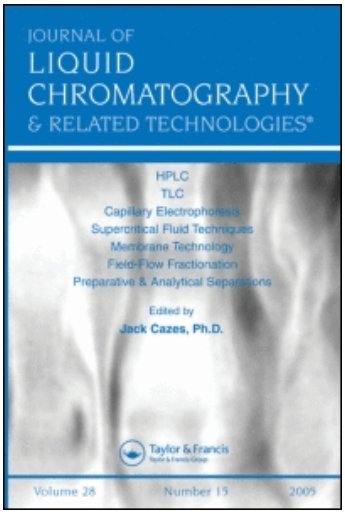

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MICELLAR LIQUID CHROMATOGRAPHIC DETERMINATION OF ANTI-CONVULSANT DRUGS IN PILLS AND CAPSULES

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ABSTRACT

A simple chromatographic procedure is reported for the determination of several anti-convulsant drugs in pharmaceuticals: carbamazepine, and the benzodiazepines bentazepam, halazepam, oxazepam, pinazepam, and tetrazepam. The procedure utilizes a C_{18} column, a hybrid micellar mobile phase of 0.1 M SDS-3% butanol-0.1% triethylamine-0.01 M phosphate buffer (pH 3), and UV detection (230 nm). The drugs eluted in less than 13 min, in accordance to their relative polarities, as indicated by their octanol-water partition coefficients.

The limits of detection (μg/mL), and intra and inter-day repeatabilities (%), for 4 μg/mL were: carbamazepine (0.03, 1.0, 4.1), bentazepam (0.05, 1.3, 1.6), halazepam (0.10, 2.3, 1.3), oxazepam (0.02, 0.9, 7.9), pinazepam (0.01, 0.3, 5.1), and tetrazepam (0.03, 1.1, 6.3). The results were compared with those obtained in a conventional procedure using methanol-water 70:30 (v/v). The contents evaluated with the micellar mobile phase always agreed with the declared compositions, instead, with the

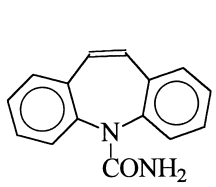
aqueous-organic mobile phase, pinazepam gave poor recoveries and halazepam did not elute at appropriate times.

INTRODUCTION

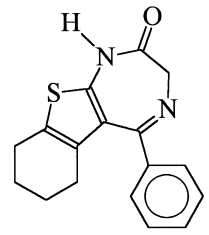
Diverse benzodiazepines, such as halazepam, oxazepam, pinazepam, tetrazepam, and bentazepam, are administered due to their tranquilizing, antidepressive, sedative and anti-convulsant properties.[1] A related compound, carbamazepine, is used as analgesic and anti-convulsant. The four former benzodiazepines are characterized by the presence of a phenyl ring fused to a partially saturated seven membered ring with nitrogen at positions 1 and 4. Bentazepam has an additional five membered ring with a sulphur atom, and carbamazepine has only a nitrogen at position 5 (Figure 1).[2]

The determination of benzodiazepines has been extensively studied, especially in physiological fluids.[3] Originally, these drugs and carbamazepine were determined by UV spectrometry, but nowadays conventional reversed-phase liquid chromatography (RPLC) with aqueous-organic mobile phases is routinely applied. Particularly, the procedures reported for the analysis of pharmaceutical formulations containing oxazepam,[4-7] halazepam,[8] and carbamazepine,[9-12] employ C_{18} and C_8 columns with binary mixtures of methanol-water, and acetonitrile-water, and ternary mixtures of acetonitrile-methanol-water and tetrahydrofuran-methanol-water, as mobile phases. Most of these procedures utilize internal standards such as diazepam, ibuprofen, penitoin sodium, guaiphenesin, phenazone, and *N*-desmethyldiazepam. There is only one report of an RPLC procedure for the determination of bentazepam, which was applied to plasma samples and employs a C_{18} column and a methanol-water mobile phase, with diazepam as internal standard.[13] We have not found any reference to RPLC analysis of the other two drugs (tetrazepam and pinazepam).

