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High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization

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Abstract: A chromatographic procedure with precolumn derivatization to form the N-(1-naphthyl)ethylenediamine dihydrochloride azodyes is proposed for the analysis of several sulphonamides (sodium sulphacetamide, sulphadiazine, sulphaguanidine, sulphamerazine, sulphamethizole, sulphamethoxazole, sulphanilamide and sulphathiazole) in pharmaceutical preparations (tablets, pills, capsules, suspensions and drops). The separation is performed with a 0.05 M sodium dodecyl sulphate/2.4% pentanol eluent at pH 7. The precolumn derivatization improved the resolution in the chromatograms and increased the selectivity in the determination of mixtures of sulphonamides and in preparations where other drugs were present. The derivatization reaction was readily performed in a micellar medium of SDS at pH 1, leading to a rapid and simple procedure. The recoveries were in the 97–104% range with relative standard deviations usually below 3%.

Keywords: *Sulphonamides; pharmaceutical quality control; azodye precolumn derivatization; HPLC; micellar eluent.*

Introduction

The pharmaceutical industry commercializes a great variety of formulations that contain sulphonamides, used as antibacterial agents in medicine and veterinary practice. Since a high urinary concentration of a sparingly soluble sulphonamide may produce crystalluria during therapy, oral preparations containing combinations of two or three sulphonamides in a low concentration are also used. With the introduction of more soluble derivatives, sulphonamide–sulphonamide combinations are becoming less frequent in oral preparations, however, they are still common for vaginal use. The quality control of sulphonamides in pharmaceutical preparations requires the availability of analytical procedures that permit their determination in the presence of a number of accompanying compounds, as well as the resolution of mixtures of sulphonamides.

The analytical procedures reported to perform this quality control are mainly spectrophotometric and chromatographic. Diazotization and coupling with the Bratton–Marshall reagent (N-(1-naphthyl)ethylenediamine dihydrochloride, NED), combined with spectrophotometric measurement is perhaps one of the most popular procedures. The amine group of the sulphonamide is diazotized with sodium nitrite, the excess nitrite is eliminated with sulphamic acid, and the diazonium ion produced is coupled to NED to form an azodye (Fig. 1), with an absorption maximum close to 550 nm and a high molar absorptivity (40 000–50 000 mol^{-1} l cm^{-1}) at pH <4 [1]. This maximum shifts to 490 nm at pH >4. A derivative spectrophotometric method based on the Bratton–Marshall reagent for the analysis of sulphonamide mixtures has also been developed [2]. It has been demonstrated that the spectrophotometric procedure is largely improved in a micellar medium [3, 4].

Liquid chromatographic techniques, usually with methanol–water mobile phases, have also been reported to evaluate the contents of sulphonamides in pharmaceuticals. In most

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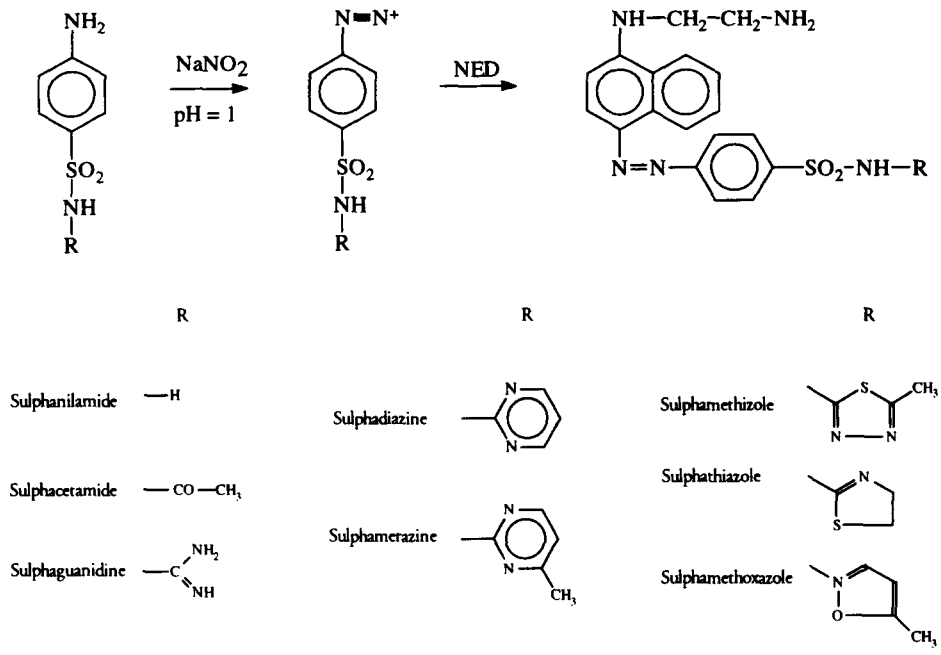

