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Micellar liquid chromatographic determination of five antianginals in pharmaceuticals

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Abstract

A procedure was developed for the determination of five antianginals (diltiazem, nadolol, nifedipine, propranolol and verapamil), using hybrid micellar mobile phases of sodium dodecyl sulphate (SDS) and pentanol, a C18 column and UV detection. All possible combinations of antianginals were resolved and determined using a mobile phase of 0.05 M SDS–5% pentanol with an analysis time of 9 min. Repeatabilities and intermediate precision were evaluated at four different drug concentrations in the 2–20 μg/ml ($n = 5$) range. Limits of detection were in the range 0.028 μg/ml for diltiazem and 0.130 μg/ml for verapamil. The range of the limit of quantitation was from 0.092 to 0.431 μg/ml for the same compounds. Antianginal drugs were studied in pharmaceuticals with no interference from related compounds. The results of the analyses of pharmaceuticals formulations were in agreement with the declared compositions.

Keywords: Column liquid chromatography; Micellar liquid chromatography; Antianginals; Pharmaceuticals

1. Introduction

Angina occurs when the muscular wall of the heart does not get enough oxygen. Antianginal agents (AAs) typically increase blood flow by either increasing the oxygen supply or decreasing oxygen demand by the heart. These drugs are administered in different forms. They can be taken regularly over a long period to reduce the number of attacks or just before some activity to prevent an attack, or even when an attack begins in order to relieve the pain/pressure. Six groups of drugs [1] (nitrates, β-blockers, calcium channel blockers, potassium channel openers, antiplatelet agents and cholesterol lowering agents) are used in the management of angina and are frequently administered in combination. It is always preferable to add a small dose of a second drug rather than increase the dose of the original drug. This allows both the first and second drug to be used in the low dose range that is more likely to be free of side effects. Some of these drugs relieve angina, some prevent episodes of angina, and a few of them reduce the risk of a heart attack and sudden death [1].

If treatment of angina with calcium channel blockers or β-blockers alone in monotherapy fails, an association of both can be effective and safe even in the paediatric age [2–11]. β-Blockers diminish the heart rate, resulting in decreased myocardial oxygen demand and increased oxygen delivery to the heart, and also decrease myocardial contractility, thus helping to conserve energy or decrease demand. Calcium channel blockers are first-line agents for the treatment of angina, hypertension and supraventricular tachycardia. They cause peripheral arterial vasodilation and reduce myocardial contractility (negative inotropic action), which results in a lower myocardial oxygen demand. The combination involves one β-blocker and one calcium channel blocker (e.g., propranolol and diltiazem; propranolol and verapamil; nadolol and nifedipine, etc.).

It is therefore necessary to have a fast, reliable and selective method for the simultaneous quantification of a mixture of these compounds in pharmaceuticals. The use of high performance liquid chromatography (HPLC) has been reported for the analysis of combinations of different AAs in several matrices, using UV [12–14] or MS detection [15]. Capillary electrophoresis [16], thin layer chromatography [17] and gas chromatography [18] have also been used.

Micellar liquid chromatography [19] (MLC), which uses a surfactant solution with a concentration above the critical micel-

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lar concentration as the mobile phase, constitutes an alternative to conventional HPLC [20–22]. The simultaneous elution of hydrophobic and hydrophilic analytes is possible [23,24] without the need for a gradient elution, and direct injection of physiological samples becomes feasible due to the solubility of proteins in the micelles. In addition, the surfactant monomers appear to displace the drug bound to the protein, releasing it for partitioning in the stationary phase. The compatibility with conventional reversed-phase column packings is particularly attractive. The stable and reproducible behaviour of micellar mobile phases allows the accurate prediction of the retention of solutes with a model that can also be used to optimise the separation of mixtures of solutes [23]. MLC has proved to be a useful technique in the determination of diverse groups of compounds [25–29].

