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Determination of sulfadimethoxine, sulfamethoxazole, trimethoprim and their main metabolites in porcine plasma by column switching HPLC*

M. J. B. MENGELERS,†‡ M. B. M. OORSPRONG,‡ H. A. KUIPER,‡ M. M. L. AERTS,‡
E. R. VAN GOGH and A. S. J. P. A. M. VAN MIERT§

‡ *State Institute for Quality Control of Agricultural Products, P.O. Box 230, 6700 AE Wageningen, The Netherlands*
§ *Department of Veterinary Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, State University of Utrecht, The Netherlands*

Abstract: A HPLC method for the determination of sulfadimethoxine, sulfamethoxazole, trimethoprim and their main metabolites in porcine plasma is reported. The metabolites under investigation were the *N*4-acetyl sulfonamides and 3′- and 4′-demethyl trimethoprim. In order to obtain a sensitivity of 25–50 ng ml^{-1}, the application of column switching HPLC was investigated. An on-line preconcentration of the drugs and metabolites was preceded by an off-line sample pre-treatment. Parent compounds and metabolites were separated by reversed-phase HPLC followed by UV-detection. The mean recoveries for 4′-demethyl trimethoprim were >80% while the mean recoveries for the other compounds were >90%. Application of the method for analysis of plasma samples obtained from pharmacokinetic studies is described.

Keywords: *Sulfadimethoxine; sulfamethoxazole; trimethoprim; metabolites; determination in plasma; column switching HPLC.*

Introduction

Combinations of sulfonamides and trimethoprim are used in human and veterinary medicine for the treatment of specific bacterial infections. In order to investigate the pharmacokinetics of sulfadimethoxine (SDM), sulfamethoxazole (SMX) and trimethoprim (TMP) in healthy and diseased pigs, a method had to be developed to determine these drugs and their main metabolites in plasma by HPLC. The major metabolic pathway of most sulfonamides in pigs is supposed to be *N*-acetylation and of trimethoprim, *O*-demethylation with subsequent conjugation [1–4]. The molecular structures of the investigated drugs and metabolites are depicted in Fig. 1.

To our knowledge, no methods have been published on the liquid chromatographic determination of SDM and *N*4-acetyl SDM in biological fluids. The determination of

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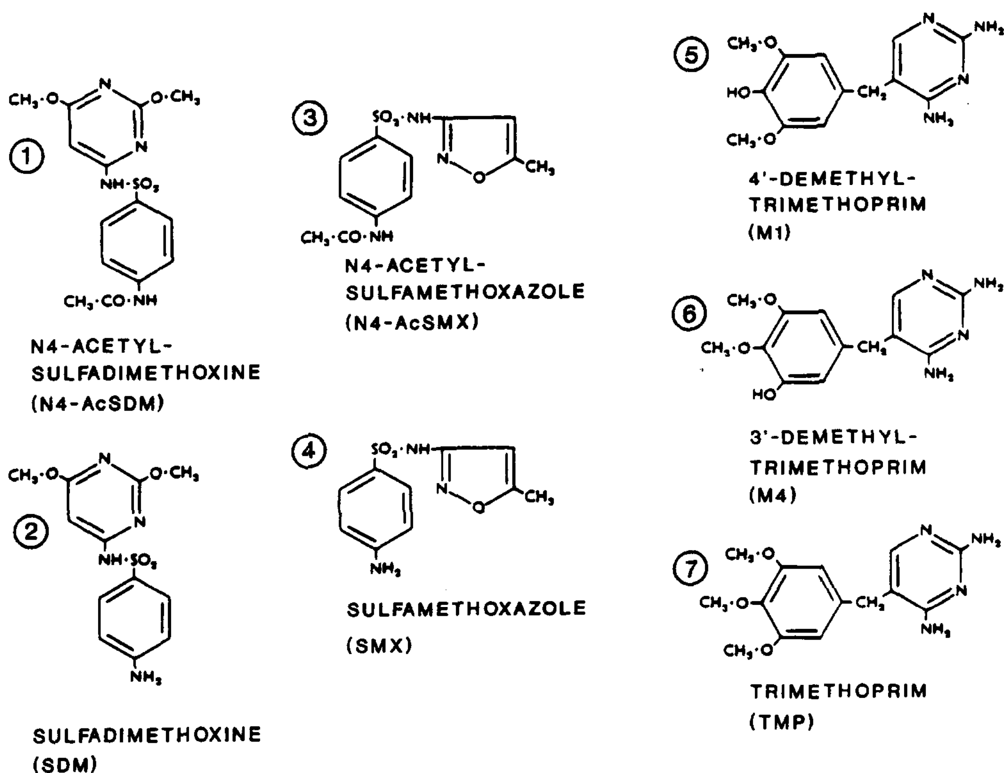
†To whom correspondence should be addressed.