Figure 1
Diazotization of sulphonamides and coupling with N-(1-napthyl)ethylenediamine dihydrochloride.

chromatographic procedures the underivatized sulphonamides are separated and detected in the UV [5–9]. Precolumn [10] and postcolumn [11] derivatization with fluorescamine, and fluorimetric detection at 495 nm have also been recommended.

Recently, much attention has been paid to the use of micellar eluents in reversed-phase chromatography [12]. These mobile phases offer several advantages over the use of conventional hydro-organic eluents. Micellar media facilitate the solubilization of organic samples and eliminate previous separation steps. They are also less expensive, toxic and volatile when compared with the usual mixed organic-aqueous solvent systems.

In this work, a chromatographic procedure for the determination of sulphonamides in pharmaceutical preparations after precolumn derivatization to form the NED azodyes is proposed, where a micellar eluent of sodium dodecyl sulphate (SDS) and pentanol is used. The precolumn derivatization improved the resolution in the chromatograms and increased the selectivity in the determination of mixtures of sulphonamides and in preparations where other drugs were present. The derivatization reaction was readily performed in a micellar medium of SDS at pH 1, leading to a rapid and simple procedure to control these compounds in pharmaceutical preparations.

Experimental

Reagents and apparatus

Sodium nitrite, sulphamic acid (Fluka, Buchs, Switzerland), N-(1-naphthyl)ethylenediamine dihydrochloride, sodium dodecyl sulphate (Merck, Darmstadt, Germany), *n*-pentanol, propanol, methanol, monosodium phosphate, phosphoric acid, hydrochloric acid (Panreac, Barcelona, Spain) were used. The micellar mobile phases were prepared by mixing the aqueous surfactant solution with the alcohol to obtain the working concentration (v/v percentage). The pH was adjusted with 0.1 M phosphate buffer before the addition of the alcohol. The stability of the mobile phase was checked by the reproducibility of the retention times of a sulphonamide, which remained unchanged during several months.

The sulphonamides studied were: sodium sulphacetamide, sulphadiazine, sulphaguanidine, sulphamerazine, sulphamethizole, sulphamethoxazole, sulphanilamide and sulphathiazole (the Sigma Chemical Company, St Louis, MO, USA). Stock solutions containing 100 $\mu g\ ml^{-1}$ were prepared with Barnstead nanopure, deionized water (Sybron, Boston, MA, USA).

A Hewlett–Packard HP 1050 (Palo Alto, CA, USA) liquid chromatograph with an isocratic pump, an automatic injector, a UV-

visible detector and an HP 3396A integrator was used. The injection volume was 20 μl and the detection was performed at 550 or 490 nm, maximum wavelength of the protonated and unprotonated azodyes, respectively. The dead volume was determined by injection of water and was similar for all the mobile phases used. The mobile phase flow-rate was 1 ml min^{-1}.

Data acquisition was performed through the PEAK-96 software from Hewlett–Packard (Avondale, PA, USA). A Spherisorb ODS-2 analytical C_{18} column (5 μm particle size, 12.5 cm × 4.6 mm i.d.) and a C_{18} precolumn (3.5 cm × 4.6 mm i.d.) from Scharlau (Barcelona, Spain) were used. The mobile phase and the solutions to be injected were vacuum-filtered through 0.45- and 0.22-μm Nylon membranes, respectively (Micron Separations, Westboro, MA, USA).