The optimisation of the experimental conditions for the determination of combinations of calcium channel blockers (diltiazem, nifedipine and verapamil) and β-blockers (nadolol and propranolol), generally associated in pharmaceutical formulations, has been done to achieve a procedure in which the analysis time and resolution of five antianginals will be the best. The uses of micellar mobile phases simplify and expedite the establishment of the optimal conditions for analysis and performance of the procedures.

The purpose of this work was to develop a rapid, simple and selective MLC procedure for screening some AAs in pharmaceutical formulations using a C18 column and UV detection.

2. Materials and methods

2.1. Chemicals and reagents

The reagents used in the mobile phases were the surfactant sodium dodecyl sulphate (99% purity, Merck, Darmstadt, Germany), (Scharlab, Barcelona, Spain), the buffer salt sodium dihydrogenphosphate (Panreac, Barcelona) and NaOH (Probus, Badalona, Spain).

The antianginals (Table 1) nadolol (ND), nifedipine (NF), propranolol (PR), and verapamil (VR) were from Sigma (St. Louis, MO, USA), and diltiazem (DL) was kindly donated by Laboratorios Esteve (Barcelona).

2.2. Instrumentation and chromatographic conditions

The balance used was a Mettler-Toledo AX105 Delta-Range (Greifensee, Switzerland). The pH was measured with a Crison potentiometer (Model micropH 2001, Barcelona), equipped with a combined Ag/AgCl/glass electrode. An ultrasonic bath was used to dissolve the standards and pharmaceuticals (model Ultrasons-H, Selecta, Abrera, Barcelona, Spain).

The chromatograph used was an Agilent Technologies Series 1100 (Palo Alto, CA, USA), equipped with a quaternary pump, an autosampler (20 μl injection volume), and a UV–vis detector (190–700 nm range). Monitoring was performed at 220 nm. The micellar mobile phase recommended for the analysis of the drugs is 0.05 M SDS–5% pentanol at pH 7. An ODS-2 column Kromasil (5 μm particle size, 150 mm × 4.6 mm i.d.) was used (Scharlab, Barcelona). Injection of the solutions into the chromatograph was performed through a Rheodyne valve (Cotati, CA, USA). The flow-rate was 2.0 ml/min. The dead time was determined as the mean value of the first significant deviation from the base-line in the chromatograms of the compounds. The signal was acquired and treated by a PC connected to the chromatograph, through an HP Chemstation (Rev. A.10.01). MICHROM, an MS-DOS software application, was also used in modelling [23].

2.3. Preparation of solutions

Stock solutions containing 100 μg/ml of drugs were prepared in a few millilitres of methanol and 0.1 M SDS, and stored at 4 °C. The working standards (1–25 μg/ml) were freshly prepared from the stock solutions by dilution with the appropriate volume of 0.1 M SDS. Nifedipine is highly sensitive to light therefore some special conditions had to be taken into account: solutions were prepared in amber flasks and were protected from light with aluminium foil to avoid its extensive photochemical degradation.

Pharmaceuticals considered in this work were in the form of tablets. For the analysis, 10 units were weighed, ground to fine powder and homogenised, so as to be able to take fixed portions that were weighed separately, and each one was dissolved in a small amount of methanol and diluted with the selected micellar mobile phase. Excipients were not soluble in this medium and, hence, the sample solutions had to be filtered before injection into the chromatograph.

The micellar mobile phases, standard solutions of antianginals, and pharmaceuticals were filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA).

2.4. Mathematical treatment

The retention of the antianginals was modelled according to [30]:

