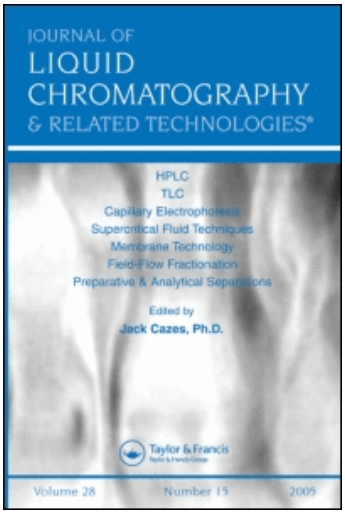

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MICELLAR CHROMATOGRAPHIC PROCEDURE WITH DIRECT INJECTION FOR THE DETERMINATION OF SULFONAMIDES IN MILK AND HONEY SAMPLES

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MICELLAR CHROMATOGRAPHIC PROCEDURE WITH DIRECT INJECTION FOR THE DETERMINATION OF SULFONAMIDES IN MILK AND HONEY SAMPLES

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ABSTRACT

The capability of liquid chromatography with micellar mobile phases of sodium dodecyl sulfate (SDS), of allowing the direct injection of biological fluids into reversed-phase columns, was applied to the determination of sulfonamides in milk and honey samples. The chromatographic behavior of a group of 15 sulfonamides was studied at pH 3.0 where the drugs showed a greater separation space. Acetonitrile was added to the mobile phase to decrease the retention of the most hydrophobic drugs and increase the efficiencies, which yielded a higher resolution. The samples were diluted with 0.10 *M* SDS to facilitate the solubilization of the matrix compounds and release the protein-bound drugs. The procedure is simple, rapid, and reliable, has a low cost, and permits screening for 11 sulfonamides (sulfacetamide,

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sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, and sulfisoxazole) with good accuracy and precision. The recoveries found for milk and honey samples spiked with 1 μg/mL of each sulfonamide, calculated from the calibration straight lines obtained in aqueous solution, were in the ranges 87–108% and 72–119% for milk and honey samples, respectively. Limits of detection were close to 0.1 μg/mL for sulfadiazine, sulfadimethoxine, sulfamethizole, sulfamonomethoxine, sulfanilamide, sulfathiazole, and sulfisoxazole in milk samples and sulfacetamide, sulfamethizole, sulfanilamide, and sulfapyridine in honey samples.

INTRODUCTION

The capability of micellar liquid chromatography (MLC) to allow the direct injection of biological fluids into reversed-phase (RP) columns (1) can be very useful for the assay of therapeutic drugs in food samples (2–4). Micellar mobile phases containing the anionic surfactant sodium dodecyl sulfate (SDS) permit the simultaneous on-line sample cleanup and separation of low molecular weight analytes (i.e., the drugs). This prevents column clogging without the need for time-consuming, hard, and repetitive precipitation of the proteins in the biological sample before column injection, which can lead to incomplete recoveries of the analytes and significant errors.

These advantages, together with complete release of protein-bound drugs and reproducible and predictable retention behavior (5), result in significant savings in labor and time, easy optimization of the analytical procedures, and enhancements in safety and low cost with respect to conventional RP liquid chromatography.

Sulfonamides are used in food-producing animals for the treatment of several diseases, but also subtherapeutically for prophylactic purposes and/or for promotion of growth (6). Residues of sulfonamides can thus appear in animal food products, such as milk, honey, eggs, and meat. The presence of residues of sulfonamides in foods can be a health hazard for consumers, owing to the carcinogenic, anaphylactic, and antithyroidal effects of some members of this group, besides the potential development of drug-resistant strains, aplasic anaemia, and changes in gastrointestinal microflora (7–9). In addition, the presence of antimicrobial agents in milk negatively affects the required microbial growth for the manufacture of yogurt and cheeses (10).

The use of sulfonamides in subtherapeutic doses is illegal in the European Union. The maximum residue limit has been established at 0.1 μg/mL for the parent drugs in bovine, ovine, and caprine milk, and in honey (11). However,

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the reports about the illegal use of these antibacterial agents are becoming more frequent. For instance, in a recent study done in Northern Ireland, sulfonamides and other drugs (chlortetracycline, penicillin, and several ionophores) were found to be the most common contaminating antimicrobials in animal food (12).

High-performance liquid chromatography (HPLC) is the most widely used technique for the determination of residues of sulfonamides in foods of animal origin. Owing to the complexity of the food matrix, a major problem is the detection of these drugs in the presence of potentially interfering compounds. A general approach in conventional HPLC with RP columns includes drug extraction and sample cleanup before HPLC separation. Usually the mobile phase is a buffered mixture of water and organic solvent (methanol or acetonitrile) (6). For detection, either ultraviolet (UV) variable-wavelength or photodiode array detectors are used. Electrochemical detection is used less frequently (6,13). In some instances, sulfonamides are derivatized for fluorimetric (14) or photometric (15) detection.

