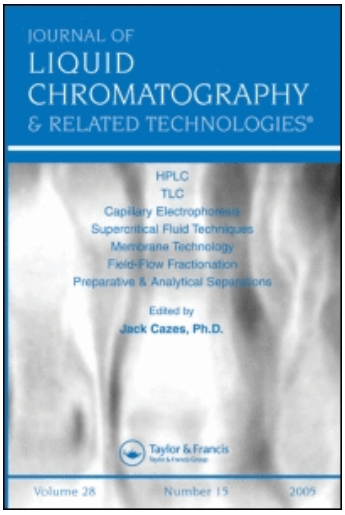

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Determination of Anticonvulsant Drugs in Pharmaceutical Preparations by Micellar Liquid Chromatography

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ABSTRACT

A micellar liquid chromatographic method for quality control of pharmaceutical preparations (capsules, pills, tablets, injections, drops, and suppositories) containing the anticonvulsant drugs acetazolamide, carbamacepine, chlordiazepoxide, diazepam, ethosuximide, phenytoin, phenobarbital, and zopiclone has been developed. This methodology involves the use of micellar solutions of cetyltrimethylammonium bromide (CTAB) as mobile phases and UV detection. The proposed approach is rapid and reproducible. Sample preparation only requires dissolution with micellar solvent and adequate dilution with the mobile phase before injection into the chromatographic system.

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Key Words: Column liquid chromatography; Micellar liquid chromatography; Anticonvulsant drugs; Pharmaceutical preparations.

INTRODUCTION

Epilepsy should not be considered a unique pathology entity. In fact, it is widely accepted to be a group of disorders with only one feature in common: the fact that recurrent anomalous electrochemical phenomena appear in the central nervous system. Epileptic fits occur when a high frequency electric discharge takes place. Anticonvulsant drugs work by decreasing the discharge propagation in different ways, e.g., by controlling the sodium and calcium ion channels, by enhancing the action of neuroinhibitory amino acids such as γ-aminobutyric acid GABA, or by inhibiting neuroexcitatory amino acids like glutamic acid.

Anticonvulsant drugs can be classified into: (i) classic anticonvulsants of first generation: phenobarbital, phenytoin, ethosuximide, and primidone; (ii) classic anticonvulsants of second generation: benzodiazepines, carbamacepines; (iii) new anticonvulsants: felbamate, lamotrigine; and (iv) other anticonvulsants like for example acetazolamide, ACTH, and corticoids.[1]

First generation anticonvulsants have gradually been substituted for the second generation ones because of the better pharmacokinetic profile of the latter, both having similar efficiencies. In addition, new anticonvulsants have better tolerance and fewer interactions with other drugs.[1] Moreover, some of the second generation anticonvulsants are effective against epileptic seizures, which are resistant to classics anticonvulsants.

The determination of anticonvulsant drugs in pharmaceutical preparations has been performed using several analytical techniques such as spectrophotometry,[2–11] voltamperometric techniques,[12–14] thin layer chromatography,[15] supercritical fluid chromatography,[16] capillary electrophoresis,[17] micellar electrokinetic chromatography,[18] and liquid chromatography.[19–29] Most of the proposed methods consider the determination of one or two anticonvulsant drugs.

Micellar liquid chromatography (MLC) is a mode of reversed phase liquid chromatography that uses aqueous solutions of surfactants above the critical micellar concentration. In MLC, electrostatic and hydrophobic interactions between the solute and both the stationary phase and micellar aggregates in the mobile phase exist, which allows the effective separation of compounds of different nature. The mobile phases are inexpensive, easy to prepare, and have much lower polluting impact than other aqueous-organic phases. In addition, the solubilization power of micellar solutions facilitates the sample preparation step, even for complex matrixes such as biological fluids.[30–32]

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The retention of a solute in a micellar liquid chromatography system depends on the surfactant nature and concentration, mobile phase pH (in the case of ionizable compounds), and ionic strength, and/or on the nature and concentration of organic modifiers.