Derivatization procedure

An aliquot of the solution containing 1–20 μg ml^{-1} of the sulphonamide was introduced into a 25-ml volumetric flask, together with 10 ml of a 0.04 M SDS–0.15 M HCl solution and 1 ml of 0.1 M nitrite. After 5 min, 1 ml of 0.3 M sulphamic acid was added, and the mixture was allowed to react for an additional 10 min. Finally, 0.5 ml of 0.03 M NED was added, and the volume was completed to the mark with water. The azodyes were formed immediately and were stable during several weeks, even when exposed to light and oxygen.

Sample preparation

The pharmaceutical formulations analysed were tablets, pills, capsules, suspensions and drops. Five tablets or pills were weighed and powdered, a portion was taken, weighed and dissolved in 5 ml of ethanol, finally it was diluted with a 0.04 M SDS–0.15 M HCl solution. A similar procedure was followed with the contents of the capsules. An aliquot of the homogenized suspension or the drops was taken and conveniently diluted in 0.04 M SDS–0.15 M HCl. The solutions of some formulations should be filtered prior to the derivatization.

Results and Discussion

Initially, the underivatized sulphonamides were chromatographed with a 0.1 M SDS mobile phase at pH 7 and 3, in the absence and presence of 4% propanol. At pH 7 the sulphonamides eluted with the void volume. At pH 3 broad peaks with capacity factors in the $3 < k' < 4.5$ range were observed, which shifted to the $1.5 < k' < 3$ range when the alcohol was added. The analysis of some formulations containing a sulphonamide and other drugs was not possible, since overlapped peaks were obtained (Fig. 2). It was then considered that the derivatization of the sulphonamides could be convenient to increase the retention by decreasing the polarity of the solutes. The selectivity could also be improved by using chromogenic derivatization.

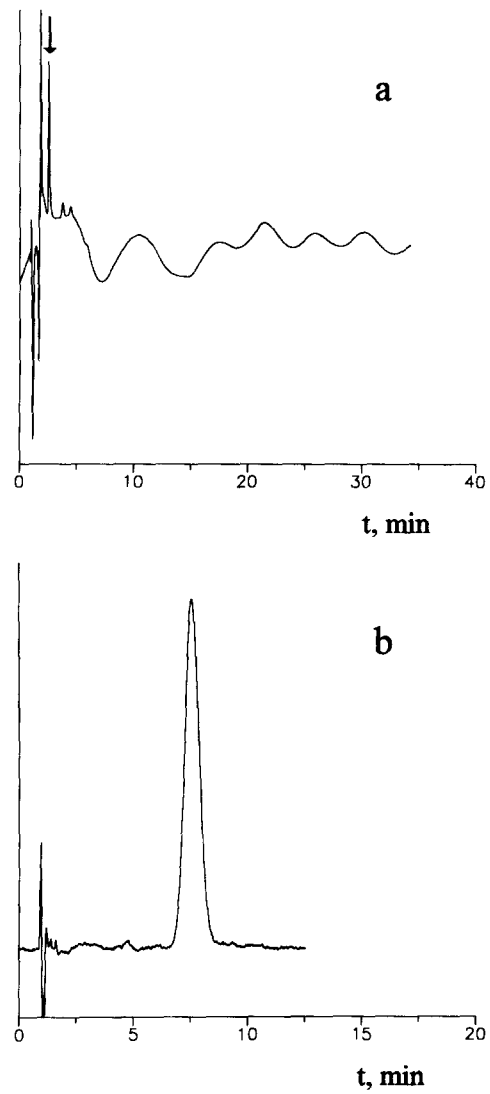

Figure 2
Chromatograms of Angileptol: (a) without derivatization (0.1 M SDS/4% propanol at pH 3, the arrow indicates the peak of sulphaguanidine); (b) after derivatization with NED (0.05 M SDS/2.4% pentanol at pH 7). The concentration of sulphaguanidine was close to 10 μg ml^{-1} for both procedures.

Both requirements were achieved by forming the NED azodye derivatives of the sulphonamides. The diazonium ions previously formed by the action of nitrous acid on the sulphonamides are coupled with NED to yield very stable azodyes. Advantages of the SDS micellar medium over the use of non-micellar media are the catalysis of the coupling reaction (which is immediate with SDS), and the higher solubility of both hydrophilic and hydrophobic azodyes. Also, the pH should not be adjusted between diazotization and coupling. In a non-micellar medium, the diazonium ion is formed at pH <1, whereas pH >2 is usually required for coupling at a reasonable speed. Finally, another modification of the pH is usually done to measure the absorbance of the azodye in its protonated form. In an SDS micellar medium, the combined effects of micellar catalysis of the coupling reaction and the earlier protonation of the NED azodyes make these pH changes unnecessary. With SDS the coupling and measurement steps can be carried out in a 0.06 M HCl solution, which results from the addition of the coupling reagent to the 0.15 M HCl solution used to diazotize the arylamines.

