Use of Micellar Mobile Phases for the Chromatographic Determination of Clorazepate, Diazepam, and Diltiazem in Pharmaceuticals

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Abstract

An ODS-2 column, a micellar mobile phase of high elution strength containing 0.1M sodium dodecyl sulfate and 3% (v/v) butanol, and ultraviolet detection at 230 nm are used for the determination of either of two benzodiazepines (clorazepate and diazepam) and a benzothiazepine (diltiazem) in pharmaceuticals. The procedure is shown to be competitive against conventional chromatography with methanol–water mobile phases, especially for diltiazem. The composition of the micellar mobile phase is selected using a predictive strategy based on an accurate retention model and assisted by computer simulation. Calibration graphs are linear at least in the 2.5 to 20 µg/mL, 4 to 20 µg/mL, and 5 to 40 µg/mL ranges for clorazepate, diazepam, and diltiazem, respectively. The intra- and interday repeatabilities (%) are clorazepate (1.7, 5.2), diazepam (0.43, 3.7), and diltiazem (0.36, 3.1). Limits of detection are well below the concentrations of the drugs found in the commercial pharmaceutical preparations analyzed. The drug contents evaluated with the proposed procedure are compared with the declared contents given by the manufacturers. The achieved percentages of label claim are usually between 95 and 104%.

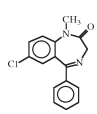
Introduction

Benzodiazepines such as clorazepate and diazepam are chemically characterized by the presence of a phenyl ring fused to an unsaturated seven-membered ring with nitrogen at positions 1 and 4 (Table I). Benzothiazepines such as diltiazem have a similar structure with a sulfur at position 1 and a nitrogen at position 5. Clorazepate and diazepam are used as psychotherapeutic agents with hypnotic, antidepressive, anticonvulsant, and tranquilizing properties (1). Diltiazem is a vasodilator and has been used as a cardiac drug with calcium-blocking activity.

Conventional high-performance liquid chromatography with aqueous–organic mobile phases has been extensively used for the determination of benzodiazepines and benzothiazepines. Particularly, the procedures reported for clorazepate (2), diazepam (3–7), and diltiazem (8,9) employ C_{18} and C_8 columns, binary methanol–water and acetonitrile–water, or ternary methanol–acetonitrile–water mobile phases with ultraviolet (UV) detection at a fixed wavelength or at several wavelengths using a diode array detector.

Micellar mobile phases can replace in many instances conventional aqueous–organic mixtures in the chromatographic control of pharmaceutical preparations with good results. Adequate separations have been reported for cases in which conventional methods do not work (10,11). The technique is an interesting alternative because of the lower cost and toxicity, the often improved selectivities, and the separation of compound mixtures of diverse polarity without requiring gradient elution. Analytical

Table I. Structures, Maximum Wavelengths, and Molar Absorptivities of the Three Drugs Studied

Compound	Manufacturer	Structure	λ (nm)	ε (1 $mol^{-1}cm^{-1}$)
Clorazepate	Sanofi Winthrop (Barcelona, Spain)		228	38,000
Diazepam	Lasa (Barcelona, Spain)		230	31,000
Diltiazem	Dr. Esteve (Barcelona, Spain)		237	24,000

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procedures have been reported for the determination of acetylsalycilic acid (12), diuretics (13–15), sulfonamides (16), steroids (17), catecholamines (18), caffeine (19), β-blockers (20,21), and other drugs.

The stable and reproducible behavior of micellar mobile phases in liquid chromatography (LC) permits an accurate prediction of the retention of solutes after obtaining a model equation that only requires the data from four or five mobile phases (22,23). This model can be very useful in the selection of the best elution conditions. In this study, this approach is applied in order to find the most appropriate mobile phase composition for the separation of clorazepate, diazepam, and diltiazem. It is demonstrated that mobile phases containing sodium dodecyl sulfate (SDS) and a small amount of butanol exhibit a high elution strength and are competitive against conventional methanol–water mobile phases in the analysis of pharmaceuticals containing either of the three drugs, especially diltiazem.