	SMX	N4-AcSMX	SDM	N4-AcSDM	TMP	M1	M4
pKa-1	± 2	± 2	± 2	± 2	7.2	± 7.2*	± 7.2*
pKa-2	5.7	5.0	6.3	6.0	-	± 9.5*	± 9.5*

* estimated pKa-values

Figure 1
Molecular structures and pK_a-values of the investigated drugs and metabolites.

SMX, TMP and their main metabolites in biological fluids by high-performance liquid chromatography (HPLC) has been described by several investigators [5–17]. Both normal phase and reversed-phase separation have been applied in combination with UV-detection. Post-column derivatization of sulfonamides with fluorescamine or *p*-dimethylaminobenzaldehyde has been applied to enhance both the selectivity and sensitivity [18–20]. However, derivatization of the *N*-4 amino group of the sulfonamides excludes detection of the *N*4-acetyl metabolites [21]. SMX and TMP were also determined by HPLC using fluorescence detection [15, 16, 22]. However, the native

fluorescence of TMP and SMX is relatively low and is less sensitive compared to variable wavelength UV-detection [16, 22]. Trimethoprim has also been determined in plasma and urine by HPLC using electrochemical detection [17, 18]. An improved sensitivity was achieved when a high oxidation potential (1200 mV) was used, but at the cost of an increased base-line noise.

For the determination of SMX, TMP and metabolites in biological fluids by HPLC a number of off-line sample pre-treatment procedures have been described. Methods implying only a deproteinization of the plasma or serum without enrichment would not have been sensitive enough for our purposes [5–9]. Mostly, a liquid–liquid extraction was applied to the (deproteinized) biological fluids. In view of the different pK_a-values of the investigated compounds (see Fig. 1), a simultaneous extraction of the sulfonamides, trimethoprim and their metabolites with an organic solvent could never result in high recoveries (i.e. >80%) for all compounds [10–13], as had been clearly demonstrated by Spreux-Varoquaux and co-workers [11].

The purpose of this study was to develop a method consisting of a combination of an off-line sample pre-treatment and an on-line preconcentration by means of column switching HPLC. Application of this method to the analysis of plasma samples obtained from animals treated with these drugs is described.

Experimental

Chemicals and reagents

Sulfadimethoxine (SDM), sulfamethoxazole (SMX) and trimethoprim (TMP) were obtained from Sigma (St. Louis, MO, USA). *N*4-acetyl sulfadimethoxine (*N*4-AcSDM) and *N*4-acetyl sulfamethoxazole (*N*4-AcSMX) were kindly donated by Dr T. B. Vree (St. Radboud Hospital, Nijmegen, The Netherlands); 4′-demethyl trimethoprim (M1) was kindly donated by Wellcome Nederland B.V. (Weesp, The Netherlands) and 3′-demethyl trimethoprim (M4) was kindly donated by Hoffmann-La Roche B.V. (Mijdrecht, The Netherlands). All chemicals were of analytical grade, triethylamine (TEA) was purchased from Fluka Chemie AG (Buchs, Switzerland) and all other chemicals were obtained from Merck AG (Darmstadt, FRG). β-Glucuronidase (*Escherichia coli*) was obtained in vials of 1000 Sigma units from Sigma. Extrelut columns (1 ml) were obtained from Merck.

The solution of 0.5 M ammonium acetate was adjusted to pH 3.5 with concentrated hydrochloric acid. Solutions of 0.5 M KH_2PO_4 were adjusted to pH 6.6 or 6.8 with 10 M potassium hydroxide.

Standards and spiked plasma samples

Stock solutions of SDM, *N*4-AcSDM, SMX and *N*4-AcSMX, in dimethylformamide (DMF) were prepared at a concentration of 20 mg ml^{-1}. Stock solutions of TMP, M1 and M4 contained 2 mg per ml in DMF. Standard solutions of sulfonamides and metabolites were prepared by diluting the stock solutions with 0.05 M potassium phosphate pH 7.5 to concentrations of 2000, 200, 20 and 2 μg ml^{-1}. Standard solutions of TMP, M1 and M4 were prepared by diluting the stock solutions with 0.05 M potassium phosphate pH 6.5 to concentrations of 200, 20 and 2 μg ml^{-1}. Stock and standard solutions of all compounds were kept at 5°C and were freshly prepared every month, with the exception of the standard solution of SDM at a concentration of 2 mg ml^{-1} which had to be prepared fresh for each analysis because storage at 5°C resulted in

the precipitation of the drug. Working standard solutions were prepared by diluting an aliquot of a standard solution with 0.05 M potassium phosphate pH 4.6 (for sulfonamides and metabolites) or with 0.05 M potassium phosphate pH 6.8 (for trimethoprim and metabolites).