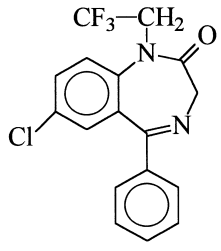
One interesting alternative to the aqueous-organic mobile phases is the use of solutions of surfactants above the critical micellar concentration.[14-16] In this technique, in addition to the formation of micelles, the reversed-phase column packings are covered with a layer of monomers of surfactant that protects and modifies the underlying alkyl-bonded silica phase. Since the solutes partition between three phases (bulk aqueous medium, micelles and stationary phase), the chromatographic behaviour is more complex than in traditional RPLC. A small amount of an organic modifier is usually added to the mobile phases to increase the elution strength and chromatographic efficiencies. Some attractive advantages of micellar mobile phases are that they are non-toxic, non-flammable, biodegradable, and less expensive, in comparison to aqueous-organic solvents, and compounds of diverse polarity can be analyzed under isocratic conditions. The stable behaviour of micellar chromatographic systems permits the accurate prediction of the retention, based on simple models.[17]



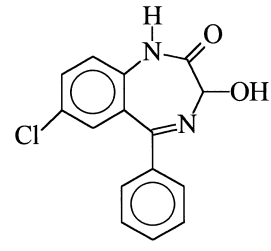
carbamazepine



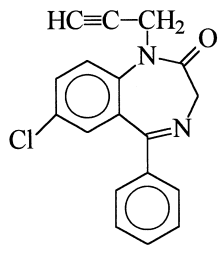
bentazepam



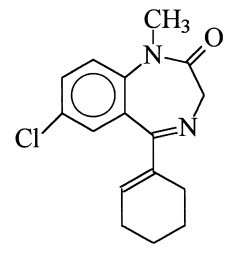
halazepam



oxazepam



pinazepam



tetrazepam

Figure 1. Compounds studied in this work.

In the literature, there are some references on the micellar liquid chromatographic (MLC) determination of carbamazepine in serum, together with other drugs of distinct therapeutical behaviour.[18-21] A cyano column was employed with pure sodium dodecyl sulphate (SDS),[18,19] and Brij-35 mobile phases.[20] A surfactant-mediated column-switching procedure was also reported for carbamazepine, where a C_8 column and a 0.04 M SDS-14% acetonitrile

mobile phase were used in the clean-up step, and a C_{18} column and a 0.04 M SDS-methanol 45:55 (v/v) mobile phase at pH 3, in the analytical separation.[21]

In this work, an MLC procedure with a mobile phase of high elution strength of SDS and butanol is developed for the rapid analysis of pills and capsules containing carbamazepine, bentazepam, halazepam, oxazepam, pinazepam, and tetrazepam. The performance of MLC is compared with a conventional procedure using binary mixtures of methanol-water.

EXPERIMENTAL

Reagents

The reagents used in the mobile phases were: the surfactant sodium dodecyl sulphate (99% purity, Merck, Darmstadt, Germany), the modifiers 1-propanol, 1-butanol, 1-pentanol (Scharlau, Barcelona, Spain) and triethylamine (Fluka, Buchs, Switzerland), and the buffer compounds sodium dihydrogenphosphate (Panreac, Barcelona) and HCl or NaOH (Probus, Badalona, Spain). The mobile phases were filtered through 0.45 μm Nylon membranes (Micron Separations, Westboro, MA, USA). Methanol (Scharlau) was used to condition the column.

The analytes were: carbamazepine (Novartis Farmacéutica, Barcelona), bentazepam (Knoll, Madrid, Spain), halazepam (Shering Plough, Madrid), oxazepam (Boehringer Ingelheim, Barcelona), pinazepam (Tedec-Meiji Farma, Madrid), and tetrazepam (Sanofi Winthrop, Barcelona). The drugs were kindly donated by the cited pharmaceutical laboratories. Stock solutions containing 100 μg/mL were prepared by dissolving the compounds in a few mililiters of methanol, with the aid of an ultrasonic bath (Selecta, Model 617, Barcelona). Nanopure deionized water (Barnstead, Sybron, Boston, MA, USA) was used throughout.