Various analytical procedures that use anionic and cationic micellar mobile phases for determining drugs in pharmaceutical formulations[33–39] have been proposed. In this work, eight anticonvulsant drugs (acetazolamide, carbamacepine, chlordiazepoxide, diazepam, ethosuximide, phenytoin, phenobarbital, and zopiclone) have been determined in several pharmaceutical formulations (capsules, pills, tablets, injections, drops, and suppositories) using micellar mobile phases of cetyltrimethylammonium bromide (CTAB) and UV detection. The sample preparation in the proposed methods is rapid, and the results provided by this approach are reproducible and adequate for quality control of drugs in several dosage forms.

EXPERIMENTAL

Instrumental and Measurement

The chromatographic system was composed of a Hewlett-Packard 1100 chromatograph equipped with an isocratic pump and a UV-visible detector (Palo Alto, CA). Data acquisition and processing were performed on an HP Vectra XM computer (Amsterdam, The Netherlands) equipped with HP-ChemStation software from Agilent Co., 2000 version (Waldbronn, Germany).

The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA), with a 20 μL loop. A Kromasil octadecyl-silane C_{18} column (5 μm, 150 × 4.6 mm i.d.) (Scharlau, Barcelona, Spain) was used. The mobile phase flow rate was 1 mL min^{-1}. The detection was performed at 240 nm. All the assays were carried out at room temperature.

A micropH 2000 pH-meter (Crison, Barcelona, Spain) was used for pH adjustment.

Reagents and Standards

Cetyltrimethylammonium bromide (CTAB) (Acros Chimica, Geel, Belgium) was dissolved in 0.05 M dihydrogen phosphate (analytical reagent, Panreac, Barcelona, Spain) solutions. Then, the micellar solution pH was adjusted before the addition of 1-butanol (reagent grade, Scharlau, Barcelona, Spain) in order to obtain the working CTAB:1-butanol concentration ratios (v/v).

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Barnstead E-pure, deionized water (Sybron, Boston, MA) was used throughout. The mobile phases and the solutions injected into the chromatograph were vacuum-filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA).

Anticonvulsants were obtained from several sources: acetazolamide, carbamacepine, chlordiazepoxide, diazepam, and phenytoin from Guinama S.L (Valencia, Spain), ethosuximide from Sigma (St. Louis, MO). Other drugs were kindly donated by different laboratories: phenobarbital (Bayer, Barcelona, Spain) and zopiclone (Aventis Pharma, Madrid, Spain).

Stock standard solutions of anticonvulsants were prepared by dissolving the compound in 0.04 M CTAB. The solutions were stored at 4°C. Working solutions were freshly prepared by dilution of the stock standard solutions with mobile phase and injected into the chromatograph.

Sample Preparation

For the analysis of tablets and pills, five units were weighted, ground in a mortar, and finally, dissolved in 0.04 M CTAB buffered solution with the aid of an ultrasonic bath. If pharmaceuticals were presented as capsules, two were taken, dissolved in CTAB solution by magnetic stirring followed by ultrasonication. This procedure allowed a total solution of all components of the capsule including the cover. In the case of injections and drops, an aliquot was taken and diluted in CTAB solution, while for the analysis of suppositories, one was taken, weighted, and diluted in CTAB buffered solution by ultrasonication. In all cases, after appropriate dilution with the mobile phase, working solutions were injected into the chromatographic system through a 0.45 μm nylon filter. For each pharmaceutical, triplicate determinations were performed.

RESULTS AND DISCUSSION

In order to determine the detection wavelength, the absorption spectra of compounds in CTAB micellar medium were obtained. The absorption spectra of all compounds showed absorption bands in the UV region with maximum absorption wavelength at 240 nm.

Retention Behavior of Compounds

Table 1 shows the structure, the logarithm of the protonation constants ($\log K$), and octanol-water partition coefficient ($\log P$) for the anticonvulsant drugs studied.

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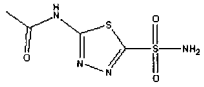
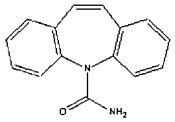
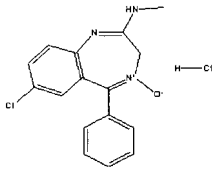
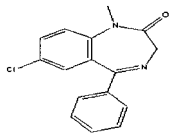
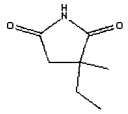
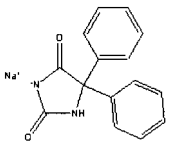
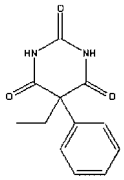
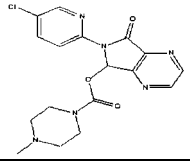
Table 1. Structure, logarithm of the protonation constants ($\log K$), $\log P$ and maximum wavelength of the compounds studied.