Aliquots of the standard solutions ($\leq$50 μl) were used to spike 1.0 ml of blank plasma. After mixing on a Vibrofix mixer the spiked plasma sample was left to stand for 30 min to allow possible protein binding of the drugs and metabolites before the extraction was carried out.

Instrumentation and chromatographic conditions

The liquid chromatographic system consisted of a thermostatically cooled autosampler (Applied Biosystems, ABI, Maarssen, The Netherlands), two solvent-delivery pumps, model SF-400 (ABI), an automatic six-port switching valve (ABI), a thermostatically controlled column oven (ABI), an UV-vis detector, model SF-783 (ABI), a solvent programmer, model SF 450 (ABI). The analytical column was a Chromsep C18 cartridge, 5 μm, 200 × 3.0 mm I.D. (Chrompack, Middelburg, The Netherlands). The column temperature was kept at 30°C. The detection was performed at 270 nm (sulfonamides and their metabolites) or at 230 nm (TMP and its metabolites).

For SDM the mobile phase was a mixture of 0.05 M potassium phosphate pH 6.5 and acetonitrile (85:15) and for SMX a mixture of 0.05 M potassium phosphate pH 5.6 and acetonitrile (88:12). The eluent for TMP consisted of a mixture of 0.05 M potassium phosphate, to which 0.2% triethylamine was added, pH 6.0, and acetonitrile (90:10). The eluents were filtered and degassed with helium before use, flow rates were 0.8 ml $\min^{-1}$.

Preconcentration was carried out on a small stainless steel column (10.0 × 3.0 mm I.D.) dry packed with PLRP-S (15–25 μm, 100 Å, Polymer Laboratories, Church Stretton, Shropshire, UK) or Bondapak C-18/Corasil (37–50 μm, Millipore, Etten-Leur, The Netherlands). SDM, SMX and their acetyl metabolites were preconcentrated with a 0.05 M phosphate solution pH 4.6 and for TMP and its metabolites a slightly basic 0.05 M phosphate solution pH 8.2 was used. The phosphate solutions were filtered and degassed with helium before use, flow rates were 1.0 ml $\min^{-1}$. The injection volume was 200–500 μl. After a flush period of 4 min with a 0.05 M phosphate solution the valve was switched and the concentrated sample was backflushed for 2 min to the analytical HPLC column with one of the HPLC eluents. The valve was then switched back to the original position.

Manual mixing was done with a Vibrofix mixer, model VF 1 (Janke and Kunkel GMBH, Staufen, FRG). Mechanical mixing of, up to 56, plasma extracts simultaneously was done with a Vortex-mixer, model 3-2201 (Buchler Instruments Inc., Fort Lee, NJ, USA).

Sample pre-treatment

The plasma sample (1.0–2.0 ml) was pipetted into a centrifuge tube and an equal volume of acetonitrile was added. The stoppered tube was shaken vigorously for 15 s on a Vibrofix mixer and allowed to stand for 10 min to ensure complete protein precipitation. The solution was then mixed again and centrifuged at 10,000*g* for 10 min. The clear supernatant was divided into two equal parts for the determination of the sulfonamides (including metabolites) and trimethoprim (including metabolites), respectively.

Sulfonamides and metabolites. One part of the deproteinized plasma sample was adjusted to pH 4.5 with 0.5 M ammonium acetate pH 3.5 (approximately 100 μl buffer per ml supernatant), mixed on a Vibrofix mixer and 1.0 ml of the solution was immediately applied onto an Extrelut column, thereby preventing turbidity of the sample. After 10 min the column was eluted with 6 ml dichloromethane (DCM) and 1.0 ml 0.05 M KOH was added to the eluate. The glass tube was stoppered and the two phases were mechanically mixed on a Vortex mixer for 10 min. For complete phase separation the samples were centrifuged at 300*g* for 2 min. The aqueous layer was transferred to another glass tube and traces of DCM were evaporated by percolation of a nitrogen stream for 10 s. An aliquot (600 μl) of the aqueous sample was pipetted into a vial and adjusted to pH 4.0–5.0 with approximately 25 μl 1 M phosphoric acid. After mixing, 200–500 μl were injected onto the preconcentration column.