In this work, we show a rapid and simple procedure for determination of sulfonamides in milk and honey. The samples were directly injected into the chromatographic system and separated using mobile phases of SDS and acetonitrile. The application of *MICHROM* (16), a software program that allows the global treatment of chromatographic data of compounds eluted with micellar mobile phases, facilitated the optimization of mobile phase composition.

EXPERIMENTAL

Reagents

Stock standard solutions of 100 μg/mL of 15 sulfonamides (Fig. 1) were prepared by dissolving the reagents with a small amount of ethanol (Scharlab, Barcelona, Spain), and dilution with 0.10 *M* SDS (99% purity, Merck, Darmstadt, Germany). The drugs were from Sigma (St. Louis, MO, USA), except for sulfamethazine which was from Aldrich (Milwaukee, WI, USA). Bovine casein, α-lactoalbumin, β-lactoglobulin, maltose (Sigma), lactose, fructose (Merck), glucose (Panreac, Barcelona, Spain), and saccharose (Probus, Barcelona, Spain) were studied as interferents.

The mobile phases contained SDS at concentrations above the critical micellar concentration (cmc $= 8.1 \times 10^{-3}$ *M*) and the organic modifiers 1-propanol or acetonitrile (Scharlab). The pH was buffered with citric acid (Sigma) and sodium hydroxide (Panreac). Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used to prepare all the solutions. The mobile phases and standard solutions were filtered through 0.45-μm nylon membranes (Micron Separations, Westboro, MA, USA) to eliminate any particulate matter.

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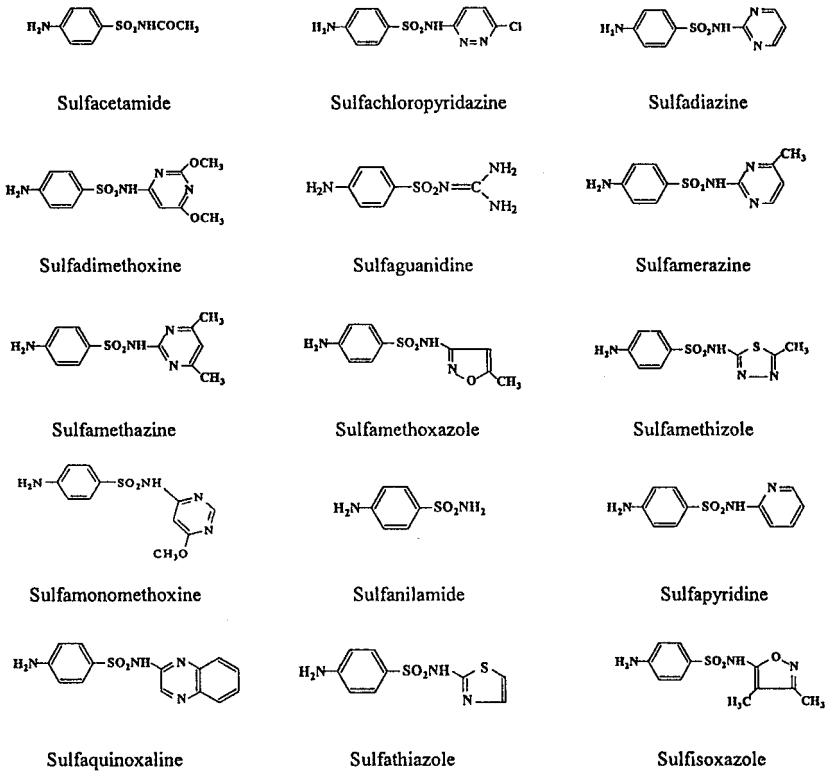

Figure 1. Sulfonamides studied in this work.

Apparatus

A diode array spectrophotometer (model HP 8452A; Agilent Technologies, Palo Alto, CA, USA) was used to obtain the spectra of the drugs. The liquid chromatograph (model HP 1050; Agilent Technologies) was equipped with an isocratic pump, an autosampler (model HP 1100), a UV-visible detector set at 275 nm, and a personal computer connected to the chromatograph through an integrator (model HP 3396A). An ODS-Hypersil column [5-μm particle size, 100 mm $\times$ 4.6 mm inside diameter (i.d); Agilent Technologies, Waldbronn, Germany] was placed after a C_{18} Nucleosil guard column (30 mm $\times$ 4.0 mm i.d., Scharlab) that saturated the mobile phase with silica. The flow rate was 1.0 mL/min and the injection volume was 20 μL. The chromatographic runs were made at room temperature.

The software programs *PEAK-96.* (Agilent Technologies; Avondale, PA, USA) and *MICHROM* (16) were used to acquire and process the chromatographic

data, respectively. *MICHROM* includes features that permit the prediction of chromatograms, which were used to find the most suitable mobile phase composition.

