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Determination of trimethoprim in bovine serum by high-performance liquid chromatography with confirmation by thermospray liquid chromatography–mass spectrometry

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ABSTRACT

A high-performance liquid chromatographic (HPLC) method with a detection limit of 5 ng/ml was developed for the analysis of trimethoprim in bovine serum. Trimethoprim and the internal standard, ormetoprim, under alkaline conditions, were first extracted into dichloromethane and then back-extracted into dilute sulphuric acid (0.15 *M*) and cleaned-up on a C_{18} cartridge. Trimethoprim was quantified on a C_{18} column using a triethylammonium acetate–acetonitrile–methanol (16:3:1, v/v/v) mobile phase at a flow-rate of 1.5 ml/min, with ultraviolet detection at 225 nm. This method was used to verify the accuracy of test responses obtained with the Brilliant Black Reduction test, a rapid screening method, for trimethoprim levels in the serum of steers treated with Trivetrin. Confirmation of the presence of trimethoprim in the sample extract was obtained by thermospray HPLC–mass spectrometry.

INTRODUCTION

Trimethoprim (TMP), [5-(3,4,5-trimethoxybenzyl)pyrimidine]-2,4-diamine (Fig. 1), is used in the treatment of urinary tract infections, *Pneumocystis carinii* pneumonia, otitis media, shigellosis, salmonellosis, and chronic bronchitis in humans, animals and poultry [1–3]. It interferes with folate synthesis by blocking the enzyme dihydrofolate reductase, which converts dihydrofolic acid to tetrahydrofolic acid in a number of organisms [2]. TMP, distributed as Trivetrin, Borgal or Tribrissen, is available to food animal producers in Europe and North America, mostly

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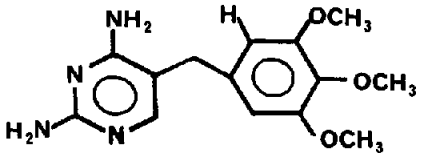

Fig. 1. Structure of trimethoprim.

as injectable formulations. The active ingredients of Trivetrin (Coopers Agropharm) and Borgal (Hoechst) are TMP and sulphadoxine (SDX), and of Tribrissen (Coopers Agropharm) are TMP and sulphadiazine (SDZ), in the ratio of 1:5 in each formulation. Together, these ingredients effect a double blockage on bacterial metabolism, giving a level of activity many times greater than that obtained for either drug alone. The recommended withdrawal period for Trivetrin in cattle and swine is ten days in meat and 96 h in milk when the recommended dose of 16 mg/kg body weight or 3 ml per 45 kg per day is administered intramuscularly or intravenously [4].

The use of TMP in animal production may result in residues of this antimicrobial drug in dairy products and tissues of animals intended for human consumption. Such residues may be of concern to individuals such as the chronically ill, pregnant women, the malnourished and the elderly who may be under folate stress [5]. It has also been reported that a female dog being treated with TMP–SMX for suspected endometritis developed hepatic necrosis and died as a result of this treatment [6]. It is, therefore, prudent to monitor the concentration of TMP in tissues of food-producing animals at slaughter to prevent the possible ingestion by consumers of TMP-contaminated meat.

Regulatory agencies have used rapid tests such as the Swab Test On Premisess (STOP) [7], Live Animal Swab Test (LAST) [8] and Sulfa-On-Site (SOS) test [9] as part of their inspection procedures at abattoirs to screen for antimicrobial residues in biological fluids and tissues at slaughter. The majority of these tests have been used to monitor the incidence of frequently detected drug residues such as penicillin G, tetracyclines, sulphonamides and streptomycin. There is currently no regulatory program that monitors the incidence of TMP in animal tissues of food-producing animals. Although TMP is always marketed in combination with sulphonamides, its presence or absence has not been monitored, even in cases where violative levels of sulphonamides have been detected. In an attempt to address this problem, our laboratory is currently adapting the Brilliant Black Reduction (BR) test, a rapid test developed initially for antimicrobial screening in milk [10], for screening pre-slaughter sera of food-producing animals and tissues of animals at slaughter for TMP and other antimicrobials. A simple and sensitive laboratory method for the routine analysis and confirmation of TMP in animal sera is, therefore, required to provide the analytical data to verify the results of the test responses obtained with the BR test.