When a surfactant solution is used as mobile phase in reversed-phase chromatography, the retention of the solutes can be adequately controlled through the addition of a small amount of alcohol. The retention of the sulphonamide azodyes was excessive when eluted with a purely micellar SDS mobile phase, with retention times larger than 40 min. The addition of propanol to the SDS eluent still led to high retention times (>25 min). Therefore, an alcohol giving a higher eluent strength, such as *n*-pentanol, should be added. This alcohol gives rise to mixed micelles in an SDS solution [13].

The primary amine group of sulphonamide azodyes is protonated in acid media, whereas in an anionic micellar solution of SDS, the protonation of the aryl-alkyl secondary amine group in *para* position with respect to the azo bridge takes place usually in the 3.5–4.5 pH range, one to two pH units higher than in a non-micellar medium [4]. Thus, in the presence of SDS, the single and double charged cationic forms of the azodyes predominate at pH <9 and pH <4, respectively.

The retention of the azodyes increased at a decreasing pH in an SDS mobile phase. The higher retention indicated that at a lower pH the solute interacted more strongly with the anionic modified stationary phase than with the anionic micelles in the eluent. The stronger interaction decreased the efficiency of the chromatographic peaks. Thus, pH 7 was selected for the preparation of the mobile phase, in spite of the lower sensitivity obtained in the spectrophotometric detection.

When a hybrid SDS-pentanol mobile phase was used, an increasing SDS concentration gave lower retention, but also lower efficiencies, as observed for other compounds with these eluents. On the other hand, an increasing pentanol amount, gave lower retention, together with increased efficiencies.

The solute-micelle association constants, K_{AM}, and stationary phase–water partition coefficients multiplied by the phase ratio (ratio of the volumes of stationary and mobile phases), ϕP_{SW}, of several sulphonamides were obtained at three concentrations of pentanol in SDS mobile phases (Table 1). These parameters were calculated by fitting the retention data to the function $1/k'$ vs concentration of micelles [12]. In Table 1 the sulphonamides have been ordered according to the values of K_{AM} in an SDS mobile phase containing 1.5%

Table 1
Solute-micelle binding constants (K_{AM}) and partition coefficients between stationary phase and water, multiplied by the phase ratio (ϕP_{SW}), in mobile phases containing increasing amounts of pentanol

	1.5% Pentanol		2.5% Pentanol		3.5% Pentanol	
Compound	K_{AM}	ϕP_{SW}	K_{AM}	ϕP_{SW}	K_{AM}	ϕP_{SW}
Sulphacetamide	133	53	17	8	7	5
Sulphamerazine	57	72	46	49	41	37
Sulphadiazine	50	55	38	34	32	25
Sulphathiazole	44	52	51	50	59	49
Sulphamethoxazole	39	29	44	29	48	30
Sulphanilamide	39	39	53	44	72	51
Sulphamethizole	29	32	27	24	23	17
Sulphaguanidine	23	15	11	9	8	6

pentanol. It may be observed that this order was altered when the amount of pentanol was increased. The incorporation of pentanol into the micelles changes the affinity of the solutes towards the micelle. A decrease in K_{AM} indicates that the interaction between the solute and the micelles decreases. The modification of the association constants with the amount of pentanol shows a correlation with the molecular structures.

Other authors have reported decreases in K_{AM} for SDS mobile phases with an alcohol added as modifier. The diminution observed was larger as the concentration of modifier (methanol, propanol or butanol) increased, especially for those solutes showing the higher hydrophobicity [14, 15]. We observed before for some diuretics, that K_{AM} decreased when a small amount of pentanol was added to the SDS mobile phase, but increased for a larger amount of alcohol [16].