Apparatus

The pH of the mobile phases was measured with a Crison potentiometer (Model micropH 2001, Barcelona), provided with a combined Ag/AgCl/glass electrode. UV spectra and absorbance measurements were obtained with a Perkin Elmer UV-Vis-NIR spectrophotometer (Model Lambda 19, Norwalk, CT, USA). Maximum wavelengths and molar absorptivities of the drugs are given in Table 1.

A Hewlett-Packard (Model HP 1100, Palo Alto, CA, USA) chromatograph was used, which was provided with a quaternary pump, an autosampler and a UV-visible detector set at 230 nm. The signal was acquired by a PC computer

Table 1

Maximum Wavelengths, Molar Absorptivities and log $P_{a/w}$ Values of the Drugs

Compound	λ(nm)	ε(L/mol cm)	log $P_{a/w}$*
Carbamazepine	286	19000	1.98
Bentazepam	250	31500	3.36
Halazepam	226	36500	4.47
Oxazepam	230	32000	2.10
Pinazepam	229	36000	3.40
Tetrazepam	227	23500	3.90

* From Ref. 2.

connected to the chromatograph through an HP Chemstation. Measurement of peak properties and optimization of mobile phase composition were assisted by the *MICHROM* software.[22] An ODS-2 column (Scharlau, 5 μm particle size, 120 mm × 4.6 mm i.d.) was placed after a guard 30-mm long pre-column of similar characteristics (Scharlau). The flow-rate was 0.7 mL/min, and the injection volume, 20 μL.

Chromatographic Analysis

The composition of the micellar mobile phase recommended for the determination of the drugs studied in this work is: 0.1 M SDS, 3% butanol, 0.1% triethylamine, and 0.01 M sodium dihydrogenphosphate. The pH was adjusted at 3 with HCl before the addition of the modifiers. The results were compared with a procedure that uses an aqueous-organic mobile phase of methanol-water 70:30 (v/v).

The analyzed pharmaceuticals were in the form of pills and capsules. For the analyses, ten pills were weighed, ground and homogenized, several portions were taken, weighed and dissolved in 10 mL of methanol with the aid of an ultrasonic bath. Finally, dilution was made with water to give a final concentration of 1 to 10 μg/mL. The capsules were weighed before and after being carefully emptied, to obtain the accurate mass of the capsule contents. Subsequently, the procedure given above was followed. The excipients were not soluble in methanol-water medium, hence, the sample solutions should be filtered before injection into the chromatograph. The standard solutions of the drugs were also filtered. Filtration was always performed directly into the autosampler vials through 0.45 μm Nylon membranes of 13 mm diameter.

RESULTS AND DISCUSSION

Nature of the Modifier

The polarities of carbamazepine and benzodiazepines are low. Consequently, their retention on a C_{18} column was excessive when eluted with pure micellar mobile phases of SDS, and after the addition of the modifiers methanol or propanol, even at high concentrations. Elution at appropriate retention times was finally achieved by the addition of an alcohol with a longer chain, such as butanol or pentanol. There are only two references in the MLC literature on the use of pentanol as a modifier in analytical procedures. This alcohol permitted the elution of diverse hydrophobic compounds, such as steroids (0.1 M SDS-7% pentanol mobile phase)[23] and sulphonamides (0.05 M SDS-2.4% pentanol mobile phase).[24] Butanol has not been employed before in MLC analytical applications, but it has been compared with other modifiers in physico-chemical studies.[25,26]

Butanol yielded better efficiencies and larger retentions than pentanol. The peaks of carbamazepine and oxazepam, on the one hand, and those of bentazepam, halazepam, and tetrazepam, on the other, could not be resolved with pentanol. Butanol was thus preferred to optimize the separation of the six drugs.

pH Selection

No reference was found in the literature on the protonation constants of the studied drugs, but for other benzodiazepines at least two acid-base equilibria with log K_1 ~ 8-11 and log K_2 ~ 2-3 have been reported.[2] Log K_2 is expected to increase in the presence of the anionic SDS micelles, owing to stabilization of the positive charge of the protonated drugs. Thus, at pH 3, benzodiazepines should be partially protonated at the N-4 position (Figure 2).