Several HPLC methods for the determination of TMP in animal serum or plasma, alone or in combination with a sulphonamide, have been published (Table I). Most of the published methods [3,11–18] required the application of either complicated solvent-switching techniques or extensive and laborious sample treatment prior to analysis, thus rendering them unattractive for routine laboratory use. The objective of this study was to develop a simple and sensitive laboratory method for the analysis of TMP in serum of food-producing animals which could be routinely used to verify the qualitative responses obtained with the BR test. This paper describes an LC method developed for the determination of TMP in serum, with ormetoprim (OMP) used as internal standard. The results of an evaluation of the method for the routine determination of TMP in serum obtained from steers administered Trivetrin are also presented.

EXPERIMENTAL

Instrumentation

The chromatographic system for UV detection of the analyte consisted of a Waters 610 pump, a 600E system controller, a 994 diode-array detector and an automatic injector (Waters Millilab Workstation with a 50-μl fixed loop, Waters

TABLE I

PUBLISHED HPLC METHODS FOR THE DETERMINATION OF TMP IN ANIMAL SERUM OR PLASMA

Study	HPLC column, TMP detection wavelength, applicable matrix	Detection limit (ng/ml), authors and reference
Method development	C_{18}, 240 nm, plasma of broilers	10, Loscher *et al.* [3].
Method development	C_{18}, 280 nm, plasma of pigs	100, Nordholm *et al.* [11].
Pharmacokinetics and metabolism	C_{18}, 280 nm, plasma of neonatal and young pigs.	100, Friis *et al.* [12].
Method development and pharmacokinetics	C_{18}, 230 nm, porcine plasma	25, Mengelers *et al.* [13].
Pharmacokinetics	C_{18}, 280 nm, serum, peritoneal and cerebrospinal fluids of mares	15, Brown *et al.* [14].
Method development and pharmacokinetics	C_{18}, 201 nm, rat blood and plasma	100, Chen *et al.* [15], Tu *et al.* [16].
Method development	Cyano, 230 nm, chinchilla[a] middle ear effusion fluid and serum	50, Erdmann *et al.* [17].
Pharmacokinetics and therapeutics	C_{18}, 254 nm, plasma of dwarf goats.	50, Knoppert *et al.* [18].

[a] Family name of two species of rodents.

Chromatography, Mississauga, Canada). Reversed-phase HPLC was performed on a 250 mm × 4.6 mm I.D., 5 μm particle size Spherisorb ODS(2) column (Phenomenex, Torrance, CA, USA) with a C_{18} pre-column filter. Data acquisition and sample injections were controlled by a Waters 820 chromatography workstation. Mobile phase flow-rate was 1.5 ml/min and the analytes were detected at a wavelength of 225 nm at a sensitivity setting of 0.020 a.u.f.s.

For LC–mass spectrometric (MS) analysis, a VG TRIO 2 mass spectrometer (VG Biotech, Altrincham, UK) was coupled to a Waters 600-MS multisolvent delivery system (Waters) through a thermospray–plasmaspray LC interface. The capillary probe tip in the ion source was held at 300°C. TMP was analysed on a 5-μm, 250 mm × 4.0 mm I.D. C_8 Inertsil column (Lab Link, Rockford, IL, USA) with a 20% acetonitrile–80% ammonium formate–trifluoroacetic acid buffer (pH 3) mobile phase at a flow-rate of 1 ml/min.