The different elution behaviour shown by the sulphonamide azodyes (different values of K_{AM} and different dependency of this parameter with the concentration of pentanol) suggested a large variation of the selectivity with the composition of the mobile phase. A mobile phase containing a low concentration of SDS is recommended due to the higher efficiencies. A 0.05 M SDS/2.4% pentanol mobile phase at pH 7 was selected for the analysis of the sulphonamides in the pharmaceutical formulations. With this mobile phase the sulphonamide azodyes were eluted in less than 15 min, following the elution order (k' values in parentheses): sodium sulphacetamide (4.5), sulphamethizole (5.0), sulphaguanidine (9.1), sulphamethoxazole (10.5), sulphadiazine (11.8), sulphanilamide (12.1), sulphathiazole (14.1) and sulphamerazine (15.1). For the analysis of some sulphonamides, another eluent with a higher elution strength can be more convenient.

Analytical figures

Table 2 shows the fitting parameters of the calibration plots obtained for each sulphonamide using the 0.05 M SDS/2.4% pentanol mobile phase, together with the peak area repeatability and limits of detection. The reproducibility was also evaluated from series of ten aliquots of sulphamethoxazole solutions, which were independently derivatized and injected into the chromatograph, being 6.8 and 1.0% for 1 and 20 μg ml^{-1}, respectively. The limits of detection were determined by injecting 1 μg ml^{-1} sulphonamide solutions (eight replicates) and using the 3s criterion.

Table 3 shows the composition, recoveries and reproducibilities achieved in the analyses of several Spanish formulations, containing sulphonamides. The analyses were performed by derivatizing five aliquots of the dissolved pharmaceutical. The recoveries with respect to the composition given by the manufacturers were usually close to 100%. The chromatograms of Angileptol obtained without and with derivatization are shown in Fig. 2. The chromatograms of other derivatized formulations are given in Fig. 3, where it is observed that the accompanying compounds did not give any peak at the detection wavelength.

Benzocaine, an arylamine found in the formulation Angileptol, together with sulphaguanidine, also forms a red azodye, being an interference in the non-chromatographic determination. When benzocaine was injected not later than 4 h after derivatization, no peak was

Table 2
Analytical figures

	$y = b\,C^* + a$		Repeatability† (%)		
Compound	Slope (b)	Intercept (a)	1 μg ml^{-1}	20 μg ml^{-1}	LOD‡ (μg ml^{-1})
Sulphacetamide	11.9 ± 0.4	7.7 ± 5.9	6.5	0.4	0.2
Sulphamerazine	15.23 ± 0.7	−0.09 ± 0.85	6.1	0.8	0.2
Sulphadiazine	16.4 ± 0.1	0.7 ± 1.6	7.0	1.0	0.02
Sulphathiazole	21.6 ± 0.4	7.5 ± 5.9	5.0	0.7	0.2
Sulphamethoxazole	16.5 ± 0.2	9.6 ± 2.2	2.6	1.4	0.1
Sulphanilamide	18.9 ± 0.6	9.1 ± 7.4	6.3	4.3	0.3
Sulphamethizole	16.40 ± 0.09	−0.2 ± 1.2	6.2	1.0	0.2
Sulphaguanidine	12.4 ± 0.4	−1.4 ± 4.8	7.8	3.8	0.1

*Concentration in μg ml^{-1}.
†For five to eight replicates of the same azodye solution at the concentration indicated.
‡Limit of detection.

Table 3
Determination of sulphonamides in pharmaceutical formulations. Contents declared by the manufacturers, recovery and reproducibility