Experimental

Reagents

Clorazepate dipotassium, diazepam, and diltiazem were kindly donated by several pharmaceutical laboratories (Table I). Stock 100-µg/mL solutions were prepared by dissolving the compounds in a few milliliters of methanol (Scharlau, Barcelona, Spain) with the aid of an ultrasonic bath (Selecta, Model 617, Barcelona, Spain) and diluted with water to prepare the standard solutions for calibration. The stability of the drugs was checked for solutions stored at 4°C. In these conditions, the decomposition was less than 2% and 5% for one and two weeks after their preparation, respectively. Stock solutions were thereafter prepared weekly and kept at low temperature.

Micellar mobile phases containing the surfactant SDS (99% purity) (Merck, Darmstadt, Germany); the modifiers 1-propanol, 1-butanol, or 1-pentanol (Scharlau); and triethylamine (TEA) (Fluka, Buchs, Switzerland) were assayed. The pH was adjusted with sodium dihydrogenphosphate (Panreac, Barcelona, Spain) and HCl or NaOH (Probus, Badalona, Spain) before the addition of the organic modifiers. Distilled deionized water (Barnstead, Sybron, Boston, MA) was used throughout. The mobile phases were filtered through 0.45-µm nylon membranes (Micron Separations, Westboro, MA).

The micellar mobile phase selected for the determination of the drugs was 0.1M SDS–3% (v/v) butanol. A 70:30 (v/v) methanol–water mobile phase was also used to validate the analysis of the pharmaceuticals.

Apparatus

Absorbance measurements were obtained with a PerkinElmer UV–visible (vis)–near-infrared spectrophotometer (Model Lambda 19, Norwalk, CT). Maximum wavelengths and molar absorptivities of the analytes are given in Table I.

A chromatograph that Hewlett-Packard (Model HP 1100, Palo Alto, CA) provided with a quaternary pump, an autosampler, and a UV–vis detector was used. An ODS-2 column (5-µm particle size, 125- × 4.6-mm i.d.) was placed after a 30-mm guard column with similar characteristics (Scharlau). The columns were washed weekly with 60 mL of water, followed by 60 mL of methanol. Monitoring was performed at 230 nm. The injection volume was 20 µL and the flow rate 0.7 mL/min. The dead time was determined as the mean value of the first deviation of the baseline obtained in each chromatogram after the injection of the compound solutions.

The signal was acquired through an HP Chemstation. The chromatographic data were treated with MICHROM—an MS-DOS software package developed in our laboratory and commercialized by Marcel Dekker (24).

Procedures

The pharmaceuticals were presented as bags of powder, pills, capsules, or enemas. For the analyses, the powder contents from 10 bags were weighed and homogenized. Several portions were taken, weighed, and dissolved in 10 mL of methanol with the aid of an ultrasonic bath. Dilution was made with water to give a final concentration of 10 to 20 µg/mL. For the pills and capsules, ten units were also taken, and the capsules were carefully emptied to obtain the accurate mass of the contents. The pill and capsule contents were ground to fine powder and homogenized, and the same procedure as for the powder was subsequently followed. The contents of five units of the enemas were mixed, and aliquots of 5 mL were taken and diluted with methanol.

The excipients were not soluble in a methanol–water medium, thus sample solutions were filtered before their injection into the chromatograph through the 0.45-µm nylon membranes. The standard solutions of the drugs were also filtered. The filtration was always performed directly into the autosampler vials.

Results and Discussion

Selection of pH and modifier

The three drugs clorazepate, diazepam, and diltiazem were highly retained in a C_{18} column. The elution strength of the micellar mobile phases of SDS without any modifier or with added propanol (even at high concentration) was not sufficient to elute the drugs in times below 30 min. The addition of alcohols with a greater chain length such as butanol or pentanol was required.

The retention of diltiazem with a protonation constant of $\log K$ = 7.7 was approximately constant in the working pH range of a C_{18} column. Diazepam and clorazepate exhibited in contrast an acid–base equilibrium in an acid medium at the N4 position and changed their retention with pH. The protonation constant of diazepam has been reported to be $\log K = 3.3$ in an aqueous nonmicellar medium, the constants for other benzodiazepines are similar or lower (25). However, the cationic protonated species of benzodiazepines should be electrostatically attracted to the anionic SDS micelles and thus stabilized. This interaction together with the hydrophobic association with the micelles will increase the log K values of the drugs. Therefore, at low pH, the cationic species will dominate. Accordingly, the retention of the two benzodiazepines was observed to increase to a small extent at pH < 4.5 in mobile phases of 0.1M SDS–3% butanol and 0.1M SDS–3% pentanol.