Trimethoprim and metabolites. The other part of the deproteinized plasma sample was adjusted to pH 8.0 with 0.5 M potassium phosphate pH 6.6 (approximately 20 μl buffer per ml supernatant), mixed on a Vibrofix mixer and 1.0 ml was applied onto an Extrelut column. After 10 min the column was eluted with 6 ml dichloromethane–*i*-propanol (95/5, v/v) and 1.0 ml 0.05 M phosphoric acid was added to the eluate. The glass tube was stoppered and the two phases were mixed mechanically on a Vortex mixer for 10 min. For complete phase separation the samples were centrifuged at 300*g* for 2 min. The aqueous layer was transferred to another glass tube and traces of dichloromethane–*i*-propanol were evaporated by percolation of a nitrogen stream for 30 s. An aliquot of the aqueous sample (600 μl) was pipetted into a vial and adjusted to pH 6.0–7.0 with approximately 45 μl 1 M KOH. After mixing, 200–500 μl were injected onto the preconcentration column.

For quantification of M1-glucuronide and M4-glucuronide, the plasma samples were divided into two parts before deproteinization. One part (1.2 ml) was transferred to a vial containing β-glucuronidase and 300 μl 0.5 M potassium phosphate pH 6.8 was added. After mixing, the solution was incubated at 37°C overnight. Standards and spiked plasma samples were also kept at 37°C overnight. The other part of the plasma sample was used for the determination of the sulfonamides and their metabolites. Subsequent sample preparation was carried out as described above.

Quantification

Calibration curves were obtained by plotting the peak areas of each compound versus concentrations. The studied concentration ranges were 0.05–10 $\mu g\ ml^{-1}$ for TMP and its metabolites and 0.05–100 $\mu g\ ml^{-1}$ for sulfonamides and their metabolites. With each sample series three spiked plasma samples of different concentrations and their corresponding standards were analysed to check linearity and recovery. Concentrations of plasma samples from treated pigs were determined from the calibration curves.

Recovery

Known amounts of the analytes were added to blank porcine plasma. The plasma samples were extracted as previously described and standards were correspondingly diluted in 0.05 M potassium phosphate pH 4.6 (for sulfonamides and metabolites) or 0.05 M potassium phosphate pH 6.8 (for trimethoprim and metabolites). The peak areas of spiked plasma samples were compared to those of the standards.

Stability

The stability of stock and standard solutions kept at 5°C and frozen (−20°C) plasma samples as well as frozen plasma extracts was checked.

Little is known on the stability of the demethyl metabolites of trimethoprim at different pH and temperature. Therefore, standard solutions of TMP, M1 and M4 (1 μg ml^{-1} in 0.05 M potassium phosphate pH 8.2, 6.8 and 4.6) were analysed immediately, or after 16 hr at room temperature (20°C), or after 24 hr at 5°C. In order to simulate the circumstances of deglucuronidation an aliquot of the standard solution at pH 6.8 was kept at 37°C for 16 h. The analysis of all the standard solutions was carried out without on-line preconcentration, e.g. by direct injection of the standard solutions on the analytical column.

Animal experiments

Combination preparations of a sulfonamide and trimethoprim were administered orally to healthy piglets weighing 30–40 kg. Two groups of six piglets received a suspension of the drug combination (single dose) 1 h after feeding. One group was given 25 mg kg^{-1} SMX in combination with 5 mg kg^{-1} TMP and the other group received 25 mg kg^{-1} SDM and 5 mg kg^{-1} TMP. Seven days before and during the pharmacokinetic study all animals were given non-medicated feed. Venous blood samples (10 ml) were collected at scheduled intervals up to 72 h after administration of the drugs. Heparinized blood samples were centrifuged at 3000*g*, plasma was separated, frozen and stored at −20°C until analysed.