Procedures

The mobile phases were prepared by first dissolving surfactant and citric acid (0.01 *M*) in water. The pH was then adjusted with NaOH. The organic solvent was added to obtain the working concentration (v/v) and, after strong shaking, water to the mark was added. The optimal mobile phase composition was 0.019 *M* SDS/5.8% acetonitrile at pH 3.0. The column was kept in methanol. Before changing to the micellar mobile phase, water was substituted for this solvent. The system was equilibrated by circulating the micellar mobile phase during 1 h.

Milk and honey samples were mixed with a micellar solution of 0.10 *M* SDS and sonicated during 10 min to solubilize proteins and fats and release the bound drugs. The samples were filtered into the autosampler vials through 0.45-μm nylon membranes and injected into the chromatograph.

RESULTS AND DISCUSSION

Selection of pH and Nature of the Modifier

Sulfonamides exhibit amphoteric properties due to the acidic N–H linkage adjacent to the sulfonyl group (pK_1) and the basic character of the *para*-amino group (pK_2) (Table 1). For most compounds, the cationic species dominates at pH < 2–3 and the anionic species at pH > 5.5–7.5. This behavior produces a significant

Table 1. Acid-Base Dissociation Constants[a] and Maximum Wavelengths for Several Sulfonamides

Compound	pK_1	pK_2	λ_{max} (nm)	Compound	pK_1	pK_2	λ_{max} (nm)
Sulfacetamide	1.78	5.38	270	Sulfamethizole	2.20	5.45	274
Sulfachloropyridazine		6.10	266	Sulfamonomethoxine		6.90	272
Sulfadiazine	2.00	6.48	218	Sulfanilamide		10.43	260
Sulfadimethoxine	2.02	6.70	264	Sulfapyridine	2.58	4.43	266
Sulfaguanidine	2.75	12.10	268	Sulfaquinoxaline		5.50	268
Sulfamerazine	2.26	7.06	246	Sulfathiazole	2.36	7.23	284
Sulfamethazine	2.36	7.38	246	Sulfisoxazole	4.62	5.00	250

[a]From ref. 17.

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dependence of the retention factors, *k*, with the pH of the mobile phase in the usual working pH range of a C_{18} column (3–7).

Figure 2 shows the variation of *k* values with pH for the 15 sulfonamides eluted with a pure micellar mobile phase of 0.075 *M* SDS (without modifier).

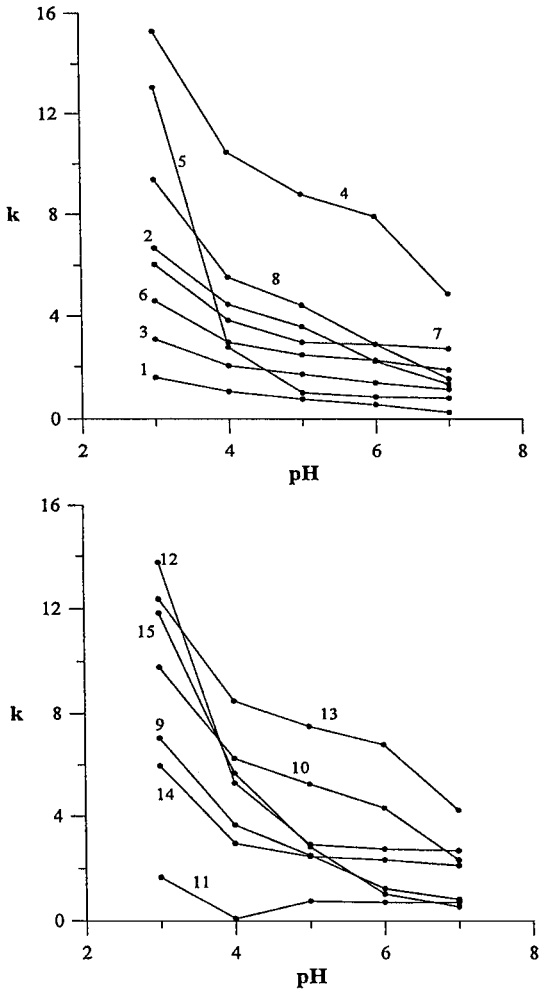

Figure 2. Acid-base behavior for the 15 sulfonamides eluted with mobile phases of 0.075 *M* SDS in the pH range 3–7. Sulfonamides: 1) sulfacetamide, 2) sulfachloropyridazine, 3) sulfadiazine, 4) sulfadimethoxine, 5) sulfaguanidine, 6) sulfamerazine, 7) sulfamethazine, 8) sulfamethoxazole, 9) sulfamethizole, 10) sulfamonomethoxine, 11) sulfanilamide, 12) sulfapyridine, 13) sulfaquinoxaline, 14) sulfathiazole, and 15) sulfisoxazole.

