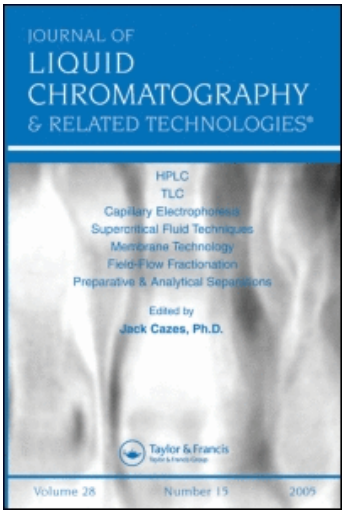

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CONCOMITANT HPLC METHOD FOR DETERMINATION OF LAMOTRIGINE, CARBAMAZEPINE, AND 10,11-CARBAMAZEPINE EPOXIDE IN PLASMA USING DUAL UV 240–220 NM WAVELENGTH DETECTION

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ABSTRACT

This paper presents a rapid, low cost, specific, high-performance liquid chromatographic (HPLC) method using an internal standard (pipamperone: PIP) for concomitant determination of lamotrigine (LMG), carbamazepine (CBZ), and its major metabolite, 10,11-carbamazepine epoxide (EPOCBZ). Drug detection is performed using a dual wavelength detection (200-240nm) to check the purity of chromatographic peaks.

Mean retention times for LMG, EPOCBZ, PIP, CBZ were 3.73, 4.34, 5.41, and 7.93 minutes, respectively. Recovery ratios averaged 88.1%±1.0 for LMG, 101.1%±1.4 for CBZ, 101.0%±1.6 for

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EPOCBZ, and 97.2%±2.5 for PIP , respectively (n=6). In the range investigated, both the intra-day and the inter-day % relative standard deviation were less than 6.5% (n=6). Absorbance ratios of 240 versus 220 nm (AR 240/220nm) averaged 0.401±0.0037 for LMG, 0.227±0.0046 for EPOCBZ, 0.525±0.0042 for CBZ, and 3.234±0.115 for PIP, respectively (n=4). Albeit, no interference was detected with any of the drugs (or metabolites) tested, which might be associated with LMG or CBZ; determination of AR 240/220nm is of interest in laboratory routine while minimizing the risk of possible interferences without additional cost and waste of time.

INTRODUCTION

Lamotrigine (LMG, 6-(2-3 dichlorophenyl)-1,2,4-triazine 3,5 diamine) is a new antiepileptic drug.(1,2) Lamotrigine pharmacological activity is mainly due to the inhibition of voltage sensitive sodium current.(1,2) Its common side effects include neurological, gastrointestinal, and dermatological effects. Severe rashs could be observed, thus, it is recommended to intiate LMG progressively.(1,3,4) LMG is currently prescribed, either as monotherapy or as adjunctive therapy with other anticonvulsivant drugs, such as valproic acid or carbamazepine.

LMG pharmacokinetics is characterized by a complete absorption after oral administration (absolute bioavailibility of 98%), with a peak plasma concentration observed 1 to 3 hours after oral administration. LMG, mainly metabolised by N-glucuronidation, has a mean plasma half life of 29 hours.(1,2)

Pharmacokinetic investigations of LMG have shown considerable variability between subjects. LMG metabolism is affected by addition of other anticonvulsivant drugs like hepatic enzyme inducing agents (carbamazepine, phenobarbitone, phenytoin), or inhibitors of hepatic microsomal enzymes (valproic acid).(5,6) A tentative target range for LMG therapeutic drug monitoring has been proposed (1-4 μg/mL).(7) Nevertheless, plasma concentrations of LMG higher than 10μg/mL have been well tolerated.(8) LMG plasma determinations have been recommended in case of a compliance problem and might be helpful to disclose drug interactions.

Rapid and automatic methods, widely used like immunoassay, are available for plasma determination of many antiepileptic drugs, such as phenobarbitone, phenytoine, and valproic, acid which is not the case of lamotrigine and carbamazepine epoxyde. This article presents a new, specific, rapid, and low cost high performance liquid chromatography concomitant assay for LMG, carbamazepine (CBZ) and its 10-11 epoxide (EPOCBZ). This method takes advantages of dual

wavelength detection to minimize the risk of interference due to the frequent comedication of epileptic patients.

EXPERIMENTAL

Chemicals and Drug Solutions

LMG was donated by Glaxo Wellcome (Paris, France), CBZ and its 10-11 epoxide (EPOCBZ) by Novartis (Basel, Switzerland). The internal standard, pipamperone (PIP), was obtained from Janssen (Antwerpen, Belgium). HPLC methanol, dichloromethane, and sodium hydroxide were obtained from Merck-Clevenot Chemical (Nogent Sur Marne, France). Purified water was purchased from Aguettant (Lyon, France).