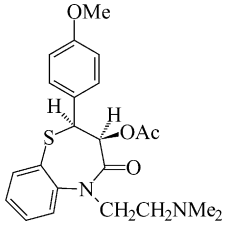
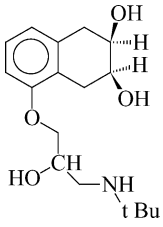
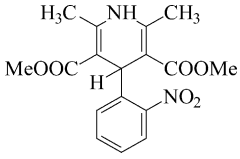
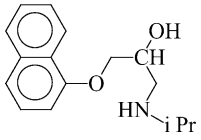
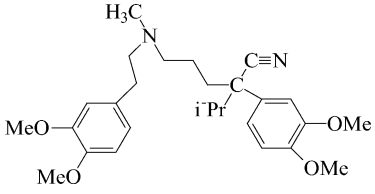
$$k = \frac{K_{AS}(1/1 + K_{AD}\varphi)}{1 + K_{AM}(1 + K_{MD}\varphi/1 + K_{AD}\varphi)[M]} \quad (1)$$

where [M] and φ are the concentrations of surfactant and modifier, K_{AS} and K_{AM} correspond to the equilibria between solute in bulk water and stationary phase or micelle, respectively; K_{AD}, K_{SD}, and K_{MD} measure the relative variation in the concentration of solute in bulk water, stationary phase and micelles due to the presence of modifier, as compared to a pure micellar solution (without any modifier).

The optimisation of the resolution of mixtures of compounds was performed by measuring the overlapping fractions of each chromatographic peak, and the shape of the chromatographic peaks was also modelled to obtain the overlapping fractions and predict chromatograms, according to equations developed by Lapassio et al. [31]. Efficiencies of the peaks were evaluated with the equation suggested by Foley and Dorsey [32].

Values of retention times for the drugs were otained for several mobile phases (SDS–pentanol) using Eq. (1). Two studies

Table 1
Structure log $P_{o/w}$ and pK_a values of the antianginal agents that were studied: calcium channel blockers (DL, NF and VR) and beta-adrenergic antagonists (ND and PR)

Compound	Structure	log $P_{o/w}$	pK_a
Diltiazem (DL)		2.70	7.70
Nadolol (ND)		0.23	9.39
Nifedipine (NF)		3.13	–
Propranolol (PR)		3.56	9.45
Verapamil (VR)		3.53	8.92

were performed keeping one parameter constant, so that the influence of the concentration of surfactant or alcohol on the drug behaviour could be observed. Results of these values were taken and adjusted using the following equation:

$$\frac{1}{k} = c_0 + c_1[\mathrm{M}] + c_2\varphi \quad (2)$$

in which c_0, c_1 and c_2 are adjustment coefficients.

2.5. Method validation

Validation of method was performed in the selected mobile phase, 0.05 M SDS–5% pentanol at pH 7. This study includes precision, linearity, limit of detection (LOD) and limit of quantification (LOQ) determinations. Recovery of compounds in pharmaceuticals was determined at different concentration levels.

3. Results and discussion

3.1. Elution behaviour in SDS–pentanol mobile phases

The equilibria between the monoprotonated (BH^+) and non-protonated (B) antianginals (acid-base constants, pK_a = 7.7–9.45, see Table 1) take place outside the working pH range of a C18 column (3–7). For these compounds, the retention was thus the same using mobile phases of SDS at pH 3 and 7. For this reason the subsequent work was carried out using mobile phases buffered with sodium dihydrogenphosphate at pH 7.

The association of the protonated antianginals to an SDS-modified C18 column was too strong, as indicated by the long retention times obtained using pure micellar eluents of surfactant (without organic modifiers). Owing to the high degree of hydrophobicity of the compounds (except ND, which is a hydrophilic drug) pentanol was selected to expedite the elution