Materials

TMP was purchased from Sigma (St. Louis, MO, USA) and stored at 5°C. OMP was donated by Dr. Müller (Laboratorium Enterotox, Krefeld, Germany). Sodium dihydrogenphosphate and sodium phosphate were obtained from BDH (Toronto, Canada) and Fisher Scientific (Edmonton, Canada), respectively. All other reagents were of HPLC grade and were used without further purification. Newborn bovine serum was purchased from Bocknek (Rexdale, Canada). Water was obtained from a Barnsted RO/Nanopure ultrafiltration unit (Sybron, Boston, MA, USA). C_{18} solid-phase extraction cartridges (Sep-Pak, 500 mg/3 ml capacity with ≥17% carbon loading) were purchased from Waters and 0.45-μm Acrodisc filters were obtained from Gelman Science (Quebec City, Canada). BR test kits were obtained from Glengarry Biotech (Cornwall, Canada).

Preparation of standard solutions and mobile phase

Stock solutions (100 μg/ml) of each of TMP and OMP were prepared in 100 ml of methanol, and working standard solutions (10 μg/ml) of each compound were prepared by appropriate dilution with water.

A 0.25% triethylammonium acetate solution (pH 4) was prepared by pipetting 2.5 ml of tri-

ethylamine into a 1-l volumetric flask containing about 900 ml of deionized water. Glacial acetic acid (5 ml) was added, and the solution was mixed and diluted to volume with deionized water. The mobile phase was prepared by mixing 800 ml of the 0.25% triethylammonium acetate solution, 150 ml of acetonitrile and 50 ml of methanol thoroughly. It was filtered and degassed under vacuum through a 0.45-μm filter.

Preparation of serum samples for analysis

Blank serum (5 ml) was pipetted into each of four 50-ml polypropylene centrifuge tubes and spiked with the 10 μg/ml TMP standard solution to produce serum samples containing 10, 50, 100, and 200 ng/ml TMP, respectively. To each sample were added 50 μl of the 10 μg/ml OMP standard. For incurred or test serum, 5-ml samples were pipetted into a polypropylene centrifuge tube, and 50 μl of the 10 μg/ml OMP standard were added. [Note: for test samples with TMP concentrations ≥200 ng/ml, it is recommended that smaller sample volumes (1–4 ml) be used instead of 5 ml.] Each sample was vortex-mixed at high speed for 15 s and allowed to sit for about 5 min. Sodium dihydrogenphosphate (2 ml; pH 11, adjusted with 5 *M* sodium hydroxide) was added to each sample and vortex-mixed for 10 s. Dichloromethane (30 ml) was added to the sample and the centrifuge tube was stoppered and shaken for 10 min on a shaker (Eberbach flatbed, Ann Arbor, MI, USA). The contents of the centrifuge tube were transferred into a 125-ml separatory funnel. The organic layer was drained into a 250-ml separatory funnel containing 10 ml of 0.15 *M* sulphuric acid solution. The aqueous phase in the 125-ml separatory funnel was drained back into the original sample tube along with 20 ml of dichloromethane added to the separatory funnel to rinse the glass walls. The centrifuge tube was stoppered and shaken for 10 min on the shaker. The contents of the centrifuge tube were transferred into the 125-ml separatory funnel, and the organic layer was added to the organic fraction from the first extraction. The 250-ml separatory funnel was stoppered and its contens were shaken for 1 min. The organic layer was discarded and 8.0 ml of the remaining aqueous phase were pipetted into a 50-ml polypropylene centrifuge tube containing 10 ml of phosphate buffer (pH 6, adjusted with 1 *M* sodium hydroxide) and vortex-mixed for 10 s. The buffered extract was loaded onto a C_{18} cartridge previously conditioned with 10 ml of methanol followed by 10 ml of water under a vacuum of 10–20 kPa at a flow-rate of *ca.* 3 ml/min making sure not to allow air into the cartridge to dry it before loading the sample onto the cartridge. Water (10 ml) was run through the cartridge and air was drawn through the cartridge for 1 min. The analytes were eluted with 1 ml of methanol into a 10-ml glasss centrifuge tube, placed into a 50 ± 1°C water bath, and the methanol was evaporated under a gentle stream of high-purity nitrogen. The residue was reconstituted in 1 ml of mobile phase solution, filtered through a 0.45-μm Acrodisc, and 50-μl aliquots were injected into the LC system for either LC–UV or LC–MS analysis.