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As observed, the differences in acid-base behavior produce some changes in elution order when the pH varies. The resolution of mixtures of sulfonamides was optimized at pH 3 since the separation space at larger pH values was too small.

The retention of the sulfonamides changed remarkably with the concentration of surfactant in the mobile phase. However, in pure micellar mobile phases the resolution of the mixtures was very poor due to the low efficiencies of the chromatographic peaks, except for sulfaguanidine (Fig. 3). We, therefore, added an organic solvent to the mobile phase, which is known to usually improve the efficiencies (18). Two organic solvents were examined: 1-propanol and acetonitrile, which have an intermediate elution strength among the conventional modifiers used in MLC (higher for 1-propanol as shown in Fig. 3). Acetonitrile was finally selected for the screening of sulfonamides due to considerable higher efficiency and resolution.

Optimization of Mobile Phase Composition

The concentrations of SDS and acetonitrile were optimized using the chromatographic data of five mobile phases buffered at pH 3.0: 0.025 *M* SDS, 0.125 *M* SDS, 0.025 *M* SDS/6.0% acetonitrile, 0.125 *M* SDS/6.0% acetonitrile, and 0.075 *M* SDS/3.0% acetonitrile. After obtaining the optimal composition, a sixth mobile phase (0.060 *M* SDS/6.0% acetonitrile) was added to the design to test the reliability of the predictions and eventually obtain a better description of the retention in the region of maximal resolution.

The retention behavior was modeled according to (5):

$$k = \frac{K_{AS}\frac{1}{1+K_{AD}\varphi}}{1+K_{AM}\frac{1+K_{MD}\varphi}{1+K_{AD}\varphi}\,[M]} \tag{1}$$

where [M] and φ are the concentrations of surfactant and modifier, K_{AS} and K_{AM} describe the association equilibria between the eluted solute in bulk water and the stationary phase or micelle, respectively, and K_{AD} and K_{MD} are constants that measure the influence of the organic solvent in the partition of the solute between bulk water and micelles, referred to a pure micellar solution. The parameters of Equation (1) and the mean relative prediction errors for the six mobile phases are given in Table 2 for each sulfonamide.

As observed, some parameters are negative or have extremely high values, which does not make any physicochemical sense. The negative values achieved for K_{MD} and K_{AD} (sulfadiazine, sulfadimethoxine, and sulfanilamide) should be considered as zero within experimental error. The negative or high values for K_{AS} and K_{AM} (sulfadimethoxine, sulfamethazine, sulfapyridine, and sulfaquinoxaline) arise from the extrapolation to concentrations close to the cmc. The intercepts

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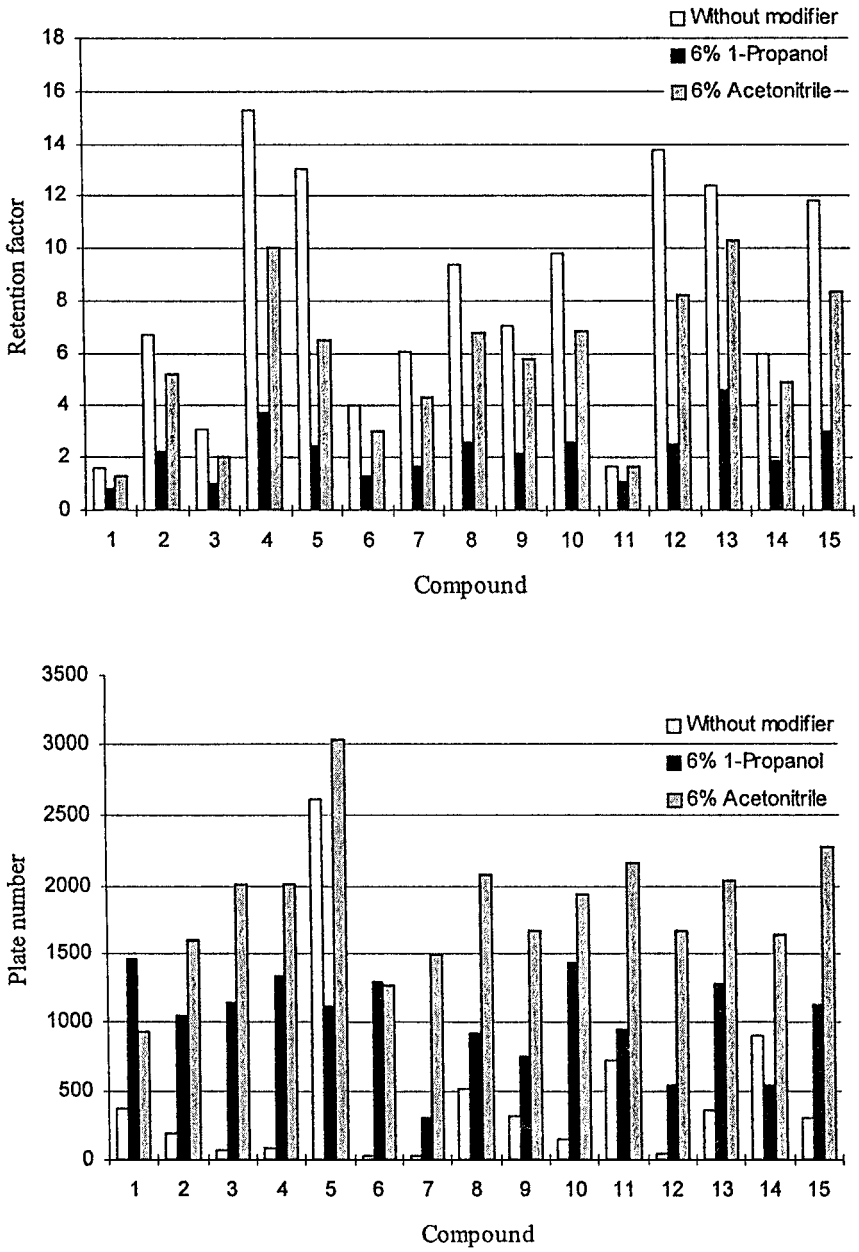

