussion

A total ion chromatogram and matched positive ion spectrum (*m/z* range 330–380) of a standard solution of TPM in mobile phase are shown in Fig. 1. The ion at *m/z* 357.2, corresponding to the ammonium adduct cation of TPM (molecular mass 339.3) dominated the spectrum. The peak at *m/z* 340.2 represents the undissociated form of TPM; peaks at *m/z* 362 and 377.9 are the sodium and potassium adduct cations of TPM, respectively. Typical chromatograms obtained from a blank plasma and a patient's plasma are shown in Fig. 2. There were no endogenous interferences in the TPM elution region for all of the blank pools tested. Moreover, from analyses of patient samples containing drugs frequently co-prescribed with TPM (Table 1) no peak was detected within 40 min of a run.

The results of the method validation analyses are summarized in Table 2. Calibration curves showed a linear correlation between concentration and peak area; the equation of the regression lines (mean±standard deviation, $n = 20$) was: $y = 20\,273(\pm 3021) + 660\,506(\pm 108\,727)x$ ($r = 0.999$), where x is the analyte concentration expressed in μg/ml, and y is the peak area of the analyte expressed in arbitrary area units. The intra-assay precision ($n = 6$) at 1 and 10 μg/ml was 2.2 and

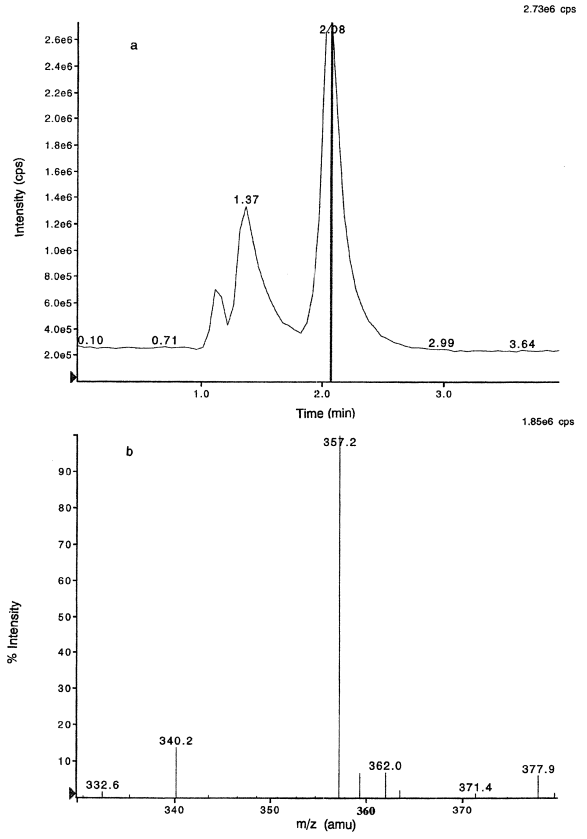

Fig. 1. Total ion chromatogram (m/z 330–380 by 0.5 amu) (a) and positive ion turbo ion spray mass spectrum (b) selected from the TPM peak (retention time 2.08 min) obtained by injecting 20 μl of a standard solution of TPM, 10 μg/ml, in mobile phase. The ion at m/z 357.2 corresponds to the ammonium adduct cation of TPM.

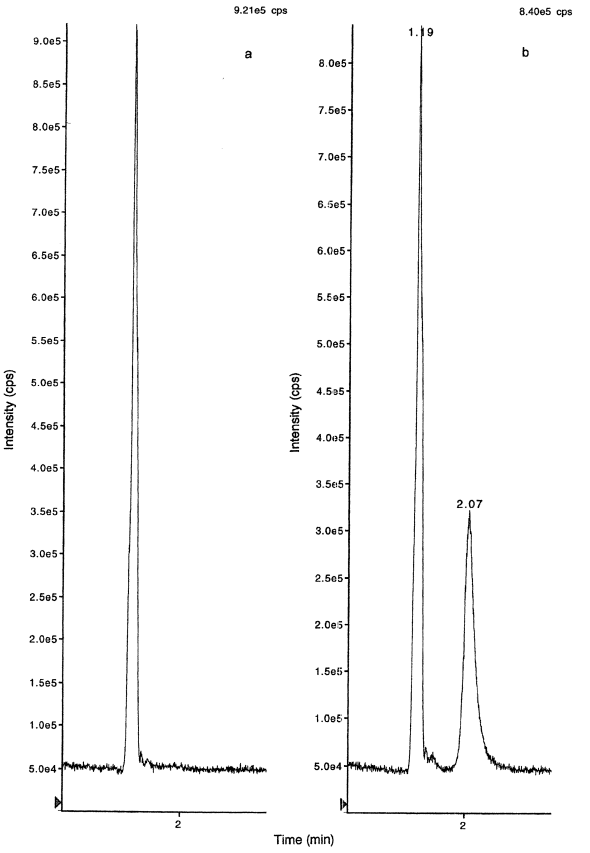

Fig. 2. Liquid–turbo ion spray mass spectrometric chromatograms obtained by injecting 20 μl of (a) deproteinized blank plasma and (b) a patient's plasma (TPM, 5 μg/ml), in the centroid mode (m/z 356.9–357.6 by 0.1 amu).

1.5%, respectively. The inter-assay precision (n = 18) for samples of the same concentration was 3.6 and 2.5%, respectively. The accuracy of the method (n = 18) was 3.6% at 1 μg/ml and 0.75% at 10 μg/ml. The absolute recovery (n = 6) averaged 93% (range 92–95%). Since only deproteinization and direct injection of samples are involved in this assay, the addition of an internal standard was found to be unnecessary. The lower limit of quantitation was 0.25 μg/ml. The method quantitation range of 0.25–20 μg/ml proved to be adequate for TDM purposes: in a cohort of 100 patients with epilepsy treated with a TPM dosage ranging from 25 to 600 mg/d, combined with different AED cotherapy, we found steady-state TPM plasma trough concentrations of 0.3–18 μg/ml.

The main advantage of the present analysis compared with previously reported methods [8–13] is the simple and rapid procedure of sample pre-treatment, which allows a large series of plasma specimens to be processed in a short time. Turbo ion spray MS detection is sensitive and highly selective and the chromatographic separation is very rapid, allowing TPM determination in plasma of patients also receiving complex AED co-medication in about 2 min. Compared with the only HPLC–MS method for TPM plasma analysis published so far [12,13], this assay significantly simplifies sample purification by omitting time-consuming extraction and drying steps.

Table 2
Characteristics of topiramate assay in plasma

Linearity range (μg/ml) ($n = 20$)	Amount added to blank plasma (μg/ml)	Calculated conc. (mean±SD, $n = 18$) (μg/ml)	Precision (RSD%)		Accuracy (%) ($n = 18$)	Recovery (%) ($n = 6$)	LLQ (μg/ml)
			Intra-assay ($n = 6$)	Inter-assay ($n = 18$)			
1–20 $y = 20\,273(\pm 3021) + 660\,506(\pm 108\,727)x$ ($r = 0.999$)	1	1.04±0.037	2.2	3.6	3.7	93	0.25
	10	10.1±0.252	1.5	2.5	0.75		

Moreover, it shows a greater sensitivity (LLQ 0.25 μg/ml vs. 2 μg/ml) and in routine use it proved suitable for TDM even in patients receiving low TPM daily dosages during clinically recommended slow titration regimens [4].

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