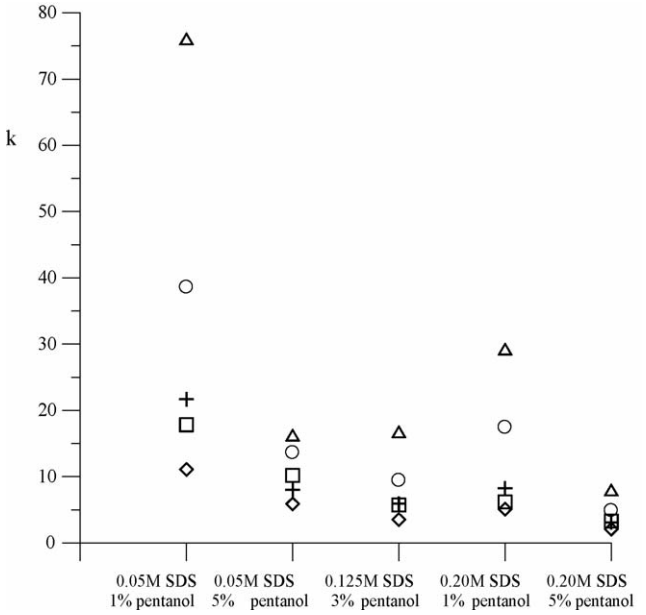


Fig. 1. Retention behaviour of the five antianginals studied in the experimental mobile phases. Compounds: DL: +; ND: ◊; NF: □; PR: △; and VR: ◯.

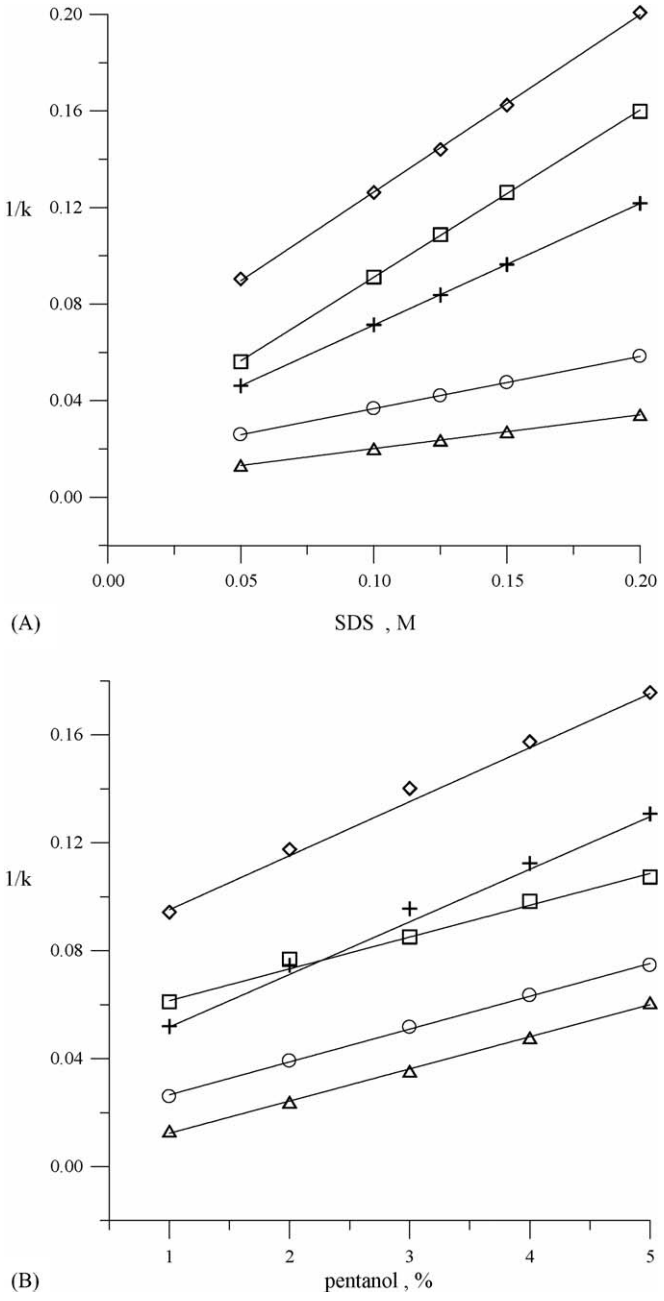


Fig. 2. Retention behaviour of the antianginals when (A) surfactant concentration or (B) organic modifier changes. For drug identification see Fig. 1.

of the antianginals. MLC is useful to perform some separations involving mixtures of hydrophilic and hydrophobic compounds using only one mobile phase. As in the case for a conventional HPLC method, an elution gradient should be applied.