Figure 3. Retention factors and efficiencies for the 15 sulfonamides eluted with mobile phases of 0.075 *M* SDS at pH 3.0. The identity of the compounds is given in the legend to Figure 2.

Table 2. Parameters of the Retention Model (Eq. 1)

Compound	ε_r (%)	K_{AS}	K_{AM}	K_{MD}	K_{AD}
Sulfacetamide	2.2	4.35 ± 0.28	22.1 ± 3.8	0.1 ± 3.5	14.8 ± 3.1
Sulfachloropyridazine	3.5	160 ± 21	356 ± 53	3.0 ± 2.4	96.4 ± 4.8
Sulfadiazine	1.8	14.7 ± 1.3	57.0 ± 9.7	-1.6 ± 2.3	40.7 ± 6.5
Sulfadimethoxine	2.4	-420 ± 94	-466 ± 44	7.4 ± 4.6	-74 ± 70
Sulfaguanidine	5.1	573 ± 928	588 ± 1046	7.8 ± 11.0	304 ± 580
Sulfamerazine	3.2	55.3 ± 6.3	176 ± 25	4.0 ± 5.2	71 ± 22
Sulfamethazine	2.9	$(3.1 \pm 0.3) \times 10^{11}$	$(8.3 \pm 0.7) \times 10^{11}$	3.4 ± 2.0	$(2.1 \pm 0.4) \times 10^{11}$
Sulfamethoxazole	3.8	236 ± 78	357 ± 89	6.1 ± 7.7	121 ± 42
Sulfamethizole	4.1	371 ± 151	770 ± 336	3.3 ± 4.8	202 ± 17
Sulfamonomethoxine	3.2	585 ± 69	905 ± 108	6.2 ± 2.7	247 ± 69
Sulfanilamide	2.1	5.29 ± 0.31	25.2 ± 3.6	-2.9 ± 2.3	15.1 ± 2.2
Sulfapyridine	4.3	$(4.4 \pm 9.7) \times 10^{13}$	$(4.8 \pm 0.8) \times 10^{13}$	14.3 ± 4.7	$(1.1 \pm 0.2) \times 10^{13}$
Sulfaquinoxaline	2.8	$(1.6 \pm 0.2) \times 10^{8}$	$(1.8 \pm 0.2) \times 10^{8}$	7.3 ± 3.7	$(8 \pm 12) \times 10^{6}$
Sulfathiazole	4.0	267 ± 243	642 ± 641	2.7 ± 4.4	199 ± 208
Sulfisoxazole	3.7	$(1.5 \pm 1.7) \times 10^{3}$	$(1.8 \pm 2.2) \times 10^{3}$	7.4 ± 5.4	581 ± 557

of the $1/k$ vs. [M] plots are, in this case, approximately zero. This merely reflects the high compound affinity for the micellar or surfactant-coated stationary phase compared to that of the bulk aqueous component of the mobile phase. In fact, sulfadimethoxine, sulfapyridine, and sulfaquinoxaline are highly retained. The abnormal high K_{AS} and K_{AM} values give rise to a correlation between these parameters. The model (Eq. 1) is reduced to:

$$k = \frac{K_{AS}}{K_{AM} K_{MD} \varphi\,[\mathrm{M}]} = \frac{K}{\varphi\,[\mathrm{M}]} \tag{2}$$

Consequently, the fitted k values depend on the $K_{AS}/(K_{AM}K_{MD})$ ratio. However, for these compounds the model still yields acceptable predictions of the retention.