Drug solutions were prepared by dissolving pure substances in methanol. EPOCBZ, CBZ, LMG, and PIP were dissolved to a concentration of 1 mg per mL. These solutions were stored in the dark at –25°C and no instability was observed.

Liquid-Liquid Extraction

Sample preparation consisted of a liquid-liquid extraction. Serum standards containing known amounts of CBZ, EPOCBZ, LMG were prepared by spiking bovine serum (Biotrol™ bovine serum, Merck Clevenot, France) to obtain final concentrations of CBZ and EPOCBZ in the range of 2-20 μg/L, and LMG in the range of 1-10 μg/L. Prior to extraction, 100 μL of a solution containing 80 μg of PIP (internal standard) were added to 250 μL of bovine serum. 300 μL of 2N sodium hydroxide were added to enhance partitioning of the drugs into the organic layer. 2 mL of the dichloromethane solution were added and the tubes were vortexed for 3.5 minutes and then spun at 3000 rpm for 5 minutes in a table-top centrifuge. 250 μL of the organic layer were put into a 5 mL centrifuge glass tube and then evaporated to dryness (55°C) during 6 minutes. After evaporation, 250 μL of purified water were added and vortexed during 45 seconds to assure total dissolution, then 100 μL of the aqueous solution were injected into the analytical column.

Chromatography

The chromatographic system consisted of an isocratic LC 6A pump (Shimadzu, Kyoto, Japan) and a column (C18 Lichrocart, Merck Clevenot, 125/4

mm ID). For UV detection, an UV SPD 6A detector (Schimadzu) was set at different wavelengths (i.e.: 200, 220, 240, and 260 nm) in order to select the optimal conditions. Chromatograms were recorded simultaneously on a RC 6A integrator (Shimadzu) for the 240 nm detection and on a Kipp & Zonen BD 40 integrator (Touzart and Matignon, Vitry Sur Seine, France) for the other wavelengths. The mobile phase was an acetonitrile-dihydrogenophosphate buffer adjusted to pH 5.8 (30:70 volume ratio) and the flow rate was set at 1.0 mL/min.

Calculations

From recorded peak heights, the ratios of drug to internal standard were calculated. The results obtained from serum standard spiked with known amounts of CBZ, EPOCBZ, LMG were used to calibrate graphs.

RESULTS

Chromatography

The mean retention times for LMG, EPOCBZ, PIP, CBZ were 3.73, 4.34, 5.41, and 7.93 minutes, respectively. A typical chromatogram of extracted standard is illustrated in Figure 1.

Recovery and Linearity

The peak heights of LMG, CBZ, EPOCBZ, and PIP were measured in chromatograms obtained after manual injection of the compounds dissolved in mobile phase without extraction at 240 nm. The peak heights were compared with those obtained with blank serum spiked with known amounts of the three compounds (LMG: 5 μg/mL, CBZ and EPOCBZ: 10 μg/mL). The % recovery values given in Table 1 are similar to those described in other studies.(9-11)

Precision and Accuracy

Interday (within day)/Intraday (between days) relative standard deviation (%RSD) and %accuracy were determined by analysing blank serum spiked with different amounts of CBZ, EPOCBZ, and LMG at 240 nm. In the range investigated, both the intra-day and the inter-day % RSD were less than 6.5% (Tables 2 and 3).

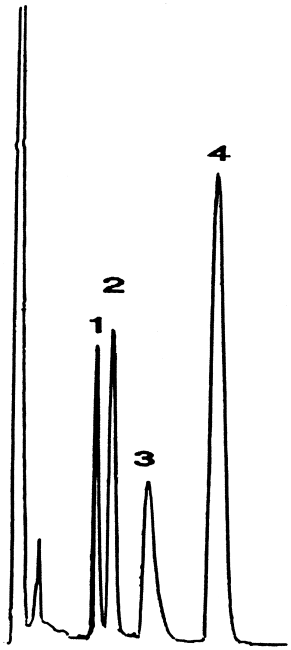

Figure 1. Chromatogram of extracted standard with lamotrigine (peak 1, retention times: 3.73 mn, concentration: 2,5 μg/mL), carbamazepine10, 11 epoxyde (peak 2, retention times: 4.34 mn, concentration: 5 μg/mL), pipamperone as internal standard (peak 3, retention times: 5.41 mn), and carbamazepine (peak 4, retention times: 7.93 mn, concentration: 5 μg/mL).

Limit of Detection, Linearity, and Specificity

240 nm has been chosen as the optimal wavelength according to the similarity of the absorbance of the drugs (LGM, CBZ, EPOCBZ) and PIP. The correlation coefficients calculated by linear regression analyses were superior to 0.9990 with 240 nm detection, but with other wavelengths, as well.