Results

Preconcentration

The preconcentration of standard solutions was tested on PLRP-S and Bondapak C-18/Corasil material, applying flush volumes of 1, 2, 4 and 6 ml respectively. Because the Bondapak C-18/Corasil material is based on silicagel, TMP and its metabolites could not be preconcentrated with a buffer solution of pH 8.2. Preconcentration with purified, distilled water resulted in a breakthrough of the metabolites of TMP after a flush volume of only about 1 ml. Preconcentration of the sulfonamides and their metabolites on Bondapak C-18/Corasil was investigated with a buffer solution of pH 4.6. SDM and *N*4-AcSDM were quantitatively retained after a flush volume of 1 ml. After 2, 4 and 6 ml the percentage of SDM lost after preconcentration was 4, 18 and 40% respectively. *N*4-AcSDM showed a breakthrough after a flush volume of 6 ml. SMX and *N*4-AcSMX showed already a breakthrough after 1 ml. Because the PLRP-S material is resistant to basic solutions the preconcentration of TMP and its metabolites was carried out with a buffer solution of pH 8.2. For preconcentration of the sulfonamides and their metabolites a buffer solution of pH 4.6 was used. All compounds investigated showed complete retention on the PLRP-S material even after a flush volume of 6 ml. Preconcentration of extracted plasma samples showed that a flush volume of 4 ml was sufficient to remove most of the matrix interferences. Increasing the flush volume did not result in a further improvement. Complete elution with an eluent containing 10% or more acetonitrile was already achieved after a backflush period of 1–2 min.

Linearity

The calibration curves were found to be linear in the concentration ranges:

0.05–100 μg ml^{-1} for SDM, *N*4-AcSDM, SMX and *N*4-AcSMX and 0.05–10 μg ml^{-1} for TMP, M1 and M4. The correlation coefficients generally exceeded 0.999.

Recovery

The recoveries obtained for the investigated drugs and metabolites are shown in Tables 1 and 2. In the concentration ranges tested, the recoveries were found to be very constant (coefficients of variation <10%, except for M1) giving average values of more than 80% for M1 and more than 90% for all other compounds.

Representative chromatograms of blank porcine plasma and plasma spiked at a concentration of 0.1 μg ml^{-1} for all compounds are given in Fig. 2.

Detection limits

The limit of detection was defined as the amount of drug which resulted in a peak-area three times that of the baseline noise in a blank sample. The calculated limits of detection were 15 ng ml^{-1} for SDM and *N*4-AcSDM, 25 ng ml^{-1} for SMX, *N*4-AcSMX and TMP, and 50 ng ml^{-1} for M1 and M4. These are conservative estimations of the detection limits as compared to the procedure proposed by the IUPAC [23] where the limit of detection is defined as the mean of the measured content of representative blank samples ($n \geq 20$) plus three times the standard deviation of the mean.

Specificity

The described off-line sample pre-treatment in combination with the on-line preconcentration gave a quantitative separation of TMP and its metabolites from the

Table 1
Mean recoveries ($n = 6$) and coefficients of variation (C.V.) of the sulfonamides and their *N*4-acetyl metabolites (C.V.s are given in parentheses)

Concentration (μg ml^{-1})	*N*4-AcSDM (%)	SDM (%)	*N*4-AcSMX (%)	SMX (%)
0.05	102 (2.4)	101 (6.9)	102 (4.5)	105 (3.4)
0.1	98.1 (2.1)	98.6 (2.8)	103 (3.4)	99.1 (4.2)
0.5	99.8 (3.5)	97.8 (1.9)	99.1 (3.1)	98.2 (4.8)
1.0	100 (1.4)	96.2 (2.8)	100 (2.6)	96.2 (3.1)
5.0	99.0 (2.3)	96.1 (1.6)	101 (2.1)	98.5 (2.3)
10.0	98.9 (1.1)	97.4 (0.9)	104 (1.1)	101 (2.2)
50.0	99.2 (1.5)	98.1 (2.1)	101 (1.2)	98.7 (1.2)
100.0	99.7 (0.9)	97.9 (1.1)	98.9 (1.1)	98.2 (1.0)

Table 2
Mean recoveries ($n = 6$) and coefficients of variation (C.V.) of trimethoprim and its demethyl metabolites (C.V.s are given in parentheses)

Concentration (μg ml^{-1})	M1 (%)	M4 (%)	TMP (%)
0.05	84.0 (10.4)	100 (4.5)	95.8 (3.4)
0.1	85.9 (6.7)	102 (3.4)	95.3 (2.4)
0.5	90.7 (3.1)	94.3 (1.7)	95.4 (0.6)
1.0	92.3 (3.0)	93.7 (1.7)	97.9 (1.3)
5.0	90.6 (0.9)	100 (0.9)	99.5 (0.9)
10.0	88.2 (1.1)	97.0 (0.7)	96.1 (0.6)