In this study, pH 3 was selected to elute the three drugs. Clorazepate underwent rapid acid mediated dehydration/decarboxylation in order to give rise to *N*-desmethyldiazepam (nordiazepam) (26,27), but the drug is relatively stable in a neutral medium and above. It has been reported that the parent drug cannot be observed in aqueous–organic mobile phases at pH < 5 because of the rapid decarboxylation rate (26). We checked that when using the micellar mobile phases at pH 3, the peaks of clorazepate (at lower retention time) and nordiazepam are well resolved. Also, apparently, clorazepate was stabilized in the micellar medium because the neutral solutions of the parent drug gave rise only to the peak at a lower retention time.

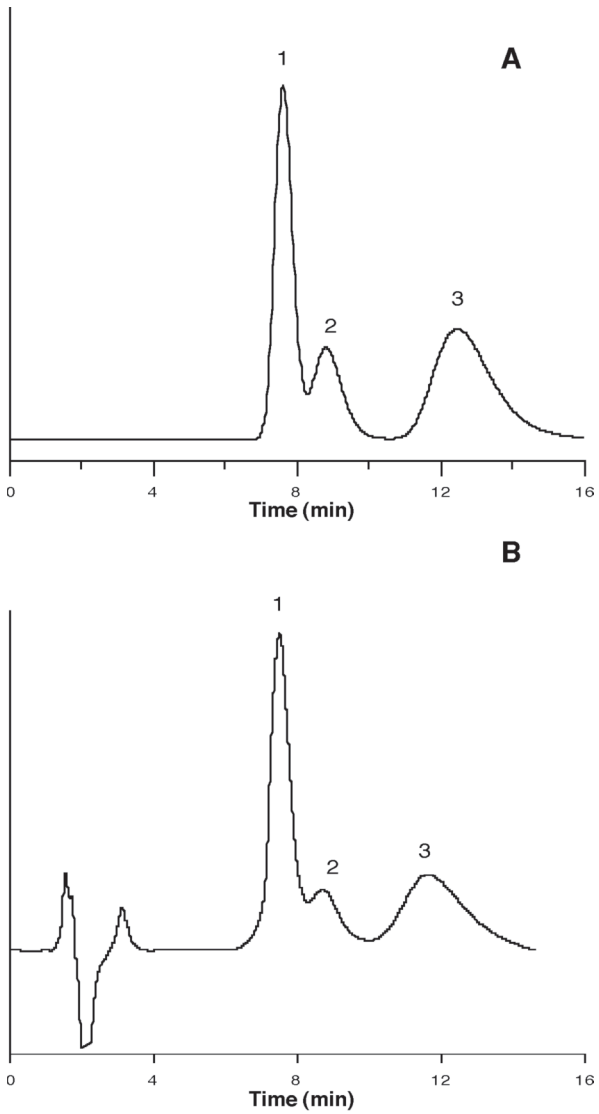

Figure 1. Predicted (A) and experimental (B) chromatograms for a mixture of clorazepate, 1; diazepam, 2; and diltiazem, 3, eluted with 0.1M SDS–3% butanol at pH 3.

Table II. Parameters in Equation 1 and Relative Global Prediction Errors

Compound	K_{AS}	K_{AM}	K_{AD}	K_{MD}	RE (%)
Clorazepate	131.9	117.5	157.6	8.4	1.3
Diazepam	90.9	58.4	69.6	16.5	1.2
Diltiazem	69.3	39.8	23.6	4.1	3.2

An adequate control of the retention was achieved by varying the concentrations of surfactant (SDS) and the organic modifier (butanol or pentanol). However, butanol was preferred to pentanol because of the smaller reduction in the retention times of the drugs and larger efficiencies, which permitted better separations. The elution strength was otherwise similar or lower for butanol than for SDS. As an example, taking as reference a mobile phase of 0.05M SDS–1% butanol, the reduction in retention times for the three drugs was approximately 25 to 30% when SDS was increased to 0.15M, whereas a reduction of approximately 15 to 30% was achieved for 3% butanol.