Precision, accuracy and recovery of the developed analytical methodology

The intra-assay precision and accuracy of the method was determined by performing five replicate analyses each of blank serum fortified with TMP at four different concentrations on the same day. These samples, together with two blind-fortified serum samples, were analyzed on four consecutive days to determine the day-to-day variation and accuracy of the method. Recoveries of TMP from serum samples over the range of concentrations studied were determined by comparing the detector responses of TMP in the fortified samples after extraction and HPLC analysis with those of external TMP standards. The accuracy of the method was evaluated by comparing the amount of TMP found from the calibration curve to the known amount added.

Analysis of antimicrobials (TMP and SDX) with the BR test

Blank bovine serum (100 μl) was pipetted into a BR test vial and labelled "negative control". Another 100 μl of blank serum were pipetted into another BR test vial. One half of a susceptibility

disk (0.01 I.U. of penicillin G) was added to this vial and the vial was labelled "positive control". A 100-μl volume of each incurred or test serum was pipetted into appropriately labelled BR test vials. The vials were allowed to sit for 30 min at room temperature for the samples to diffuse into the agar in the BR vials after which they were supported in a styrofoam floater and incubated in a 65 ± 1°C water bath until the negative control sample had changed color from purple-blue to yellow. All vials which retained the purple-blue coloration of the indicator are considered to contain detectable residues of microbial inhibitor (s), whereas vials in which the color had changed to yellow (the reduced form of the indicator) are considered to be drug- or inhibitor-free.

Applications

Ten steers in a local commercial feedlot being treated for bovine respiratory disease (BRD) were isolated for this study. The steers (average weight of 450 kg) were each treated with 30 ml of Trivetrin per day. One steer was treated for two consecutive days, eight steers were treated for three consecutive days, while one steer was treated for four consecutive days. Blood samples were collected from each steer just prior to drug administration on the first day. On the fifth day after the first treatment, blood samples were obtained from all ten steers. Blood samples were again obtained from the same steers fourteen days after the first treatment. Blood samples were centrifuged at 3000 *g* for 10 min and the serum was harvested and stored at −20°C for analysis of TMP by HPLC and the presence of microbial inhibitor(s) by the BR test. Serum samples were also analysed for the presence of SDX using thin-layer chromatography–densitometry (TLCD), our laboratory method for the analysis of sulphonamides, already described elsewhere [19].

RESULTS AND DISCUSSION

Method development

Fig. 2 shows the UV spectrum of a standard solution of TMP over the range 190–400 nm obtained with a diode-array detector after elution from the HPLC–UV system. It is seen that TMP shows maximum UV absorbance at *ca.* 225 nm. However, except for the analysis of pharmaceutical preparations, most of the previously published papers for the determination of TMP in animal serum or plasma, which have been summarized in Table I, have monitored TMP at wavelengths higher than 225 nm, presumably to increase specificity, to reduce absorbances from endogenous and exogenous components and to eliminate the need for extensive sample clean-up. In our laboratory, we decided to monitor TMP in serum at its most sensitive wavelength of 225 nm since this offered up to a ten-fold improvement in sensitivity relative to that used in most studies (see Table I).