The concentration ranges studied for the modifiers were 0.05–0.20 M for SDS, and 1–5% for pentanol. Results of the retention factor of five experimental mobile phases are shown in Fig. 1. As can be seen, using the lowest strength mobile phase (0.05 M SDS–1% pentanol) makes retention of compounds too large with an analysis time of 75 min, and PR and VR are eluted far away from the rest. Conversely, for the highest strength mobile phase (0.20 M–5%) retention is reduced for all compounds, with an analysis time around 10 min and, instead of resolution of five compounds, there are four overlapping compounds. It is easy to see which is the best mobile phase based on a compromise between resolution and analysis time, and using this premise there is only one mobile phase in which the five compounds under study are completely resolved (0.05 M SDS–5% pentanol). For other purposes, such as the individual analysis of compounds, 0.20 M SDS–5% pentanol could be a good mobile phase due to the low analysis time. In Fig. 1, we can see the change in elution order of compounds between DL and NF. Using 0.05 M SDS–1% pentanol NF elutes earlier than DL but an inversion is produced when the concentration of pentanol increases to 5%. In mobile phases consisting of 0.125 M SDS–3% pentanol and 0.20 M SDS–5% pentanol, DL and NF are eluted in the same time.

In MLC with surfactant or organic modifier a decrease in the retention of compounds with increased concentration of SDS or pentanol is typically achieved. This behaviour was followed by the antianginals. Fig. 2 plots Eq. (2) for the five compounds studied. Fig. 2A shows the curves of the equation for each compound when the concentration of SDS is changed, and correlation of the curves was always $r^2 > 0.9996$. Theoretical results of retention were taken at increasing concentrations of SDS (each step rising by 0.05) while pentanol concentration remained fixed (1%). It has to be noted that every compound decreases its retention as the surfactant concentration increases, this behaviour being larger for DL, NF, and ND, with slopes of 0.53, 0.69, and 0.74, respectively. Moreover, the rate of change in retention of hydrophobic compounds decreased more than in the case of hydrophilic substances, as can be observed in the slopes of the plots. The linearity of the relationship with elution strength and the inverse of retention factor give enough information about the effect of alcohol for each drug.

In contrast, Fig. 2B plots Eq. (2) as a function of the volume fraction of organic modifier which was taken at steps that increased by 1% (v/v) each time, and concentration of SDS was kept constant at 0.05 M. DL and NF undergo an inversion in their elution order, and the lines of DL and NF cross in 0.05 M SDS–2% pentanol. Alcohol exerts the same effect for two groups of drugs, and on one side ND and DL are more influenced by alcohol strength than on the other (NF, VR and PR), as can be seen in the slopes of the plot. Finally, for every drug and within the range examined, SDS has higher elution strength than alcohol.

3.2. Selection of the mobile phase and flow rate

We considered the possibility of using the same mobile phase to carry out these analyses of mixtures of two antianginals (calcium antagonist and β-blocker). We therefore performed an optimisation study for mixtures of all the drugs included in this work. Adequate control of the concentrations of surfactant and modifier can lead to chromatograms showing good resolution and sufficiently strong elution.

To optimise the mobile phase composition, the retention equations (Eq. (1)) of the five drugs were obtained using a reduced, selected number of mobile phases. The errors in the retention factors predicted with these equations were below 2%. Fig. 3 plots the resolution of antianginals in the selected factor space, 0.05–0.20 M SDS and 1–5% pentanol. As can be seen in Fig. 3A, there are two areas of high resolution, one in the left corner at high concentration of alcohol and low concentration of surfactant with a maximum resolution ($R = 0.999$) in a mobile phase of 0.05 M SDS–5% pentanol. The second area is situated in the right factor space at low alcohol concentration, with a low analysis time and a resolution of 0.994 and a mobile phase consisting of 0.15 M SDS–1% pentanol. Fig. 3B and C shows the chromatograms for these optimum mobile phases. It has to be noted that an inversion in the elution order with ND and DL is produced between the two mobile phases. Moreover, the analysis time in Fig. 3C is longer than in Fig. 3B, with the elution of VR and PR too separated and far away from the rest of compounds. The weak elution of these two compounds means a decrease in efficiencies as can be seen in Fig. 3C. Finally, after taking the time of analysis and the chromatographic parameters into account, a mobile phase of 0.05 M SDS–5% pentanol was selected to perform the analysis.