Drug		$\log K$[a]	$\log P$[b]
Acetazolamide		7.2	−0.3
Carbamacepine		—	2.5
Chlordiazepoxide		4.8	2.4
Diazepam		3.4	3.18
Ethosuximide		9.5	−0.33
Phenytoin		8.3	2.5
Phenobarbital		7.4	1.5
Zopiclone		—	—

[a]From Ref.[41].
[b]From Ref.[40].

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All these compounds have polycyclic structures. As can be observed in Table 1, there are basic compounds, such as: chlordiazepoxide, diazepam, and zopiclone; and acidic compounds, such as, acetazolamide, ethosuximide, phenytoin, and phenobarbital. Moreover, the compounds studied cover a wide range of hydrophobicity (with $\log P$ values between -0.3 and 3.18).

Table 2 shows the retention, efficiency, and asymmetry factors of the anticonvulsant drugs for different CTAB-1-butanol mobile phases assayed. A 0.04 M CTAB concentration in the micellar mobile phase was selected according to previous studies. The use of 0.02 M CTAB mobile phase provided too long elution times. The use of 0.06 M CTAB mobile phase provided similar retention times of all compounds as those obtained in 0.04 M CTAB. Different concentrations of 1-butanol (3, 5, and 7%) were assayed, as can be observed in Table 2. The addition of increasing amounts of 1-butanol to the mobile phase reduced retention factors and improved the separation efficiency.

The mobile phase pH is a very important variable affecting the retention of the anticonvulsant drugs, as expected from the protonation constant values. Two different mobile phase pH values were tested: pH 3 and 7. With independence of the nature of compounds, the retention of compounds decreased when the mobile phase pH decreased. This behavior can be explained for basic compounds due to the electrostatic repulsion between the ionic form of the compounds and the surfactant monomers adsorbed on the stationary phase. For acidic drugs, retention also decreases when the mobile phase was varied from 7 to 3, because of the absence of the electrostatic attraction at pH 3 between these drugs and the surfactant monomers on the stationary phase.

As can be observed in Table 2, the use of a micellar mobile phase containing 0.04 M CTAB, 0.05 M phosphate buffer pH 3, and 7% 1-butanol, gives appropriate retention times of anticonvulsants for quantitative purposes (between 2.5 min for acetazolamide and 11.7 min for phenytoin). However, under these conditions, zopiclone eluted in the void volume. For pharmaceutical preparations containing zopiclone, the use of a mobile phase containing 0.04 M CTAB, pH 7, and 3% 1-butanol provided adequate retention time (5.2 min).

Analytical Data

The calibration curve of each analyte was obtained by injections of standard solutions, containing analyte concentrations in the range 1–40 μg/mL for all compounds studied. Both peak area and height were used as dependent variables. Tables 3 and 4 show the regression statistics for the

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Table 2. Retention data (k), efficiency (N) and asymmetry factors (B/A) in different CTAB-1-butanol mobile phases.

CTAB (M)	0.04			0.04			0.04			0.04		
% 1-butanol	3			3			5			7		
pH	7			3			3			3		
Compound	k	N	B/A	k	N	B/A	k	N	B/A	k	N	B/A
Acetazolamide	7.1	3466	1.19	—	—	—	—	—	—	1.3	1122	1.54
Carbamacepine	10.4	2861	1.26	—	—	—	—	—	—	5.9	2462	1.40
Chlordiazepoxide	21.7	3278	1.29	3.3	1420	1.40	2.4	1079	1.22	2.3	1197	1.05
Diazepam	23.6	2641	1.28	20.0	3212	1.30	15.4	2796	1.37	10.1	2465	1.50
Ethosuximide	2.0	349	2.85	2.4	1014	1.39	1.4	961	1.42	1.5	1079	1.51
Phenytoin	26.0	3133	1.30	21.8	4665	1.04	15.8	2715	1.34	10.3	2894	1.29
Phenobarbital	13.3	3005	1.29	8.9	2903	1.38	6.6	2182	1.59	1.4	1695	1.38
Zopiclone	4.2	1199	1.46	2.4	1293	1.27	—	—	—	—	—	—

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Table 3. Regression statistics of the calibration curves, coefficients of variation and limits of detection (peak area).