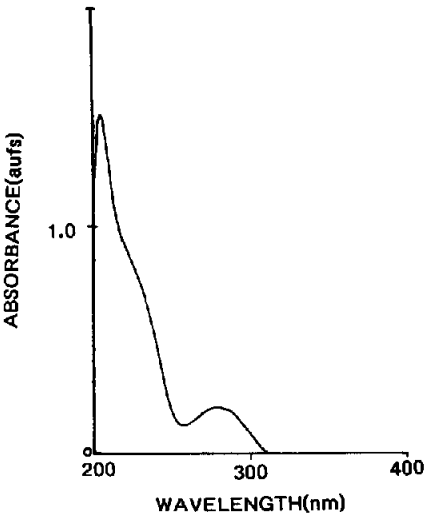

Fig. 2. UV absorption spectrum of a standard solution of trimethoprim obtained with a diode-array detector after elution from the HPLC system.

The use of solid-phase extraction, with or without sample extraction with an organic solvent, to remove co-extractives and concentrate TMP-containing serum samples, has been reported [17,18]. These approaches alone were found to be inadequate in sufficiently cleaning up serum samples from endogenous and exogenous components for the analysis of TMP at 225 nm when they were tested in our laboratory (see Fig. 3). Therefore, we investigated the extraction of the

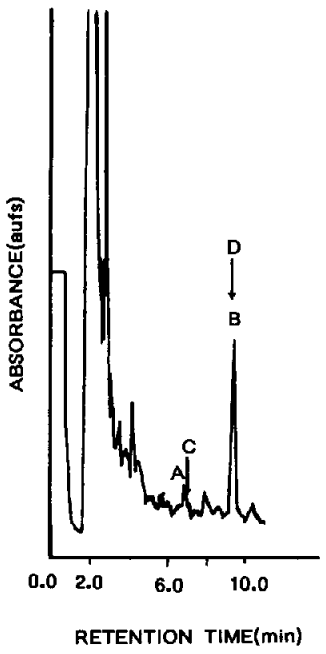

Fig. 3. Chromatogram of blank bovine serum after extraction and clean-up according to the method described in refs. 17 and 18 showing interference peaks A and B with retention times comparable to those of TMP and OMP. Arrows C and D indicate the retention times for TMP and OMP, respectively, if they are injected onto this column.

basic drugs TMP and OMP in serum with basified dichloromethane. These conditions were designed to selectively exclude the extraction of strongly and weakly acidic and water-soluble contaminants into dichloromethane. Dichloromethane was found to be the most suitable solvent for the extraction of the basic drugs TMP and OMP from serum under alkaline conditions in an experiment to find the best solvent for the extraction of the analytes from serum. Recovery of TMP and OMP from serum was highest ($\geq$90%) with dichloromethane followed by chloroform (66%). Ethyl acetate and other solvent combinations, including dichloromethane–ethyl acetate (3:1, v/v) and chloroform–ethyl acetate (3:1, v/v), showed recoveries of less than 50% for TMP and OMP from serum. Fig. 4A shows that, after subsequent clean-up on a C_{18} solid-phase extraction cartridge and chromatographic analysis with UV detection at 225 nm, the extracts provided very clean chromatograms with no interferences from endogenous or exogenous components beyond the retention time of 4 min. Fig. 4B, C and D show chromatograms of extracts from blank bovine serum fortified with the internal standard (OMP), blank bovine serum fortified with 100 ng/ml TMP and 100 ng/ml OMP, and an incurred serum found to contain 708 ng/ml TMP. TMP and OMP are well resolved from each other and from other impurities with retention times of *ca.* 7 and 9 min, respectively.

We investigated the possible chemical interference from sulpha drugs, including SDX, sulphamethazine (SMZ) and sulphadimethoxine (SDM), by injecting standard solutions of these drugs into the HPLC system. It was found that only SMZ with a retention time of less than 10 s of the retention time for TMP would interfere with TMP analysis. However, with the extraction/sample clean-up procedure described, SMZ and other sulphonamides, which are weakly acidic, are not extracted and did not, therefore, present any interferences in the analysis.