The optimization strategy pointed out that the optimal mobile phase was located outside the upper left corner of the experimental design, at 0.019 *M* SDS/5.8% acetonitrile. Figures 4a and b show the predicted and experimental chromatograms for the mixture of 15 sulfonamides eluted with this mobile phase. A large area of overlapping is observed for sulfachloropyridazine-sulfamethazine sulfathiazole, sulfamethoxazole-sulfamethizole, and sulfaguan-idine-sulfamonomethoxine. Instead, a mixture of 11 sulfonamides (the previous 15 ompounds except sulfachloropyridazine, sulfamethazine, sulfamethizole, and sulfamonomethoxine) could be resolved with almost baseline resolution (Figs. 4c and d).

In any case, the agreement between predicted and experimental chromatograms was excellent, although the experimental chromatograms showed better

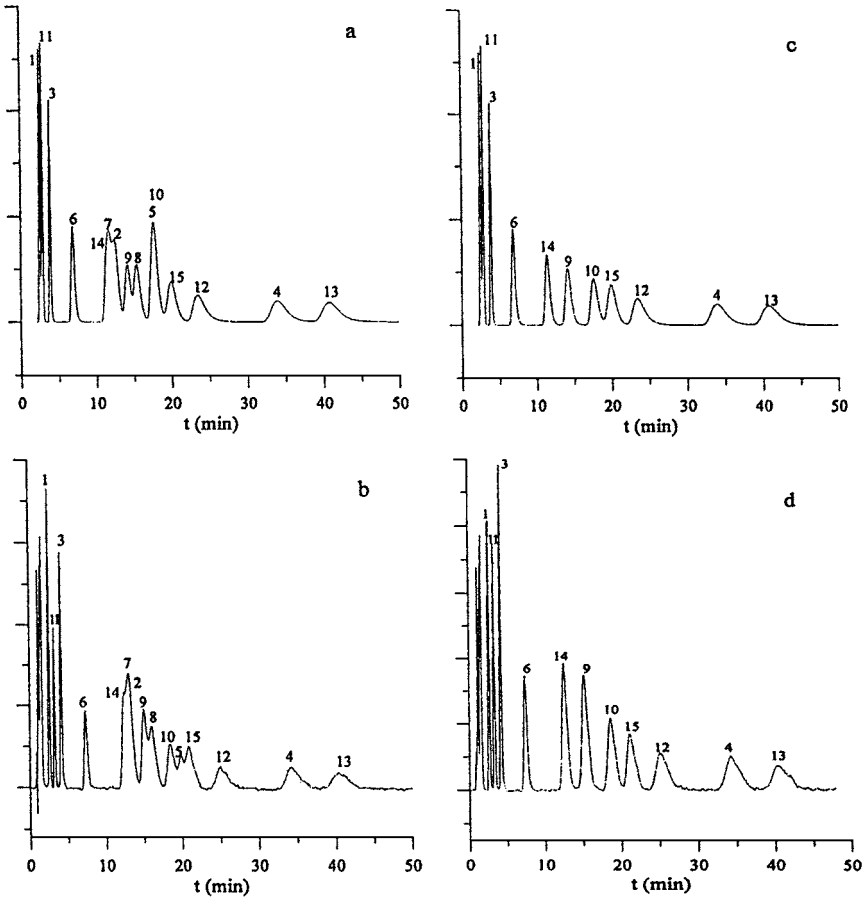

Figure 4. Predicted (a and c) and experimental (b and d) chromatograms for a mixture of 15 (a and b) (c and d) sulfonamides, eluted with the optimal mobile phase: 0.019 *M* SDS/5.8% acetonitrile at pH 3.0. See the legend to Figure 2 for peak identities.

separation for sulfacetamide and sulfanilamide. The errors obtained in the prediction of peak position for sulfaguanidine should be attributed to the large dependence of its retention with pH at low pH (see Fig. 2).

Although it can be argued that a further decrease in the concentration of surfactant could enhance the resolution, this possibility was discarded since, collaterally, it would diminish the solubilization of the proteins in the food samples. A smaller concentration of surfactant would also undesirably increase the retention times. On the other hand, the resolution was rather poor at a higher concentration of surfactant and lower concentration of modifier with respect to the optimal mobile phase.

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As observed, complete resolution was only feasible in a narrow range of the factor space. The optimization strategy used in this work found, however, the best separation conditions, easily, whereas a sequential approach would have taken much time and effort.

The retention times for the last-eluting drugs were rather high: sulfapyridine (25 min), sulfadimethoxine (34 min), and sulfaquinoxaline (40 min). These sulfonamides are the most hydrophobic among those studied and show a slow action. Therefore, they are rarely administered. Equation (1) and the parameters given in Table 2 can be used to predict the separation of mixtures containing a smaller number of sulfonamides or select a mobile phase composition that yields more adequate retention times for each drug. As an example, the retention times of sulfapyridine, sulfadimethoxine, and sulfaquinoxaline can be calculated to decrease to 9.7, 12.7, and 13.3 min, respectively, for a mobile phase of 0.045 *M* SDS/6.0% acetonitrile.