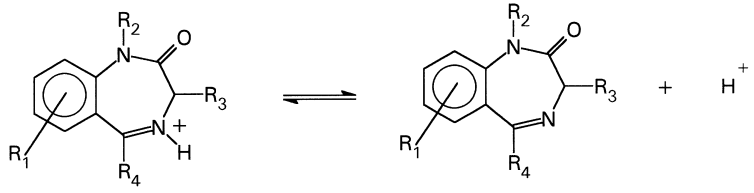


Figure 2. Benzodiazepine acid-base equilibrium.

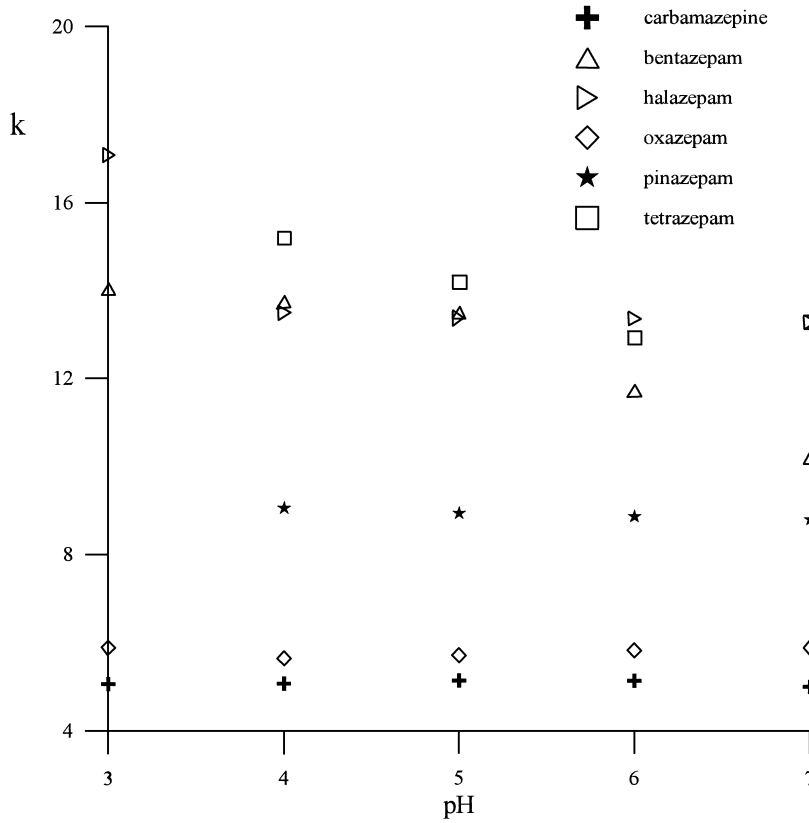


Figure 3. Retention factors *vs.* pH plot for bentazepam, carbamazepine, halazepam, oxazepam, pinazepam, and tetrazepam, eluted with 0.1 M SDS-2% butanol.

A series of measurements were carried out with 0.1 M SDS-2% (v/v) butanol mobile phases, where the pH was varied between 3 and 7. The retention was effectively somewhat larger below pH 4 for halazepam, tetrazepam, bentazepam, and pinazepam (Figure 3). Owing to the greater overlapping of halazepam and bentazepam at pH 4-5, mobile phases were next buffered at pH 3.

Concentrations of Surfactant, Alcohol, and Amine

The elution behaviour of carbamazepine and the benzodiazepines was the usual in MLC: an increase in the concentration of surfactant resulted in decreased retentions and efficiencies, and an increase in the concentration of

alcohol modifier yielded decreased retentions but enhanced efficiencies. Due to the low polarity of the drugs, the efficiencies were, however, still low with added alcohols. The elution strength for butanol was greater than for SDS, in the 1-5% butanol and 0.05-0.15 M SDS concentration ranges.