Formulation	Composition	Recovery %	RSD %
Amidrin-Bio (Fardi, Barcelona)	Per 1 ml drops: 5 mg chloramphenicol, 50 mg **sodium sulphacetamide**, 10 mg phenylmethylaminopropanol chlorhydrate, 4 mg trichloroisobutanol, excipient	100.1	4.3
Celestone S (Schering-Plough, San Agustín de Guadalix, Madrid)	Per 100 ml drops: 100 mg betamethasone, 10 g **sodium sulphacetamide**, excipient	98.3	3.6
Visu-blefarite (Merck Sharp & Dohme, Chibret, Alcalá de Henares, Madrid)	Per 1 ml drops: 100 mg **sodium sulphacetamide**, 1.5 mg betamethasone, 0.5 mg tetrazolium phosphate, excipient	100.8	0.8
Angileptol (Sigma-Tau, Alcalá de Henares, Madrid)	Per tablet: 1 mg tirotricine, 3 mg enoxolone, 50 mg **sulphaguanidine**, 4 mg benzocaine, 3 mg sodium sacarine, 300 mg sacarose, excipient	101.8	1.8
Bucodrin (Fardi, Barcelona)	Per tablet: 100 mg **sulphathiazole**, 2 mg ethacridine, 3 mg ephedrine, 1.8 g sacarose, excipient	98.9	5.2
Micturol sedante (Boots Pharmaceuticals, Alcalá de Henares, Madrid)	Per pill: 125 mg **sulphamethizole**, 50 mg phenazopyridine chlorhydrate, 3.25 mg starch, 220 mg sacarose, lactose, excipient	104.0	4.7
Bio-Hubber (ICN Hubber, Corberà de Llobregat, Barcelona)	Per tablet: 25 mg neomycin sulphate, 1000 U.I. bacitracin, 50 mg estreptomicine sulphate, 30 mg **sulphadiazine**, 50 mg pectin, 20 mg sodium menadione bisulphite, 20 mg nicotinamide, 10 mg sacarine, 50 mg starch, lactose, excipient	102.0	2.1
Amidrin (Fardi, Barcelona)	Per 1 ml drops: 4 mg **sulphanilamide**, 8 mg ephedrine chlorhydrate, 5 mg chlorbutanol, excipient	102.3	1.8
Balsoprim (Juste, Madrid)	Per 5 ml suspension: 27 mg trimethoprim, 133 mg **sulphamethoxazole**, 2.5 mg bromhexine chlorhydrate, 10 mg sodium sacarine, excipient	101.2	1.7
Bronquimucil (Uriach, Barcelona)	Per 100 ml suspension: 250 mg brovanexine chlorhydrate, 800 mg trimethoprim, 4000 mg **sulphamethoxazole**, 240 mg sodium sacarine, 50 g sacarose, excipient	99.8	1.6
Broncobactifor (Andrómaco, Torrejón de Ardoz, Madrid)	Per 7.5 ml suspension: 80 mg trimethoprim, 400 mg **sulphamethoxazole**, 4 mg bromhexine, 30 mg sodium ciclamate, 7.5 mg sacarine, excipient	102.8	2.1
Brongenit (Elfar-Grag, Fuenlabrada, Madrid)	Per capsule: 80 mg trimethoprim, 400 mg **sulphamethoxazole**, excipient	103.1	3.2
Broncorema (Septa Farma 86, Viladecans, Barcelona)	Per 5 ml suspension: 200 mg **sulphamethoxazole**, 40 mg trimethoprim, 75 mg guaifenesin, 15 mg sodium sacarine, excipient	100.8	2.5
Pulmosterin Duo (Normon, Madrid)	Per 5 ml suspension: 40 mg trimethoprim, 200 mg **sulphamethoxazole**, 2.5 mg bromhexine chlorhydrate, 25 mg guaifenesin, 20 mg sodium benzoate, 10.5 mg sodium sacarine, 2.5 mg sacarose, excipient	100.2	2.8
Traquivan (Instituto Llorente, Madrid)	Per 5 ml suspension: 27 mg trimethoprim, 133 mg **sulphamethoxazole**, 5 mg dihydrocodeine bitartrate, 2.5 mg sodium sacarine, excipient	97.3	0.3