Table 1. Absolute Recovery Rates (%RSD: Relative Standard Deviation)

Lamotrigine	10–11 Carbamazepine Epoxide	Internal Standard (Pipamperone)	Carbamazepine
88.1% (%RSD:1.1%)	101.0% (%RSD:1.5%)	97.2% (%RSD:2.6%)	101.1% (%RSD:1.4%)

Table 2. Interday Accuracy and Relative Standard Deviation (%RSD) (n=7)

Lamotrigine	10–11 Carbamazepine Epoxide	Carbamazepine
1µg/mL:	**2µg/mL:**	**2µg/mL:**
% Accuracy: 101% (1.01 µg/mL)	% Accuracy: 99% (1.98 µg/mL)	% Accuracy: 98.5% (1.97 µg/mL)
%RSD: 5.0 %	%RSD: 5.1%	%RSD:6.5 %
2.5µg/mL:	**5µg/mL:**	**5µg/mL:**
% Accuracy: 98.4% (2.46 µg/mL)	% Accuracy: 99% (4.95 µg/mL)	% Accuracy: 99,2% (4.96 µg/mL)
%RSD: 2.6%	%RSD:3.6 %	%RSD: 3.5%
5µg/mL:	**10µg/mL:**	**10µg/mL:**
% Accuracy: 98.2% (4.91 µg/mL)	% Accuracy: 98.5% (9.85 µg/mL)	% Accuracy: 99.5% (9.95 µg/mL)
%RSD: 2.9%	%RSD: 2.2%	%RSD: 2.8%
10µg/mL:	**20µg/mL:**	**20µg/mL:**
% Accuracy: 101.6% (10.16µg/mL)	% Accuracy: 100.5% (20.10µg/mL)	% Accuracy: 101% (20.21 µg/mL)
%RSD: 2.7%	%RSD: 2.0%	%RSD: 2.1%

The lower limit of detection, defined as a signal two or three times greater than baseline noise, was 0.25 µg/mL at 240 nm for LGM, EPOCBZ, and CBZ. When testing standard solutions containing various other antiepileptic or psychotropic drugs that may be given in combination, minimal interferences were found for any drug or metabolite tested (Table 4). Retention times observed with EPOCBZ and phenobarbitone, although similar, led to two distinct peaks compatible with analytical determination.

Table 3. Intraday Accurancy and Relative Standard Deviation (%RSD) (n=6)

Lamotrigine	10–11 Carbamazepine Epoxide	Carbamazepine
1µg/mL:	**2µg/mL:**	**2µg/mL:**
% Accuracy: 94% (0.94 µg/mL)	% Accuracy: 90% (1.80 µg/mL)	% Accuracy: 98% (1.96 µg/mL)
%RSD: 4.0 %	%RSD: 2.6%	%RSD:5.1%
2.5µg/mL:	**5µg/mL:**	**5µg/mL:**
% Accuracy: 102.8% (2.57 µg/mL)	% Accuracy: 103% (5.15 µg/mL)	% Accuracy: 101.8% (5.09 µg/mL)
%RSD: 2.8%	%RSD:4.2%	%RSD: 3.4%
5µg/mL:	**10µg/mL:**	**10µg/mL:**
% Accuracy: 100.8% (5.04 µg/mL)	% Accuracy: 101.2% (10.12 µg/mL)	% Accuracy: 99.1% (9.91 µg/mL)
%RSD: 1.2%	%RSD: 2.9%	%RSD: 3.0%
10µg/mL:	**20µg/mL:**	**20µg/mL:**
% Accuracy: 97% (0.97µg/mL)	% Accuracy: 99.5% (19.91µg/mL)	% Accuracy: 100.1% (20.01 µg/mL)
%RSD: 2.2%	%RSD: 2.2%	%RSD: 2.1%

Table 4. Retention Times in HPLC of Drugs Analyzed for Interference (Detection Wavelength: 240nm)

Lamotrigine	3.73 mn
Carbamazepine-10,11-Epoxide	4.34 mn
Pipamperone	5.41 mn
Carbamazepine	7.93 mn
Antiepileptic drugs	
Clonazepam	13.71 mm
Diazepam	30.88 mn
Desmethyldiazepam	17.74 mn
Ethosuccimide	2.80 mn
Gabapentine	N D
Phenobarbitone	4.62 mn
Phenytoine	9.21 mn
Tiagabine	10.99 mn
Topiramate	ND
Valproic acid	ND
Vigabatrin	ND
Other drugs	
Amitriptyline	23.72 mn
Nortriptyline	17.30 mn
Clomipramine	38.22 mn
Desmethylclomipramine	27.94 mn
Clozapine	14.69 mn
Fluoxetine	28.16 mn
Norfluoxetine	21.67 mn
Haloperidol	17.37 mn
Imipramine	18.01 mn

ND: non detected.