	Peak area					
Compound	$m \pm ts_m$	$n \pm ts_n$	r	RSD (%)[a]	RSD (%)[b]	LOD (μm/mL)
Acetazolamide	0.33 ± 0.05	−0.3 ± 1.1[c]	0.996	1.2	1.9	0.063
Carbamacepine	1.08 ± 0.03	0.4 ± 0.7[c]	0.9998	0.4	0.3	0.013
Chlordiazepoxide	0.76 ± 0.02	0.2 ± 0.4[c]	0.99993	1.1	2.4	0.109
Diazepam	1.59 ± 0.07	0.2 ± 1.0[c]	0.9998	0.2	3.6	0.098
Ethosuximide	0.0087 ± 0.0007	−0.013 ± 0.016[c]	0.998	3.0	5.2	0.079
Phenytoin	0.070 ± 0.003	0.05 ± 0.13[c]	0.9996	0.7	1.4	0.040
Phenobarbital	0.147 ± 0.009	0.2 ± 0.1[c]	0.9997	0.9	4.0	0.454
Zopiclone	0.21 ± 0.11	−0.4 ± 1.5[c]	0.98	0.8	0.8	0.030

[a]25 ppm.
[b]1 ppm.
[c]Statistically non-significant.
Key: m: slope; n: intercept value; r: regression coefficient; RSD: relative standard deviation, LOD: limit of detection (see text for details).

Table 4. Regression statistics of the calibration curves, coefficients of variation and limits of detection (peak height).

	Peak area					
Compound	$m \pm ts_m$	$n \pm ts_n$	r	RSD (%)[a]	RSD (%)[b]	LOD (μm/mL)
Acetazolamide	1.72 ± 0.03	0.58 ± 0.59[c]	0.99996	0.4	1.8	0.077
Carbamacepine	3.00 ± 0.11	0.5 ± 2.3[c]	0.9998	0.2	1.7	0.068
Chlordiazepoxide	4.79 ± 0.09	2 ± 2[c]	0.99994	1.1	4.7	0.210
Diazepam	2.67 ± 0.14	0.5 ± 1.9[c]	0.9998	0.9	1.4	0.048
Ethosuximide	0.0709 ± 0.0012	−0.01 ± 0.03[c]	0.99996	1.5	4.8	0.443
Phenytoin	0.123 ± 0.008	0.04 ± 0.17[c]	0.9994	1.6	2.2	0.550
Phenobarbital	0.44 ± 0.02	0.2 ± 0.3[c]	0.9998	0.5	3.2	0.295
Zopiclone	0.5 ± 0.2	0 ± 3[c]	0.98	3.5	3.5	0.274

[a]25 ppm.
[b]1 ppm.
[c]Statistically non-significant.
Key: *m*: slope; *n*: intercept value; *r*: regression coefficient; RSD: relative standard deviation, LOD: limit of detection, (see text for details).

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calibration curves of each compound. The curves showed adequate regression coefficients ($r > 0.99$) over the working range. The obtaining of intercepts statistically equal to zero reveals the absence of systematic errors.

Tables 3 and 4 also show some of the analytical parameters obtained for the set of anticonvulsants studied. The repeatability expressed as the relative standard deviation (RSD) of five successive injections of the same solution, was evaluated at two concentration levels: 1 and 25 μg/mL, ($n = 5$). The coefficients of variation ranged in general between 0.2% and 3.5% for the higher concentration level. At the lower concentration level, the relative standard deviation of the method was, as expected, slightly higher than at the 25 μg/mL. In any case, the relative standard deviation was always lower than 5.2% for all the analytes with both areas and heights. So, from these results, it is possible to conclude that the precision of the proposed method is adequate for quality control of pharmaceuticals.

The limits of detection (LOD) were calculated according to the 3s criterion from the standard deviation related to peak area or height, obtained by injecting five times a solution containing 1 μg/mL of each anticonvulsant drug studied. In general, LOD values were lower than 0.5 μg/mL (Tables 3 and 4), which makes this methodology useful for quantification purposes of anticonvulsant agents in pharmaceutical preparations.