Table 2
Calibration curves ($y = a + bx$) for the antianginals

Compound	a	b	r
DL	−1.2	28.63	0.9997
ND	0.84	18.19	0.9979
NF	−0.85	31.05	0.9967
PR	−1.4	81.82	0.9999
VR	0.79	16.27	0.9997

With the aim of reducing the analysis time of the procedure, several studies based on the change of flow-rate were carried out. Flow-rate was increased by steps of 0.5 ml/min, and results showed that using a flow-rate of 1.5 ml/min increased the analysis time by 5 min and, for a flow-rate of 2 ml/min, the final analysis time was 8 min; the pressure of column was adequated.

The final conditions selected to perform the analysis of drugs, in pharmaceuticals were a mobile phase of 0.05 M of SDS–5% pentanol at pH 7, using a temperature of 25 °C, a flow-rate of 2 ml/min, and UV detection at 220 nm.

3.3. Method validation

3.3.1. Linearity

Calibration curves were built using the areas of the chromatographic peaks from triplicate injections of standards, at seven increasing concentrations in the 1–25 μg/ml range. The response for the drug was linear ($r^2 > 0.997$) in the concentration range studied. Other calibration parameters, such as slope, intercept and correlation coefficient, are shown in Table 2.

3.3.2. Precision

The results of repeatability and intermediate precision experiments are shown in Table 3. The method that was developed was found to be precise, as the relative standard deviation (R.S.D.) values of repeatability were below 1.7, and intermediate precision was below 1.2. Results of precision are given at four different concentrations. Repeatability was performed following 20 determinations covering the specified range (four concentrations at five replicates each). For intermediate precision, different analysts and different equipment were used to obtain the results.

Table 3
Repeatability and intermediate precision of the antianginals

Compound	Repeatability				Intermediate precision			
	2 μg/ml	5 μg/ml	15 μg/ml	20 μg/ml	2 μg/ml	5 μg/ml	15 μg/ml	20 μg/ml
DL	0.96	0.25	0.34	0.43	0.85	0.23	0.24	0.32
ND	1.03	0.52	0.85	0.66	1.20	0.46	0.77	0.49
NF	1.0	0.38	0.98	0.93	1.04	0.29	0.88	0.90
PR	0.54	0.23	0.78	0.18	0.48	0.33	0.57	0.25
VR	2.71	1.21	2.13	0.73	1.22	1.01	1.65	0.84