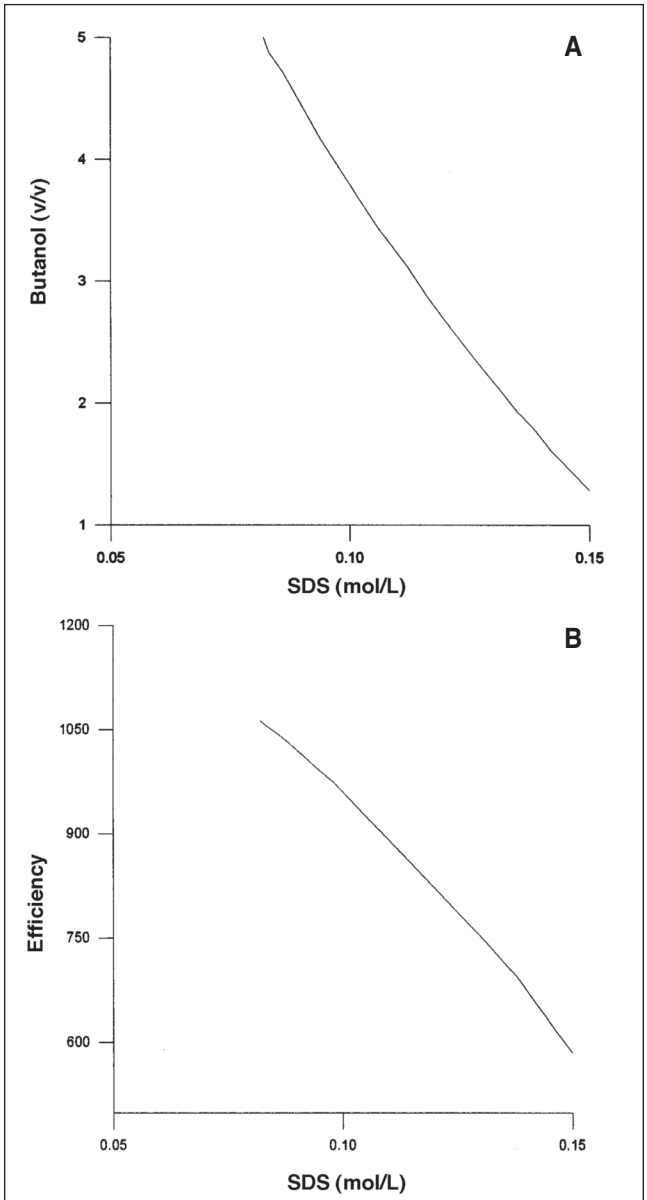

Figure 2. Mobile phase composition isoline for the elution of clorazepate in 7 min (A) and variation of the efficiency with the composition of the mobile phase (B). For B, the concentration of butanol should be read in A at each SDS concentration.

Use of an amine to enhance the efficiencies

The use of an amine (usually TEA or isopropylamine) as a component of the mobile phase to protect the silanol groups of the stationary phase and increase the efficiencies is a usual practice in reversed-phase LC for compounds having amine groups. Some chromatographic procedures with aqueous–organic mobile phases that have been reported in literature for benzodiazepines include an amine in the mobile phase (28–30). TEA has also been used to increase the efficiencies of several compounds eluted with micellar mobile phases (31–33). For this reason, we studied the influence of TEA on the chromatographic behavior of the three drugs. However, it was observed that the efficiencies scarcely increased when the concentration of TEA was modified in the 0 to 0.5% range. The amine acted as a modifier of the retention, and for larger concentrations the drugs eluted near the void volume. Because of the extra reduction in the retention, the addition of the amine was not found convenient.

Table III. Limits of Detection and Intra- and Interday Repeatabilities with 0.1M SDS–3% Butanol

Compound	LOD (µg/mL) (*n* = 10)	Intraday* CV (%) (*n* = 5)	Interday* CV (%) (*n* = 3)
Clorazepate	0.10	1.7 (4)	5.2 (4)
Diazepam	0.02	0.43 (6)	3.7 (6)
Diltiazem	1.0	0.36 (8)	3.1 (8)

* Assayed concentration in µg/mL is given in parenthesis.