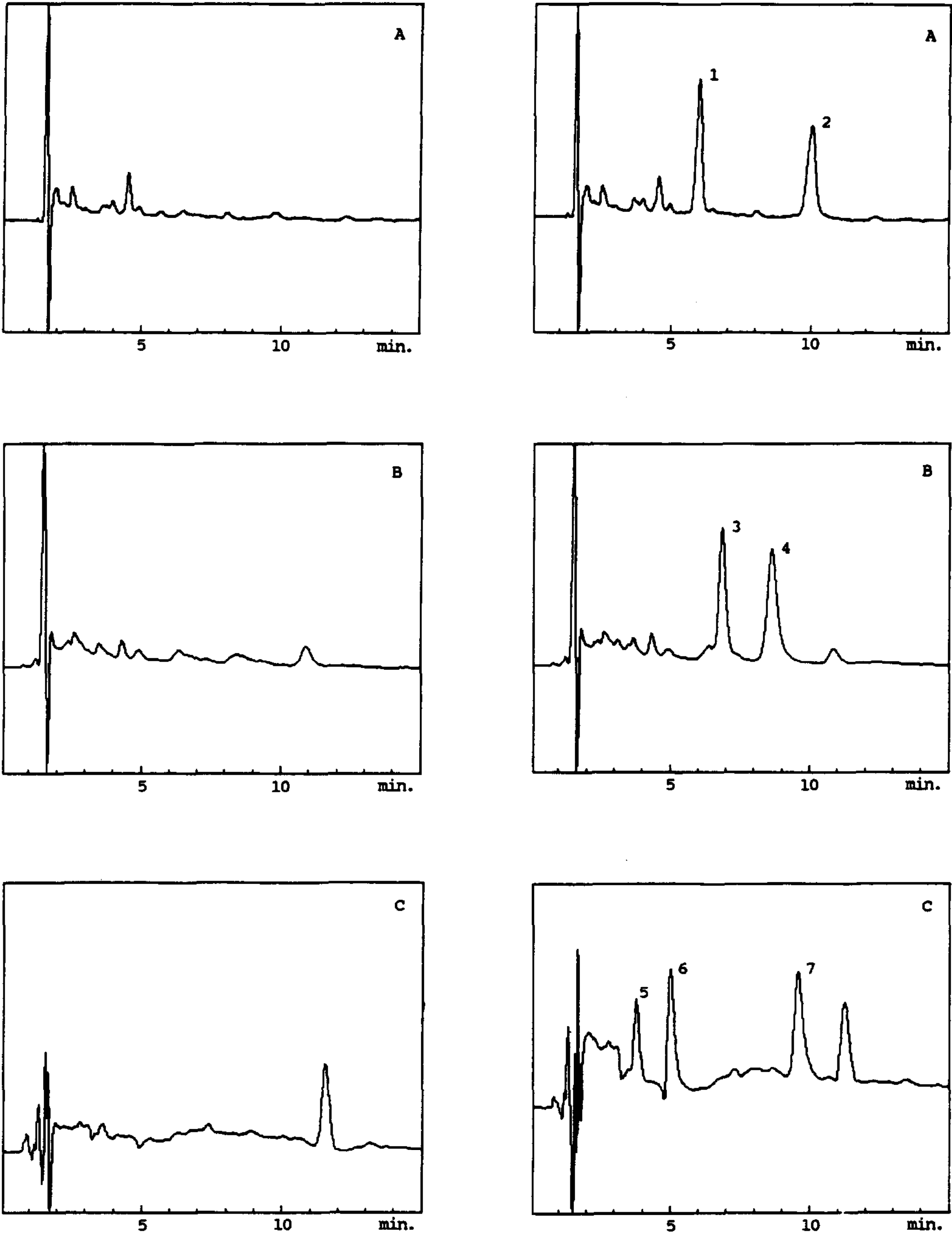


Figure 2
Chromatograms of blank (left) and spiked (right) plasma samples at 100 ng ml^{-1} for all compounds. 1=*N*4-AcSDM; 2=SDM; 3=*N*4-AcSMX; 4=SMX; 5=M1; 6=M4; 7=TMP A: 0.02 AUFS; B: 0.01 AUFS; C: 0.01 AUFS.

sulfonamides and their metabolites. However, a very small percentage of the sulfonamide (including metabolite) may co-elute with trimethoprim and its metabolites from the Extrelut column and subsequent protonation of the aromatic amino function may occur when re-extracted with 0.05 M phosphoric acid. In the case of SDM very high plasma levels (50–100 μg ml^{-1}) were obtained after medication and, in spite of an unfavourable preconcentration step, this resulted in peaks in the TMP-analysis corresponding to 10–100 ng ml^{-1} SDM. Chromatographic separation of SDM from TMP and its metabolites can be achieved when the proper eluent is used (pH 6.0).