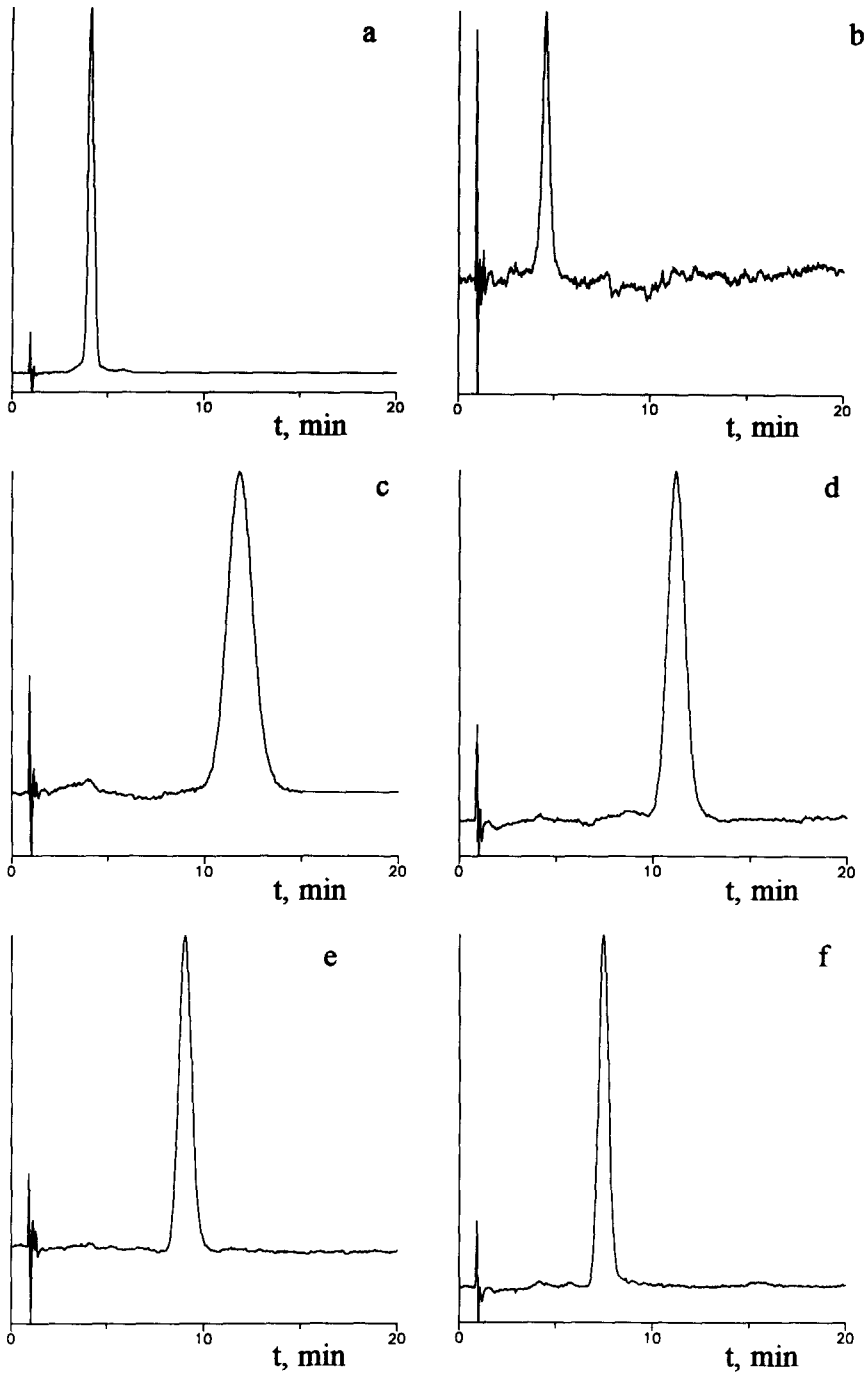

Figure 3
Chromatograms of several pharmaceutical formulations after precolumn diazotization and coupling with NED: (a) Amidrin-Bio (sodium sulphacetamide), (b) Micturol sedante (sulphamethizole); (c) Bucodrin (sulphathiazole), (d) Amidrin (sulphanilamide), (e) Bio-Hubber (sulphadiazine), (f) Bronquimucil (sulphamethoxazole). A 0.05 M SDS/2.4% pentanol mobile phase at pH 7 was used. The concentration of the sulphonamides was always close to 10 $\mu g\ ml^{-1}$.

observed in the chromatograms. After this time the benzocaine derivative showed a peak with $k' = 6.1$, which increased with the time elapsed between the derivatization and injection.