Analysis of Pharmaceutical Formulations

The proposed method was applied to the analysis of sample preparations containing anticonvulsants commercially available in Spain. These drugs are in different dosage forms (tablets, pills, capsules, suppositories, injection solution, and drops). The majority of the pharmaceutical preparations contain only one anticonvulsant as an active principle, together with other excipients such as lactose, propilenglycol, ethanol, etc. (see Table 5). But, there were several samples that contained two anticonvulsant agents and other drugs with hypnotic or antipsychotic action. The contents of each analyte in the formulations were determined by triplicate injections of each pharmaceutical.

Figure 1 shows some of the chromatograms obtained. The analyte peaks were adequately separated from other compounds present in the samples, except for the pharmaceutical Edemox (acetazolamide) in which the analyte peaks were slightly overlapped with another peak. Table 5 shows the recoveries and standard deviations obtained when area was used as a dependent variable. The results were reproducible and the recoveries ranged between 90 and 110%. The amount of drug found agree with the declared content within the limits specified by United States Pharmacopeia for these

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Table 5. Pharmaceutical preparations, composition, recoveries and USP tolerances of the compounds determined.

Preparation (presentation) source	Composition	Recoveries ± s (%)	USP tolerances
EDEMOX (tablets) Chiesi Wassermann, S.A	Acetazolamide (DCI) (250 mg) Excipients (c.s)	96.0 ± 0.9[a]	95–105
CARBAMAZEPINA ALTER EFG (tablets) Laboratorios Alter, S.A.	Carbamacepine (200 mg) Excipients (c.s)	100.4 ± 0.9	90–110
HUBERPLEX® (pills) Teofarma iberica, S.A	Chlordiazepoxide HCl (5 mg) Wheat starch (5 mg) Sacarose (44.9 mg) Lactose (43.9 mg) Other excipients (c.s)	95 ± 2	90–110
PSICO-BLOCÁN (tablets) Laboratorio Estedi, S.L	Chlordiazepoxide HCl (10 mg) Scopolamine bromide (2.5 mg) Excipients (c.s)	98 ± 2.2	90–110
ANEUROL (pills) Lacer, S.A	Diazepam (5 mg) Piridoxine HCl (10 mg) Sacarose (46.8 mg) Lactose and other excipients (c.s)	95.8 ± 1.5	90–110
COMPLUTINE (tablets) Novartis Consumer Health, S.A	Diazepam (DCI) (5 mg) Piridoxine (DCI) HCl (10 mg) Lactose and other excipients	106 ± 3	90–110
TEPAZEPAN® (capsules) Almirall Prodesfarma, S.A	Diazepam (DCI) (5 mg) Sulpiride (DCI) (50 mg) Piridoxine HCl (DCI) (5 mg) Excipients (c.s)	99.1 ± 0.3	90–110
DIAZEPAM PRODES (suppositories) Almirall Prodesfarma, S.A	Diazepam (5 mg) Excipients (c.s)	99.0 ± 0.6	90–110

(*continued*)

Table 5. Continued.

Preparation (presentation) source	Composition	Recoveries ± s (%)	USP tolerances
ETOSUXIMIDA FAES (capsules) Productos farmacéuticos FAES	Ethosuximide (250 mg) Excipients (c.s)	112 ± 2	90–110
REDUTONA (tablets) Laboratorio Faes, S.A	Phenytoin (70 mg) Phenobarbital (30 mg) Piridoxine HCl (50 mg) gamma-amino-beta-hydroxi-butíric acid (100 mg) Excipients (c.s)	105 ± 3 102 ± 2	93–107 90–110
GARDENAL® 0.05 (tablets) Aventis Pharma, S.A	Phenobarbital (DCI) (50 mg) Wheat starch and other c.s.p	98.6 ± 0.3	90–110
LUMINAL (injection solution) Química farmacéutica Bayer, S.A	Phenobarbital (200 mg/ml) Excipients: propylenglycol, ethanol (83 mg) Sodium hydroxide and water for injection	92.1 ± 1.1	90–105
GRATUSMINAL® (drops) Almirall Prodesfarma, S.A	Phenobarbital diethilamine (126 mg/ml) Excipients (ethanol)	94 ± 3	90–110
DATOLÁN® (pills) Productos farmacéuticos FAES	Zopiclone (DCI) (7.5 mg) Wheat starch Other excipients (c.s)	111.7 ± 1.5	90–110

[a]The reported recovery for acetazolamide was calculated from peak height because another sample component eluted close to acetazolamide peak end.

preparations. For the pharmaceutical Edemox, acetazolamide was slightly overlapped with some other sample components. However, the use of peak height as dependent variable lead to good recovery 96.0% of this analyte in the sample studied.