Interferences

Chromatograms of blank porcine plasma are shown in Fig. 2. Generally no interfering peaks were observed. However, the chromatographic behaviour of the sulfonamides, trimethoprim and their metabolites strongly depends on the pH of the eluent. Slight changes in the pH of the eluent have great impact on the retention times of the investigated compounds [10, 22]. Therefore, sometimes a minor adjustment of the pH was necessary to separate an interfering substance from, mainly, M1, *N*4-AcSMX and SMX.

The packing material of the preconcentration column was renewed after approximately 100 injections. Deterioration of the preconcentration column was visible on the chromatogram resulting in additional peaks or an increased base line noise. Therefore every day a buffer solution was injected onto the preconcentration column to check for interfering substances. This was more evident when the detection was carried out at 230 nm. An example of the latter can be seen in Fig. 2C comparing the baseline of the chromatograms of the blank and the spiked porcine plasma sample.

Stability

Stock and standard solutions as described under Experimental were stable at 5°C for at least one month. Frozen plasma samples as well as frozen plasma extracts were stable at −20°C for at least six months.

Standard solutions of TMP and M4 were stable at different pH and different temperatures. Standard solutions of M1 at pH 4.6 and 6.8 kept at room temperature for 16 h or at 5°C for 24 h showed no decline in concentration. A standard solution of M1 at pH 6.8 kept at 37°C for 16 h (deglucuronidation conditions) showed a decomposition of 2.4%. However, a standard solution of M1 at pH 8.2 kept at 5°C for 24 h decreased in concentration by 30.7% and by 55.5% when kept at room temperature for 16 h. Therefore, extracted plasma samples and standards containing M1 were stored at pH <7.

Animal experiments

Typical chromatograms of porcine plasma samples collected during a pharmacokinetic study are shown in Fig. 3. The analysed plasma samples were taken 12 h after oral administration of 25 mg kg^{-1} sulfonamide (SDM or SMX) in combination with 5 mg kg^{-1} trimethoprim. The calculated concentrations (in μg ml^{-1}) were: 4.4 for *N*4-AcSDM, 53.7 for SDM, 2.0 for *N*4-AcSMX, 5.6 for SMX, <0.05 for M1, 0.07 for M4 and 0.21 for TMP. The percentage of metabolites, based on the ratio of the area-undercurve of metabolite to parent drug, were: 5–10% for *N*4-AcSDM, 25–40% for *N*4-AcSMX, 5–10% for M1 and 20–35% for M4.