Amines, such as triethylamine (TEA)[27] and isopropylamine,[28] have been added to the mobile phase in some analytical RPLC procedures proposed for benzodiazepines. The use of an amine is a common practice to protect the silanol groups of the stationary phase, in order to increase peak efficiencies for compounds having amine groups. The effect of TEA on the separation of the drugs was thus examined. The concentration of TEA was varied in the 0-0.2% concentration range for a 0.1 M SDS-2% butanol mobile phase buffered at pH 3. The addition of TEA enhanced the efficiencies for bentazepam, halazepam, and tetrazepam, especially for the latter compound. However, the amine behaved as another modifier and the retention factors of the compounds were reduced. For this reason, the concentration of TEA was limited to 0.1%. The retention factors without amine and with 0.1% amine were: carbamazepine (5.1, 4.6), bentazepam (14.0, 7.9), halazepam (17.1, 10.8), oxazepam (5.9, 5.7), pinazepam (11.6, 7.2), and tetrazepam (15.6, 9.9). Note that the most hydrophobic compounds suffered the larger decrease in retention.

Optimization Strategy

The accurate prediction of the retention behaviour, based on a checked model, can expedite the process of finding the optimal composition of the mobile phase, for a given compound. The following equation has demonstrated to be adequate to describe the retention of many compounds in MLC with hybrid mobile phases, with errors in the 2-4% range:[17]

$$k = \frac{K_{AS}\,\dfrac{1}{1 + K_{AD}\,\varphi}}{1 + K_{AM}\,[M]\,\dfrac{1 + \mathrm{KsubMD}\,\varphi}{1 + K_{AD}\,\varphi}} \qquad (1)$$

where k is the retention factor, [M] and ψ are the concentrations of surfactant and modifier, K_{AS} and K_{AM} describe the association equilibria between the solute in bulk water and stationary phase or micelle, and K_{AD} and K_{MD} are constants that measure the relative variation in the concentration of solute in bulk water and micelles, due to the presence of modifier, and referred to a pure micellar solution (without modifier).

On the basis of the selected modifier, pH and concentration of TEA, an optimization study was carried out using a software developed in our laboratory

(MICHROM), which allows the rapid and reliable simulation of chromatograms based on equations that describe the retention (such as Eq. 1), and peak shape:[29]

$$h(t) = -H \exp\left(\frac{1}{2} \frac{(t - t_R)^2}{[s_0 + s_1 (t - t_R)]^2} \right) \quad (2)$$

where h(t) is height at diverse times, H the peak height, t_R the retention time, s_0 is a measurement of peak width at the maximum, and s_1 a distortion factor. These coefficients were obtained from the values of retention time, efficiency and asymmetry factor. The two latter parameters were interpolated by weighting the inverse of the distance between the predicted and available experimental mobile phases. With MICHROM, the changes in the predicted retention times with mobile phase composition can easily be observed owing to the high simulation speed. It has been checked, for several groups of compounds, that the agreement between predicted and experimental chromatograms is excellent.

The coefficients of the retention model given by Eq. 1 (Table 2) were calculated for each compound, using the retention factors obtained for a set of five mobile phases (0.05 M SDS-1% butanol, 0.05 M SDS-5% butanol, 0.1 M SDS-3% butanol, 0.15 M-1% butanol, and 0.15 M SDS-5% butanol), all containing 0.1% TEA. Peak positions and shapes were then predicted, in the whole factor space.

It was found that a single mobile phase of 0.1 M SDS-3% butanol-0.1% TEA at pH 3 (0.01 M phosphate buffer) permitted the elution of the six drugs in adequate times, although shorter retention times can be obtained by increasing the volume fraction of butanol. Anyway, the prediction of the composition of the mobile phase giving any desired retention time can be made easily using

Table 2

Coefficients of Equation (1)

Compound	K_{AS}	K_{AM}	K_{MD}	K_{AD}
Carbamazepine	27.3	5.0	38.5	42.7
Bentazepam	12.9	4.5	24.8	30.9
Halazepam	20.6	5.8	27.8	37.5
Oxazepam	30.7	6.2	31.3	46.5
Pinazepam	18.2	4.0	78.3	32.5
Tetrazepam	9.7	2.5	58.5	27.5

Eq. 1 and the coefficients in Table 2. The retention times for the selected mobile phase were the following (min): carbamazepine (6.0), oxazepam (6.8), pinazepam (8.3), bentazepam (9.0), tetrazepam (10.7), and halazepam (11.6). The logarithm of the octanol-water coefficients *(log $P_{o/w}$)* for the drugs are given in Table 1. As observed, the drugs eluted in accordance to their relative polarities.