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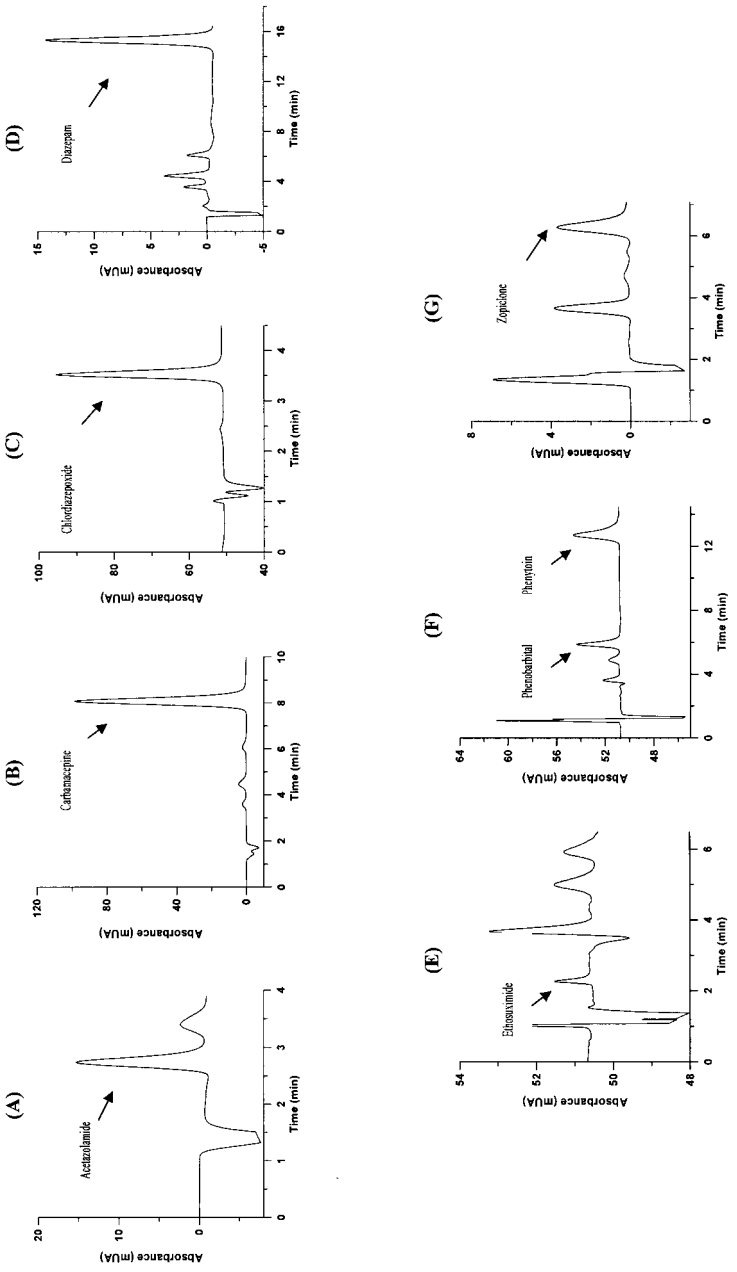

Figure 1. Chromatograms obtained for the pharmaceutical preparations (A) Edemox, (B) Carbamacepina Alter EFG, (C) Huberplex®, (D) Diazepam Prodes, (E) Etosuximida FAES, (F) Redutona, and (G) Datolán.

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CONCLUSIONS

The described method allows a rapid and reproducible determination of different anticonvulsant drugs in pharmaceutical formulations. All the preparations were easily dissolved in micellar medium 0.04 M CTAB, 0.05 M phosphate buffer pH 3, despite the form of presentation (tablets, pills, drops, and suppositories). The analytical parameters of the proposed method, reproducibility, accuracy, and linearity make it useful in quality control in the pharmaceutical industry.

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