Selection of the mobile phase

In LC, interpretive optimization strategies can be more efficient and reliable than sequential approaches. These strategies can be

Table IV. Analysis of Pharmaceuticals Containing Clorazepate or Diazepam

Compound	Pharmaceutical (laboratory)	Composition (mg)	Found* (mg)	CV (%)* (*n* = 5)	Found (mg)	CV (%) (*n* = 5)
Clorazepate	Tranxilium pediátrico (Sanofi, Girona, Spain)	per unit powder: clorazepate (2.5), sodium saccharose, lactose, and other excipients	2.3	2.1	–	–
	Tranxilium 5 (Sanofi)	per capsule: clorazepate (5) and excipients	4.9	1.3	5.1	3.2
	Tranzilium 10 (Sanofi)	per capsule: clorazepate (10) and excipients	9.7	1.6	9.1	1.1
	Tranxilium 15 (Sanofi)	per capsule: clorazepate (15) and excipients	14.7	2.8	14.5	1.2
	Tranxilium 50 (Sanofi)	per pill: clorazepate (50), lactrose, and other excipients	48.3	0.01	48.1	0.05
	Dorken 25 (Roger, Barcelona, Spain)	per pill: clorazepate (25), GABOB‡ (150), pyridoxine chlorhydrate (75), and excipients	24.9	0.61	25.1	0.82
Diazepam	Stesolid 5 (Lasa, Barcelona, Spain)	per enema: diazepam (5), benzoic acid, ethanol, and other excipients	5.0	1.4	–	–
	Stesolid 10 (Lasa)	per enema: diazepam (10), benzoic acid, ethanol, and other excipients	10.4	1.9	–	–
	Gobanal (Normon, Madrid, Spain)	per pill: diazepam (5), pyridoxine chlorhydrate (10), and excipients	5.4	1.2	4.5	1.8
	Ansium Lesvi (Lesvi, Barcelona, Spain)	per capsule: diazepam (5), sulpiride (50), and excipients	4.8	1.2	4.6	1.2
	Pacium (Uriach, Barcelona, Spain)	per capsule: diazepam (5), pyridoxine chlorhydrate (10), and excipients	5.2	3.7	5.3	4.2
	Edym sedante (Vita, Barcelona, Spain)	per capsule: diazepam (2.5), metoclopramide chlorhydrate (5), dimethicone (100), and excipients	2.6	2.3	2.4	0.08
	Vincosedam (Reig Jogré, Barcelona, Spain)	per pill: diazepam (5), pyridoxine chlorhydrate (10), and excipients	5.4	2.5	–	–

* Micellar mobile phase: 0.1M SDS–3% butanol at pH 3.
† Aqueous–organic mobile phase: 70:30 methanol–water.
‡ GABOB, 4-amino-3-hydroxybutyric acid.