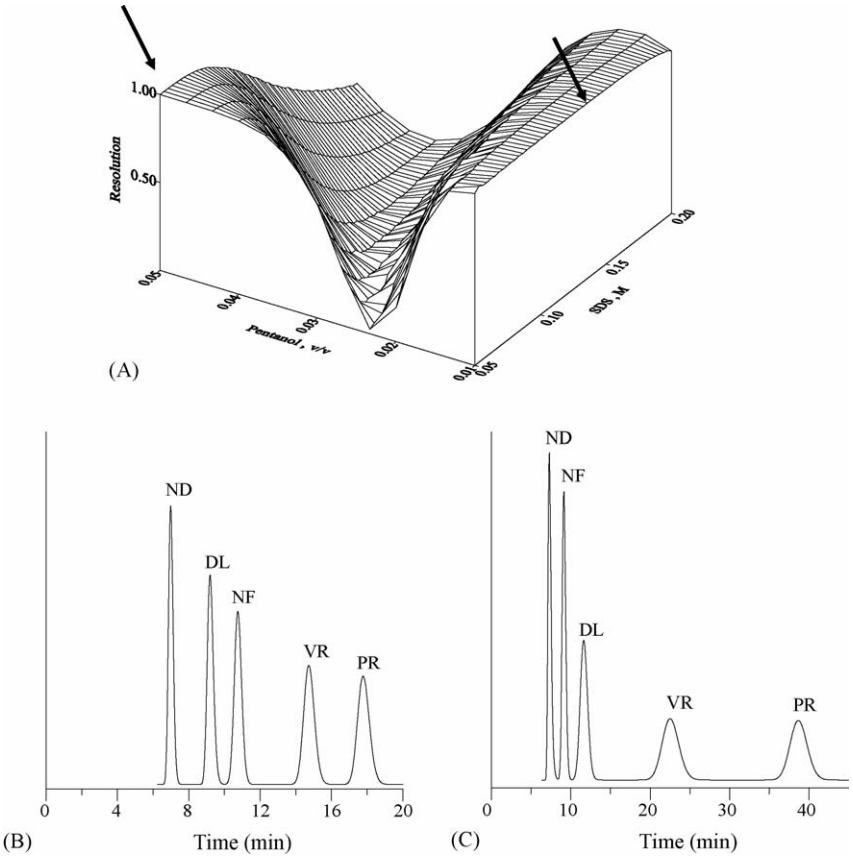


Fig. 3. 3D resolution diagram of the five antianginals (A), selected chromatograms for the two optimum mobile phases, 0.05 M SDS–5% pentanol (B) and 0.15 M SDS–1% pentanol (C).

Table 4
LOD and LOQ of the studied compounds

Compound	LOD (μg/ml)	LOQ (μg/ml)
DL	0.028	0.092
ND	0.067	0.225
NF	0.033	0.111
PR	0.042	0.140
VR	0.130	0.431

3.3.3. Detection limit and quantification limit

Limit of detection (LOD) and limit of quantification (LOQ) were obtained according to the *3 s criterion* and *10 s criterion*, respectively. Results were based on the standard deviation of the response and the slope of a specific calibration curve containing the drug in a range of concentrations close to LOQ. Both values were calculated for antianginals as can be seen in Table 4.

3.4. Application of the method to pharmaceutical analysis

The five drugs studied are all currently administered in our country. Pharmaceutical formulation was presented as tablets in the cases of Diltiazem Edigen, Solgol 40, Nifedipine Retard Bayvit, Sumial 40 and Manidón 120 Retard. Table 5 shows the compositions declared by the manufacturers, and those found according to the recommended MLC procedure. The recoveries obtained agreed with the declared compositions.

Fig. 4 shows the chromatograms of five pharmaceuticals analysed using the micellar procedure developed here. The general analysis time was below 9 min. If drug analysis is performed individually the analysis time decreases depending on the compound analysed.

To extend the application of the developed method, it could be useful to the determination of the five antianginal in bio-fluids.

Table 5
Application of the procedure to the pharmaceutical formulation ($n = 5$)

Pharmaceutical formulation (Company)	Declared composition per tablet (mg)	Found (mg)	Recovery (%)
Diltiazem Edigen (Edigen, Madrid, Spain)	Diltiazem hydrochloride (60) and excipients	58.6 ± 0.9	97.7
Solgol 40 (Sanofi-Synthelabo, Barcelona, Spain)	Nadolol (40) and excipients	39.9 ± 0.4	99.8
Nifedipine Retard Bayvit (Bayvit, Barcelona)	Nifedipine (20) and excipients	20.6 ± 0.5	103
Sumial 40 (AstraZeneca, Madrid)	Propranolol hydrochloride (40) and excipients	41.2 ± 1.12	103
Manidón 120 Retard (Abbott, Madrid)	Verapamil hydrochloride (120) and excipients	118.3 ± 2.2	98.6