Figures 4a and 4e illustrate predicted chromatograms of drug mixtures (oxazepam, bentazepam, and halazepam in Figure 4a, and carbamazepine, pinazepam, and tetrazepam in Figure 4e). The chromatograms of several pharmaceuticals containing the drugs are also shown in Figure 4.

Figures of Merit

The analysis time was below 13 min for all assayed drugs with a mobile phase of 0.1 M SDS-3% butanol-0.1% TEA at pH 3 (0.01 M phosphate buffer). Calibration curves were prepared with standard solutions of the six drugs (five points and triplicate injections). Peak areas were measured. The concentration ranges were: 2.5-20 μg/mL for carbamazepine, bentazepam, halazepam, pinazepam, and tetrazepam, and 0.5-5 μg/mL for oxazepam. The regression coefficients were always $r > 0.999$. The limits of detection (LODs), evaluated according to the 3s criterion, were in the 10-100 ng/mL range (Table 3). Intra-day and inter-day repeatabilities were evaluated from five and three replicate injections, respectively, at two concentrations (4 μg/mL and 10 μg/mL, Table 3).

Table 3

Limits of Detection, Intra-Day and Inter-Day Repeatabilities with 0.1 M SDS-3% Butanol Mobile Phase

Compound	LOD (μg/mL)	Intra-Day[a] (CV %)		Inter-Day[b] (CV %)	
		4 μg/mL	10 μg/mL	4 μg/mL	10 μg/mL
Carbamazepine	0.03	2.0	0.6	4.1	1.9
Bentazepam	0.05	1.3	0.3	1.6	1.3
Halazepam	0.10	2.3	0.3	1.3	0.6
Oxazepam	0.02	0.9	0.5	7.9	2.8
Pinazepam	0.01	0.3	0.3	5.1	3.0
Tetrazepam	0.03	1.1	0.7	6.3	3.1

[a] Assayed concentration: 4 μg/mL, (n = 5). [b]Assayed concentration: 10μg/mL, (n = 3).