Wieling et al. (19) optimized the separation of 12 sulfonamides (9 from those studied in this work: sulfacetamide, sulfachloropyridazine, sulfaguanidine, sulfamerazine, sulfamethoxazole, sulfamethizole, sulfanilamide, sulfapyridine, and sulfathiazole, plus phthalylsulfacetamide, sulfamethoxypyridazine, and sulfisomide), using a quaternary mobile phase of buffer/methanol/acetonitrile/tetrahydrofuran. There was a partial overlapping of sulfamerazine, sulfapyridine, and sulfathiazole at low retention times (less than 5 min), but the mixture eluted in only 17 min. The quaternary aqueous-organic mobile phase would not, however, allow the direct injection of the food samples into the chromatographic system.

Finally, Yang and Khaledi (3) reported the resolution of a mixture of 12 sulfonamides (9 studied in this work: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamonomethoxine, sulfaquinoxaline, and sulfathiazole, plus sulfabenzamide, sulfamethoxypyridazine, and sulfisomide). The drugs were resolved in less than 17 min using a micellar mobile phase of 0.070 *M* SDS/6.0% 1-propanol at pH 3.0, with only partial overlapping of sulfamethoxypyridazine, sulfachloropyridazine, and sulfamonomethoxine, due to the atypical high column efficiencies ($N = 7000$ plates) achieved with a hydrophilic end-capped YMC ODS-AQ 250-mm long column, which is rather unusual. As mentioned above, the resolution achieved in this work with the mobile phases of SDS-1-propanol using the ODS-Hypersil column was rather low.

Analysis of Milk and Honey

Figure 5 shows chromatograms of milk and honey samples spiked with 0.5–1.5 μg/mL of 11 sulfonamides (the same as in Figs. 4c and d), which were eluted with 0.019 *M* SDS/5.8% acetonitrile at pH 3.0. The chromatograms of the matrices are also given. The milk (whole cow's milk) and honey samples used

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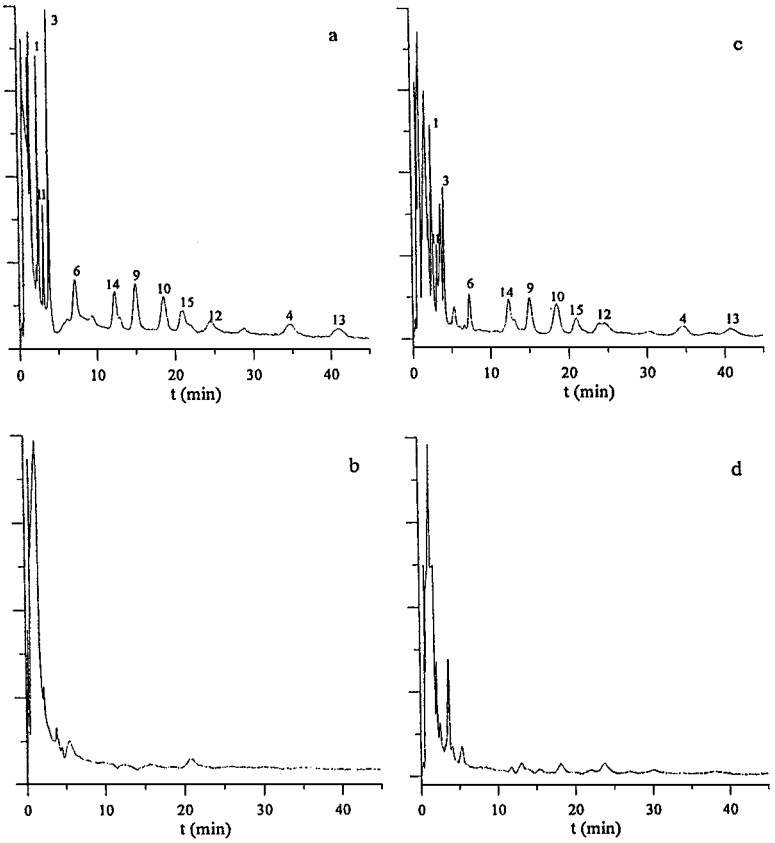

Figure 5. Chromatograms of milk (a and b) and honey (c and d) samples diluted in a 1:10 factor, injected directly into an ODS-Hypersil column and eluted with 0.019 *M* SDS/5.8% acetonitrile at pH 3.0. Milk (a) and honey (c) samples were spiked with 11 sulfonamides in the range 0.5–1.5 μg/mL. The chromatograms of the matrices (b and d) are also shown. See the legend to Figure 2 for peak identities.

for these experiments were Spanish products acquired in a local supermarket. The trademarks for the milk samples were Puleva (Granada), Pascual (Burgos), and Kaiku (Guipúzcoa) and for the honeys were San Francisco (Barcelona) and Anae (Ayora). Several chromatograms of the products were taken at different times during 1 year to reveal changes in the matrices.