It was checked that the sulphonamide azodyes were very stable, no degradation being produced during several days, except for sulphacetamide. The degradation of this azodye was observed by the diminution of the

Table 4
Recoveries in the analysis of mixtures of sulphonamides

Mixtures	Recovery %	RSD % (n = 4)
4 μg ml^{-1} sulphacetamide–	98.7	4.1
12 μg ml^{-1} sulphaguanidine	99.3	2.4
8 μg ml^{-1} sulphacetamide–	99.0	3.7
8 μg ml^{-1} sulphaguanidine	99.1	3.1
12 μg ml^{-1} sulphacetamide–	98.6	3.4
4 μg ml^{-1} sulphaguanidine	98.0	4.2
4 μg ml^{-1} sulphamerazine–	98.3	3.3
12 μ ml^{-1} sulphamethizole	99.8	2.3
12 μg ml^{-1} sulphamerazine–	98.7	2.6
4 μ ml^{-1} sulphamethizole	97.5	2.8
4 μg ml^{-1} sulphacetamide–	98.3	3.6
12 μg ml^{-1} sulphadiazine	99.4	1.1
12 μg ml^{-1} sulphacetamide–	97.4	3.8
4 μg ml^{-1} sulphadiazine	96.3	3.7
8 μg ml^{-1} sulphathiazole–	98.5	3.1
8 μg ml^{-1} sulphaguanidine	96.9	4.2
8 μg ml^{-1} sulphamethoxazole–	98.4	2.9
8 μg ml^{-1} sulphanilamide	97.7	3.1
4 μg ml^{-1} sulphamethizole–	98.3	3.2
8 μg ml^{-1} sulphadiazine–	98.8	1.8
12 μg ml^{-1} sulphamerazine	99.2	2.0
8 μg ml^{-1} sulphamethizole–	97.6	2.4
8 μg ml^{-1} sulphadiazine–	98.6	2.9
8 μg ml^{-1} sulphamerazine	99.1	2.6
12 μg ml^{-1} sulphamethizole–	98.6	2.3
8 μg ml^{-1} sulphadiazine–	98.5	3.1
4 μg ml^{-1} sulphamerazine	97.3	3.3

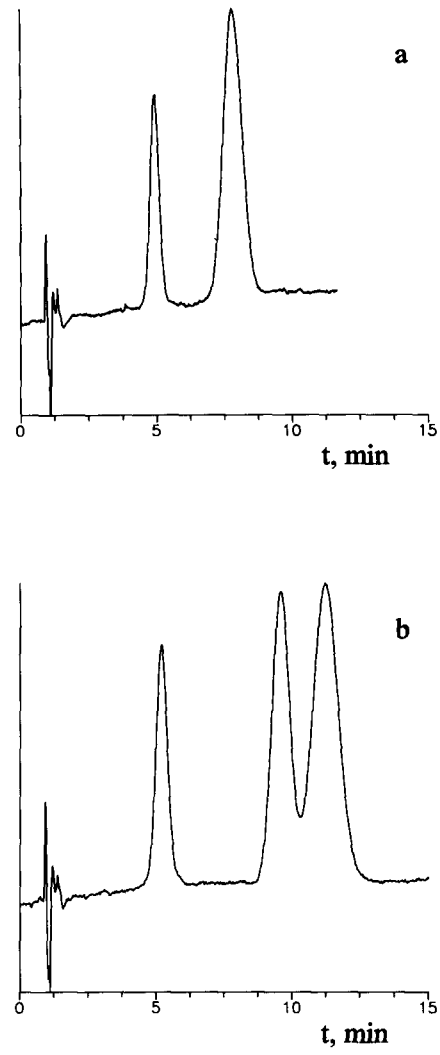

Figure 4
Chromatograms of mixtures of sulphonamides: (a) 4 μg ml^{-1} sodium sulphacetamide (4.9 min) and 12 μg ml^{-1} sulphaguanidine (7.8 min); (b) 4 μg ml^{-1} sulphamethizole (5.2 min), 8 μg ml^{-1} sulphadiazine (9.5 min), and 12 μg ml^{-1} sulphamerazine (11.2 min). A 0.05 M SDS/2.4% pentanol mobile phase at pH 7 was used.

chromatographic peak at 4–5 min, and the appearance of a second peak at a shorter retention time. The sum of the areas of both peaks was constant. If the solution of the sulphacetamide azodye was injected before 2 h from its preparation, only one peak was observed.

The results of the analyses of mixtures of sulphonamides are given in Table 4 and Fig. 4. In all cases, the mixtures were well resolved and good agreement was found between taken and found values.

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