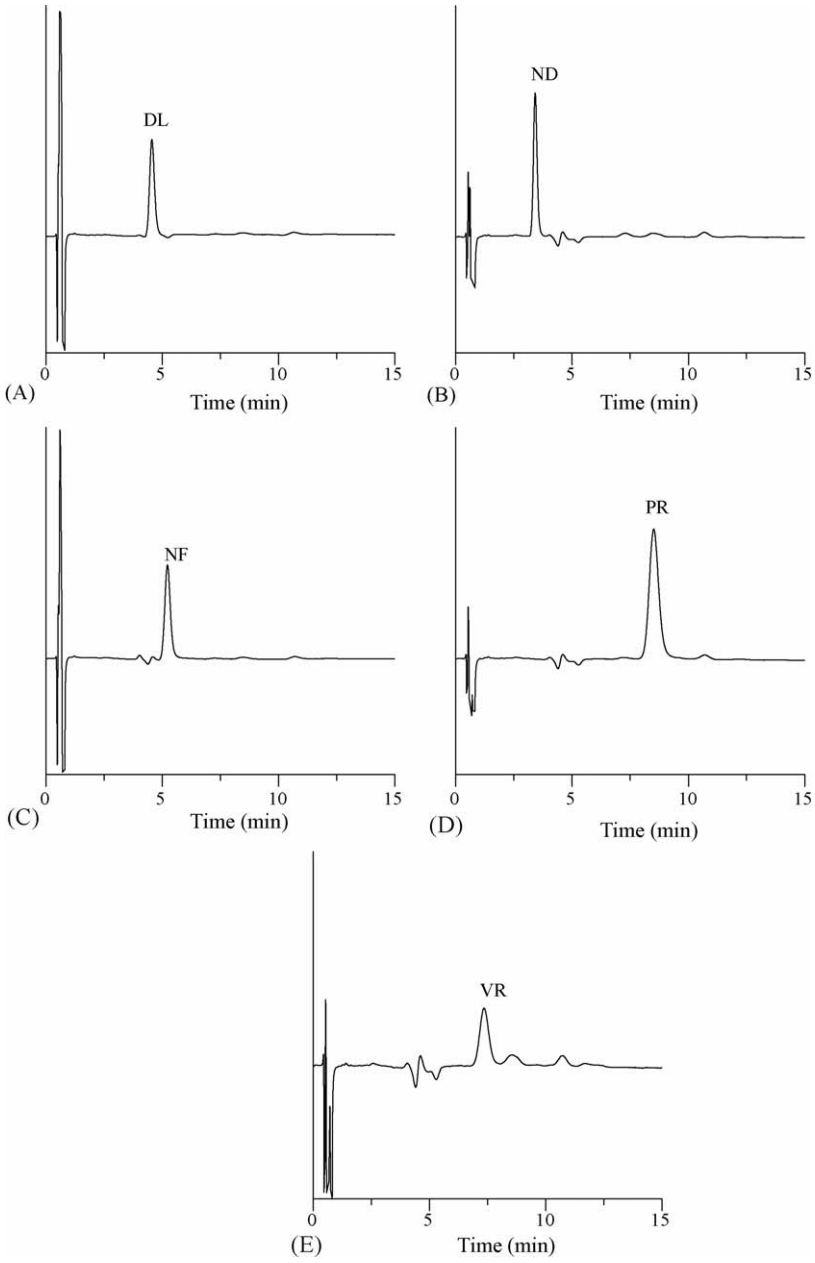


Fig. 4. Chromatograms of pharmaceuticals containing antianginals: Diltiazem Edigen (A), Solgol 40 (B), Nifedipine Retard Bayvit (C), Sumial 40 (D), and Manidón 120 Retard (E).

The use of micellar media allows the solubilization of proteins, and thus, the direct injection of biological samples.

4. Conclusion

This paper proves the feasibility of using hybrid micellar mobile phases in the determination of antianginals in pharmaceuticals. The five studied drugs were analysed directly by a fast, simple procedure in only one injection. Good resolution and low analysis time (9 min) are achieved due to the optimisation of the composition of the mobile phase using only five experimental mobile phases. The selected mobile phase contain a surfactant and a low amount of pentanol which is retained in the micellar solution, thus this media is less toxic than others used in conventional RPLC using aquo-organic solutions.

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