The chromatograms of the matrices showed a wide band at short retention times (below 4 min), which also appeared in the chromatograms of the main compounds found in milk (lactose, bovine milk fat—commercial butter, casein, β-lactoglobulin, and α-lactoalbumin), and honey samples (glucose, fructose,

saccharose, and maltose), at the normal concentrations. The chromatograms of the matrices also contained several small peaks. The presence and size of these peaks depended on the analyzed lot, especially for the honey samples.

A peak was obtained at 21 min for milk samples, which should be assigned to an endogeneous compound of unknown identity. This peak affects the quantification of sulfisoxazole with the selected mobile phase. However, the resolution of this drug and the endogeneous compound was possible by changing the composition of the mobile phase.

The samples were diluted with 0.10 *M* SDS to facilitate the solubilization of the matrix compounds (proteins and fats) and release the protein-bound drugs. Although the stationary phase is coated with surfactant monomers, this dilution is convenient to prevent damage of the chromatographic column due to protein precipitation. The injection of a large number of biological samples without dilution may be harmful, with shortening of column life or requirement of a frequent regeneration of the stationary phase.

Also, as mentioned, because there is no sample cleanup, the biological matrix can eclipse the peaks of early-eluting drugs. The width of the protein band is diminished when the food samples are diluted with the micellar solution before their injection. We observed that the retention times did not change, at least after 100 sequential injections into the chromatographic system of milk and honey samples diluted in a 1:10 factor. We also checked that a 3:10 dilution permitted a high number of injections without any change in back-pressure.

Calibration curves were obtained for the 11 sulfonamides in aqueous solutions and milk- and honey-spiked samples. Drug concentrations were 0.1, 0.5, 1.0, 2.0, and 3.0 μg/mL. The regression coefficients of the fitted straight lines were, in all cases, $r > 0.999$. The slopes of the calibration straight lines decreased, usually in the order water > milk > honey. The recoveries found for milk and honey samples spiked with 1 μg/mL of each sulfonamide, calculated from the calibration straight lines obtained in aqueous solution, are indicated in Table 3. As observed, the recoveries were in the ranges 87–108% and 72–119% for milk and honey samples, respectively.

The limits of detection (LODs, 3s criterion) were calculated by measuring the peak areas obtained by injection of six sulfonamide solutions at low concentrations (close to the LODs). The values given in Table 3 for milk and honey corresponding to the injection of samples diluted in a 1:10 factor referred to the original sample without any dilution. The LODs of sulfamerazine, sulfapyridine, and sulfaquinoxaline in milk samples and for sulfadiazine, sulfadimethoxine, sulfamerazine, sulfaquinoxaline, and sulfathiazole in honey samples were too high. However, using a lower dilution factor for the injected samples, the LODs were 2- to 3-fold lower than the values shown in Table 3. Injection of honey samples was less problematic for the column, but the presence of endogeneous (and possible added) compounds decreased the reliability of the detection of sulfonamides.

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Table 3. Recoveries, Limits of Detection, and Coefficients of Variation[a]

	Water		Milk[b]			Honey[b]		
Compound	LOD (μg/mL)	CV (%)	Recovery (%)	LOD (μg/mL)	CV[c] (%)	Recovery (%)	LOD (μg/mL)	CV (%)
Sulfacetamide	0.01	0.1	108	0.7	1.6	104	0.4	1.2
Sulfadiazine	0.04	1.0	106	0.4	1.0	89	1.0	1.6
Sulfadi-methoxine	0.10	3.7	94	0.6	2.1	82	2.0	4.9
Sulfamerazine	0.02	0.7	94	3.0	10.8	81	0.9	3.0
Sulfamethizole	0.03	0.8	96	0.5	1.4	78	0.4	1.2
Sulfamono-methoxine	0.04	1.3	93	0.3	1.1	90	0.8	2.2
Sulfanilamide	0.02	0.6	103	0.2	0.6	119	0.6	0.8
Sulfapyridine	0.03	1.1	87	0.8	3.7	95	0.1	5.2
Sulfaquin-oxaline	0.30	8.7	92	1.0	4.7	72	0.9	3.4
Sulfathiazole	0.04	1.5	90	0.5	1.6	82	2.0	5.2
Sulfisoxazole	0.04	1.3	97	0.5	1.0	77	0.7	1.9

[a] $n = 6$.
[b] LODs were obtained by injection of 1:10 diluted samples but are referred to the original samples.
[c] CV, coefficient of variation.

In conclusion, a method for the determination of residual amounts of sulfonamides in milk and honey was developed, which allows the direct injection of the samples into the chromatographic system. The method is simple, rapid, reliable, and economical and permits the screening of these drugs with good accuracy and precision.

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