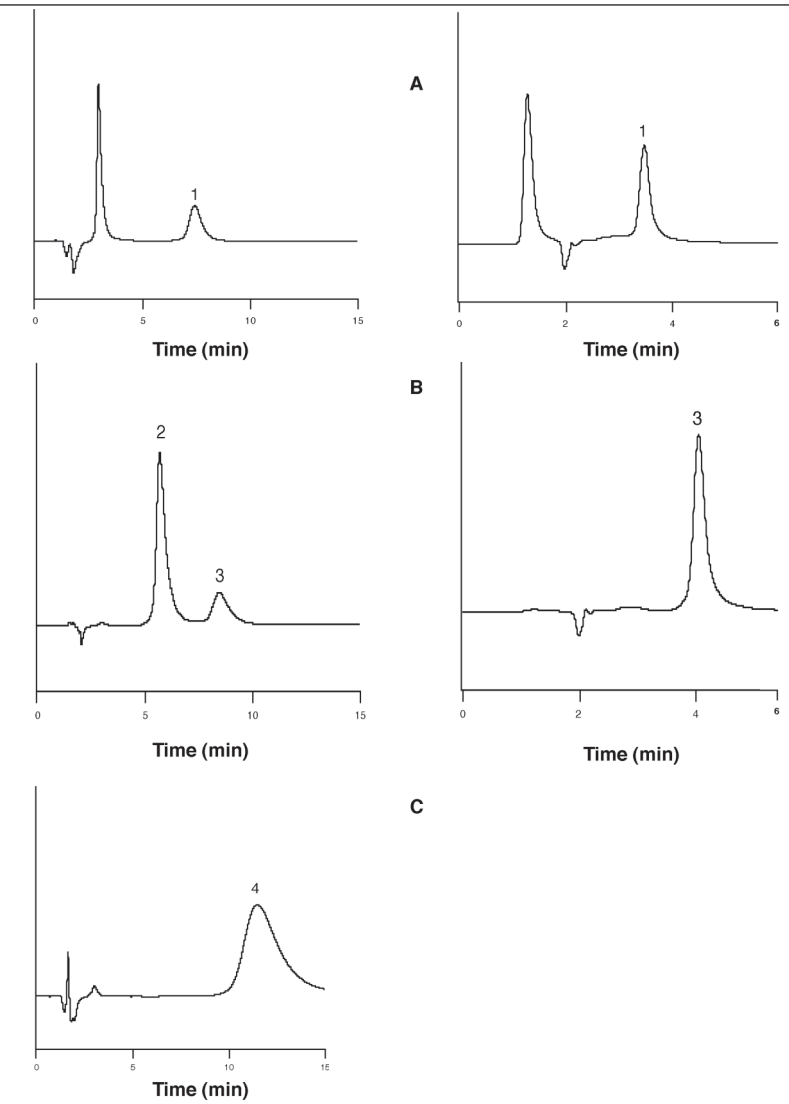

Figure 3. Experimental chromatograms for Tranxilium 50 (A), Ansium Lesvi (B), and Diltiwas 120 (C). Compounds: clorazepate, 1; sulpiride, 2; diazepam, 3; and diltiazem, 4. Mobile phases: 0.1M SDS–3% butanol at pH 3 (left), and 70:30 methanol–water (right).

assisted by computer simulation, which can mimic the methodology that is followed by experienced chromatographers with reduced time and effort. We selected the most convenient mobile phase composition with the aid of MICHROM (24). This software allows for the graphic observation of changes in the chromatograms when the user progressively varies the concentrations of the surfactant and modifier. The mathematical model used to describe the retention behavior of the eluted compounds is:

$$k = \frac{K_{AS}\dfrac{1}{1+K_{AD}\varphi}}{1+K_{AM}[M]\dfrac{1+K_{MD}\varphi}{1+K_{AD}\varphi}} \qquad \text{Eq. 1}$$

where k is the retention factor; $[M]$ and φ are the concentrations of surfactant forming the micelles and modifier, respectively; K_{AS} is the partition coefficient of the solute between water and the stationary phase multiplied by the phase ratio; K_{AM} is the association constant between the solute and micelle; and K_{AD} and K_{MD} describe the modification of the water–micelle equilibrium in the presence of a modifier. The determination of the parameters in equation 1 requires the retention data from an experimental design containing at least four mobile phases. The prediction errors with this equation are frequently in the 2 to 4% range (22).

The description of the peak shape (required for the simulation of chromatograms containing asymmetrical peaks) was performed with a previously proposed modified Gaussian model. In this model, a linear equation substitutes the standard deviation of the Gaussian curve (23):

$$h(t) = He^{-(1/2)([t-t_R]/[s_0+s_1(t-t_R)])^2} \qquad \text{Eq. 2}$$

Table V. Analysis of Pharmaceuticals Containing Diltiazem

Pharmaceutical (laboratory)	Composition (mg)	Found* (mg)	CV (%)* (n = 5)
Diltiwas 60 (Wassermann, Barcelona, Spain)	per pill: diltiazem chlorhydrate (60), lactose, and other excipients	58.0	1.4
Diltiwas Retard 120 (Wassermann)	per capsule: diltiazem chlorhydrate (120), saccarose, and other excipients	120.2	3.6
Masdil 60 (Dr. Esteve, Barcelona, Spain)	per pill: diltiazem chlorhydrate (60), lactose, and other excipients	59.6	3.8
Masdil Retard (Dr. Esteve)	per pill: diltiazem chlorhydrate (120), saccharose, and other excipients	112.4	0.44
Masdil 300 (Dr. Esteve)	per capsule: diltiazem chlorhydrate (300) and excipients	285.3	0.65

* Micellar mobile phase: 0.1M SDS–3% butanol at pH 3.

where H is the peak height, t_R the retention time, s_0 a measurement of peak width at the maximum, and s_1 a factor that quantitates peak distortion. The s_0 and s_1 coefficients were calculated by the interpolation of the efficiencies and asymmetry factors obtained in the mobile phases of the experimental design for the solute peaks.