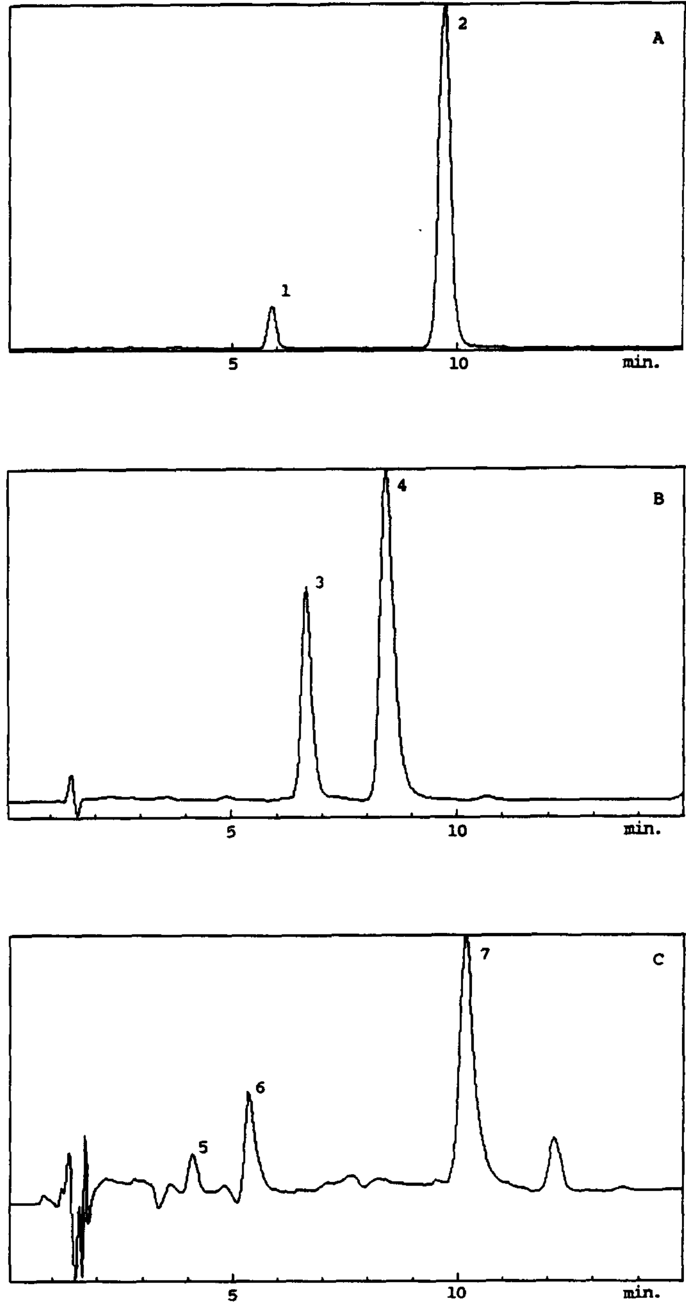


Figure 3
Typical chromatograms of plasma samples from treated pigs, 12 h after oral administration of 25 mg kg^{-1} sulfonamide (SDM or SMX) in combination with 5 mg kg^{-1} TMP. 1=*N*4-AcSDM; 2=SDM; 3=*N*4-AcSMX; 4=SMX; 5=M1; 6=M4; 7=TMP Concentrations (in μg ml^{-1}): *N*4-AcSDM: 4.4; SDM: 53.7; *N*4-AcSMX: 2.0; SMX: 5.6; M1: <0.05; M4: 0.07; TMP: 0.21.

Discussion

The pK_a-values of M1 and M4 had to be estimated because no data were available in the literature. The first pK_a-value (7.2) of the demethyl metabolites was based on the pK_a of TMP and the second pK_a-value (9.5) was estimated on account of the phenolic character of M1 and M4. The fact that M1 and M4 could not be extracted with an organic solvent at pH 10–11 confirmed the latter assumption. Therefore, the extraction of TMP, M1 and M4 was carried out at pH 8.0, as was previously described by Schwartz *et al.* and Sigel and Grace [24, 25]. During the development of our method we observed some remarkable differences between the chemical properties of M1 and M4. First of all, TMP and M4 could very well be eluted from an Extrelut column with DCM at pH 8.0. In the case of M1 however very poor recoveries were obtained. The addition of a small percentage of isopropanol (5%) improved the extraction of M1 remarkably. The solubility of M1 and M4 in DMF was also different. At a concentration of 2 mg ml^{-1} of DMF M4 could easily be dissolved but M1 only after sonication of the solution. Furthermore, M1 was less stable than M4 under slightly basic conditions and whenever the stock solution of M1 in DMF was not stored at 5°C the solution turned yellowish after a few days. The instability of M1 at pH >7 could very well explain why Nordholm and Dalgaard found a lower yield of deglucuronidated products after hydrolysis above pH 7 when compared to samples treated at pH <7 [18].

The described off-line sample pre-treatment gave a quantitative separation of the sulfonamides and their *N*4-acetyl metabolites from trimethoprim and its demethyl metabolites. Extraction of the investigated compounds at different pH resulted in very high recoveries which could not be obtained with a simultaneous extraction of SMX, *N*4-AcSMX and TMP [10–13]. The acetate buffer was adjusted to pH 3.5 with hydrochloric acid because acetic acid would rapidly elute with DCM from Extrelut columns causing poor recovery of sulphonamides and their metabolites. Application of an Extrelut column provided a simple extraction of the deproteinized plasma sample and during re-extraction no emulsions occurred.

The on-line preconcentration allowed the injection of relatively large sample volumes (0.2–0.5 ml) and a further clean-up of the extracted plasma samples. Provided that the pH of the preconcentration solution is adjusted to ensure the analytes to be in their undissociated form, the column switching technique yields good recoveries and low detection limits.

The liquid chromatographic separation of the drugs and metabolites on a reversed-phase C-18 column requires eluents of different pH. Tailing of TMP and metabolites can be suppressed by the addition of 0.2% (v/v) triethylamine to the eluent.

The described method was successfully applied to the routine analysis of plasma samples collected during pharmacokinetic studies. Low detection limits are a pre-requisite since the observed half-lives of SMX and TMP were very short (2–3 h). In the case of TMP, plasma levels dropped below the detection limit 18–24 h after an oral single dose administration of 5 mg kg^{-1}. The application of the described method to the determination of the investigated compounds in porcine urine is presently being studied at the authors' institute.

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