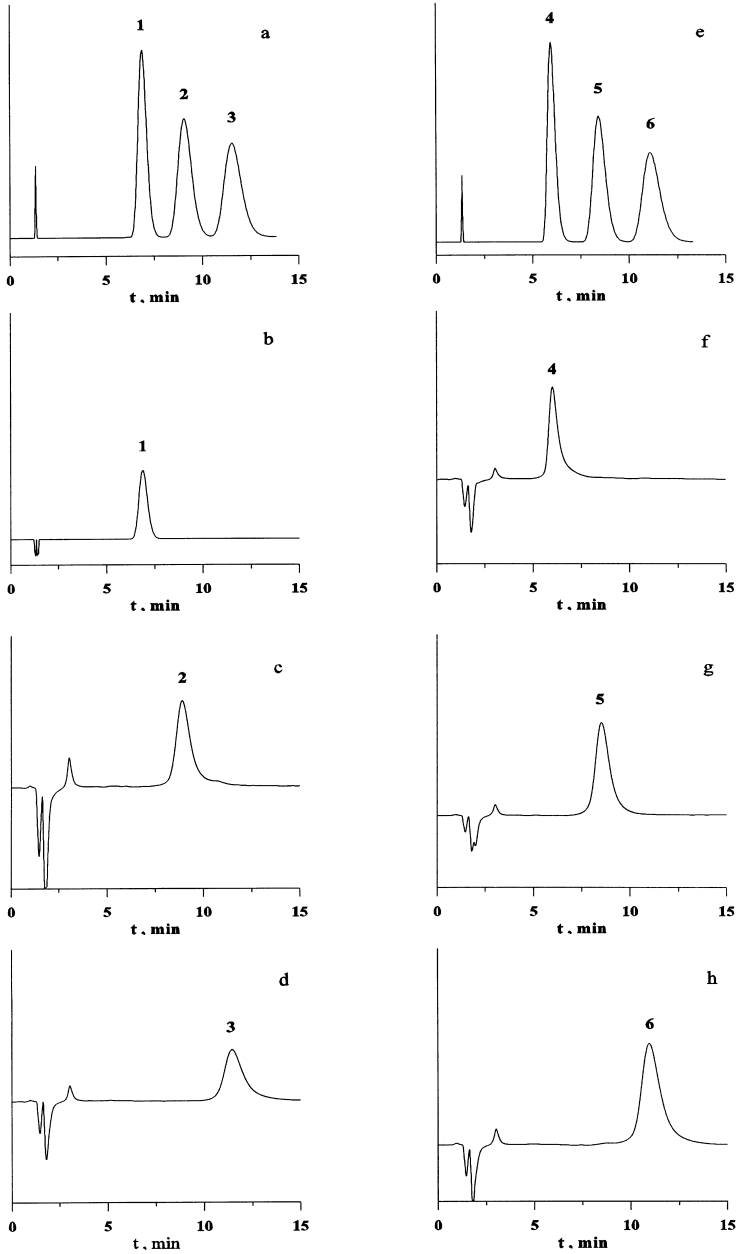


Figure 4. a,e) Predicted chromatograms for mixtures of the drugs, and experimental chromatograms for the pharmaceuticals: b) Adumbran, c) Tiadipona, d) Alapryl, f) Tegretol, g) Duna, and h) Myolastan (see Table 4 for composition). Drugs: oxazepam (1), bentazepam (2), halazepam (3), carbamazepine (4), pinazepam (5), and tetrazepam (6). Mobile phase: 0.1 M SDS-3% butanol-0.1% triethylamine at pH 3 (0.01 M phosphate buffer).

Table 4

Analysis of Pharmaceutical Samples Containing Benzodiazepines and Carbamazepine

Compound	Pharmaceutical (Manufacturer)	Composition (mg)	Found[a] (mg)	CV[a](%) (n = 5)	Found[b] (mg)	CV[b](%) (n = 5)
Carbamazepine	Tegretol 400 (Geigy, Barcelona, Spain)	per pill: carbamazepine (400) and excipients	393	0.5	372	0.3
Bentazepam	Tiadipona (Knoll, Madrid, Spain)	per pill: bentazepam (25), lactose and other excipients	24.0	1.4	21.0	1.9
Halazepam	Alapryl (Menarini, Barcelona)	per pill: halazepam (40), lactose and other excipients	39.9	1.1	---	---
Oxazepam	Adumbran (Behringer-Ingelheim, Barcelona)	per pill: oxazepam (10), lactose and other excipients	10.1	1.9	10.6	0.8
Pinazepam	Duna 2.5 (Tedec-Meiji Farma, Madrid)	per capsule: pinazepam (2.5), lactose and magnesium stearate	2.4	0.6	---	---
	Duna 5 (Tedec-Meiji Farma)	per capsule: pinazepam (5), lactose and magnesium estearate	4.8	1.7	---	---
Tetrazepam	Myolastan (Sanofi Winthrop, Gerona, Spain)	per pill: tetrazepam (50), lactose and other excipients	46.7	2.8	48.2	1.5

[a] Micellar mobile phase: SDS 0.1 M-3% butanol at pH 3 (0.01 M phosphate buffer). [b] Aqueous-organic mobile phase: methanol:water 70:30 (v/v).

Analysis of Pharmaceuticals

The developed procedure was applied to the quality control of several pharmaceuticals marketed in Spain, in the form of pills and capsules. The analyses were performed with samples prepared with ten units of the formulations. Table 4 shows the compositions declared by the manufacturers, and those found according to the recommended MLC procedure and an RPLC procedure with methanol-water 70:30 (v/v).[30]

The retention times for the methanol-water mobile phase were (min): carbamazepine (2.8), oxazepam (3.0), pinazepam (3.7), bentazepam (4.4), and tetrazepam (5.8), which also agreed with the order of polarity. The peak of halazepam was not observed, and therefore, the pharmaceuticals containing this drug could not be analyzed with the aqueous-organic mobile phase. Also, with this mobile phase, the recoveries for the pharmaceuticals containing pinazepam were below 50%. Instead, the recoveries for the micellar mobile phase agreed with the declared compositions.

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