The experimental design used for the three drugs consisted of five mobile phases—four located at the corners of a rectangular factor space and the fifth in its center. The limits of the factor space (surfactant and alcohol) were selected according to the studies described previously. The concentration of SDS and the volume fraction of butanol were in the 0.05 to 0.15M and 1 to 5% ranges, respectively. Table II shows the values of the parameters in equation 1 and the relative global errors obtained in the prediction of the retention factors. The prediction errors were below 3%.

The elution of the three drugs using the same mobile phase was first considered. Upon simulation of the chromatograms with MICHROM, it was found that adequate retention times (7.6, 8.8, and 12.5 min for clorazepate, diazepam, and diltiazem, respectively) were obtained for 0.1M SDS–3% butanol, which was selected for the analyses of the pharmaceuticals. The prediction capability of the interpretive strategy that was assessed by comparing the experimental and predicted chromatograms for a mixture of the three drugs eluted with this mobile phase was checked to be satisfactory (Figure 1). Otherwise, the retention times for clorazepate and diazepam that were eluted with 70:30 (v/v) methanol–water were 3.5 and 4.0 min, respectively. With this mobile phase, diltiazem appeared as a double deformed peak with a retention time that changed with the concentration of the drug between 15 and 18 min.

The use of a different mobile phase for each drug (yielding retention times as low as possible) was also considered. Because the micellar mobile phase had two components (SDS and butanol), there are several combinations of both that will lead to the same retention time for a given compound. However, the efficiencies of the peaks will differ because they depend on the concentrations of SDS and alcohol in the mobile phase. The predictive strategy was again useful to obtain these concentrations. As an example, Figure 2 depicts the retention isoline and the corresponding efficiencies for clorazepate eluted in 7 min. The efficiencies ranged from N = 584 for 0.15M SDS–1.3% butanol to N = 1063 for 0.082M SDS–5% butanol. Because of the higher efficiencies, the use of larger concentrations of butanol are preferable. It should be noted that the efficiencies achieved with the methanol–water mobile phase were similar.

Analysis of pharmaceutical preparations

Calibration curves (five points and triplicate injections) were prepared with standard solutions of the three drugs eluted with 0.1M SDS–3% butanol: 2.5–20 µg/mL for clorazepate, 4–20 µg/mL for diazepam, and 5–40 µg/mL for diltiazem. In all cases, the regression coefficients were $r > 0.999$. The limits of detection (LOD) according to the 3-s criterion and intra- and interday repeatabilities are given in Table III.

The developed procedure was applied to the determination of clorazepate, diazepam, and diltiazem in pharmaceuticals commercialized in the Spanish market. In each case, the analyses were performed with samples taken after mixing ten units of the preparations for the powder, pills, and capsules and five units for the enemas. Figure 3 illustrates the chromatograms achieved for three pharmaceuticals using the proposed procedure and a 70:30 methanol–water mobile phase (34). The results obtained with the micellar mobile phase were always in agreement with the declared contents, and the analyses did not present any difficulty. In contrast, several problems appeared when the analyses were performed with the aqueous–organic mobile phase.

Table IV shows the declared compositions and results for the pharmaceuticals that contained clorazepate and diazepam, which were obtained with 0.1M SDS–3% butanol and 70:30 methanol–water. The results for diltiazem with the micellar mobile phase are given in Table V. As already commented, a deformed peak was achieved for this drug with 70:30 methanol–water (also with 80:20 methanol–water), which made the quantitation unfeasible. Also, for the methanol–water mobile phase, the chromatograms obtained with Tranxilium pediátrico (clorazepate) and Stesolid 5 and 10 (diazepam) showed a large peak near the void volume, and the recoveries were below 50%. For Vincosedam (diazepam), the peak of the analyte was overlapped with the peak of pyridoxine chlorhydrate.The results of the analyses indicate that the optimized micellar procedure is adequate for the assay of the three drugs in pharmaceuticals with recoveries close to 100%. The proposed procedure with micellar mobile phases has the advantage of using a small amount of organic solvent (only 3% butanol versus 70% methanol in the conventional procedure). Butanol is also less toxic than methanol and is highly retained in the SDS micellar solution, which reduces the risk of evaporation.

Acknowledgments

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