Dual UV Detection

Absorbance ratios (AR) between 240 nm and other wavelengths were calculated with four different concentrations of LMG, CBZ, EPOCBZ, and PIP after extraction of spiked serum and are detailed in Table 5.

DISCUSSION

The present HPLC method is easy to perform, accurate, low cost, rapid, and sensitive. The recovery, linearity, precision, and accurancy were in agreement with data reported in the literature.(9,10,11) This method appears suitable for

Table 5. Ratios 240 nm Absorbance Versus Others Wavelengths Absorbance (%RSD: Relative Standard Deviation) (n=4)

Wavelength	Lamotrigine	10–11 Carbamazepine Epoxide	Internal Standard (Pipamperone)	Carbamazepine
260 nm	1.818 (%RSD:2.7%)	4.305 (%RSD:3.1%)	1.511 (%RSD:2.0%)	2.185 (%RSD:1.2%)
240 nm	1	1	1	1
220 nm	0.401 (%RSD:1.0%)	0.227 (%RSD:2.0%)	3.234 (%RSD:3.6%)	0.525 (%RSD:0.80%)
200 nm	0.232 (%RSD:4.5%)	0.135 (%RSD:2.5%)	0.507 (%RSD:1.8%)	0.536 (%RSD:5.0%)

concomitant determination of LMG , CBZ, and its major metabolite EPOCBZ and, thereby, can save considerable time in a laboratory, and limits the sample volume for the plasma determination (i.e., 250 μL).

LMG plasma determination represents an interesting tool for the physician and has several applications in order to check patient compliance and evaluate potential interactions during addition or removal of an interacting agent with LMG metabolism. Drug monitoring and obtention of therapeutic and/or toxicological threshold are more questionnable, but may be relevant with some type of population like ederly patients for which a number of studies remain limited. LMG and CBZ comedication is often used to stabilize patients, so their concomitant determination in plasma permits appraisal of the relevance of interactions. Although LMG has shown no evidence of affecting the pharmacokinetic of antiepileptic including CBZ, some authors have observed plasma increase of EPOCBZ by 10-45% with LMG,(12) but this fact remains questioned and further studies are needed to determine whether this interaction is significant or not. On the other hand, LMG metabolism is greatly affected by the use of carbamazepine.(13,14) It has been reported, that mean half life was shortened from 29 h during LMG monotherapy to 15 h in association with carbamazepine.(1) This fact should be kept in mind, and special care should be taken if CBZ is removed from a LMG-CBZ comedication.

Analytical interferences, though rare, remains a serious issue in chromatography, due to the complexity of the comedication including both antiepileptic drugs and their metabolites. Possibility of interference caused by agents absorbing in UV with identical retention times, may occur and lead to significant errors in the determination of drugs. Injection of standard solutions containing various other drugs, as has been carried out in our study, minimizes this risk. Nevertheless, drug metabolites of antiepileptics are particularly numerous and difficult to obtain from manufactors. Ramachandran et al. have reported interfer-

ence between LMG and EPOCBZ using a reversed phase high performance method.(15) In this publication reporting a concomitant determination in plasma of LMG, CBZ, phenytoin, and phenobarbitone, the chromophore was set at 307 nm to detect LMG in the presence of interfering EPOCBZ, though the other antiepileptic drugs were detected at 220 nm. Low cost, rapid, and easily usable methods are needed by laboratories for routine analysis to insure the purity of a chromatographic peak. Taking advantage of drastic changes in UV absorbance of LMG, CBZ, EPOCBZ, and PIP, 240 versus 220 nm absorbance ratio determination offers significant interest, with optimal reproductibility as shown in Table 5. McIntyre et al. have developed a similar, combined dual 220-254 nm HPLC method with antidepressive agents determination in plasma. The authors have found possible interferences between agents, which have been detected with dual UV detection.(16)

CONCLUSION

Prescription of antiepileptic drugs requires special care due to the complexity of pharmacokinetic interactions between agents, especially with drug such as lamotrigine or carbamazepine. Drug monitoring of lamotrigine, carbamazepine, and 10-11 carbamazepine epoxide appeared clinically useful, and specific methods of concomitant determination of the agents in plasma are needed. The method of plasma determination described in this article stresses the interest of dual UV detection. Albeit, no interference was detected with any of the drugs (or metabolites) tested; dual 240-220 nm detection optimizes the specifity of the method by minimizing the risk of possible interferences without extra charges or waste of time.

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