The recoveries of TMP from serum samples were estimated by comparing the peak heights of TMP from blank serum samples fortified with known amounts of TMP to the peak heights of external TMP standards. Table II shows that recoveries of more than 80% of TMP were obtained with this method over the concentration range 10–200 ng/ml. The intra-assay precision of the method ranged from 0.4 to 6%, and concentrations of TMP in fortified samples could be estimated with a margin of error ranging from -1 to $+7$%. The results for the day-to-day variation (inter-assay) of the method for serum samples are presented in Table III. The results show that the inter-assay relative standard deviation was less than 5%, and mean errors of -1% were obtained in the estimation of the concentrations for the blind-fortified samples (quality controls 1 and 2) included in the analysis. Calibration curves obtained from plots of the means of the response ratios (detector response of TMP to detector response of OMP), $\bar{y}$, *versus* the concentration, x, of TMP in serum were linear over the concentration range studied and described by the regres-

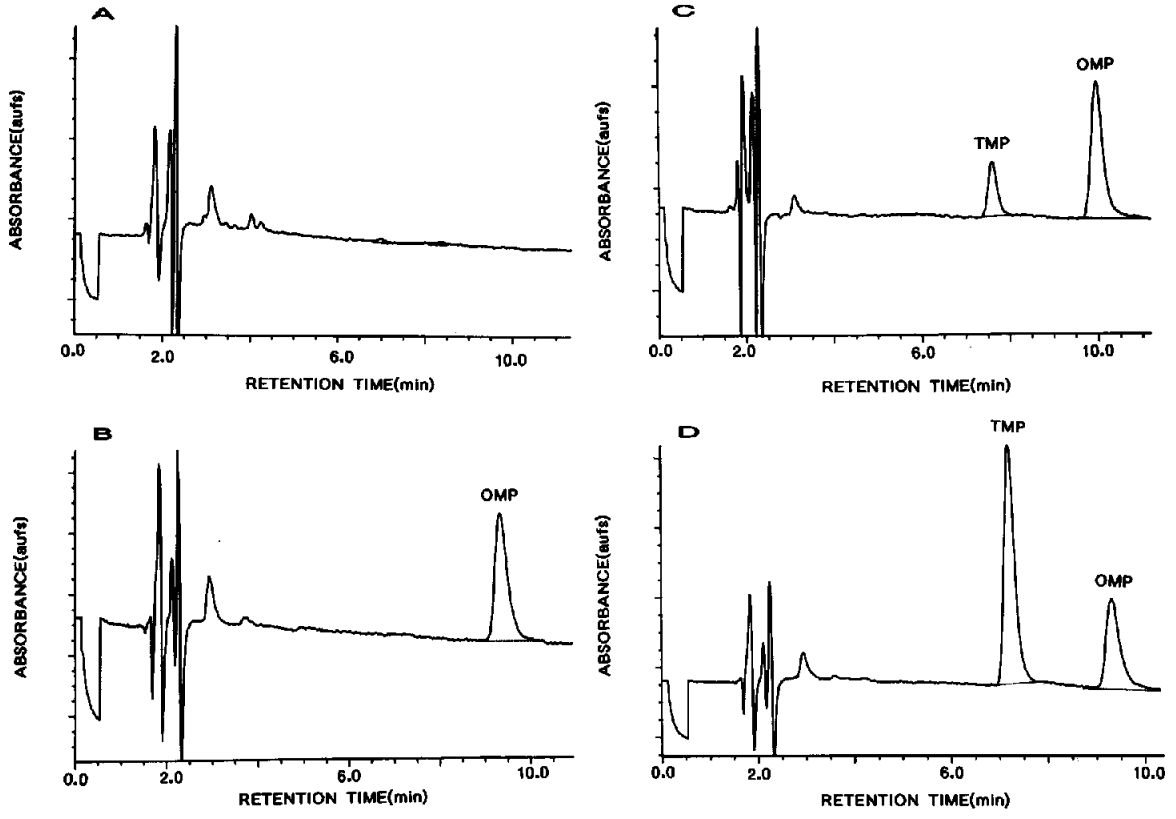

Fig. 4. Chromatograms of (A) blank bovine serum, (B) blank bovine serum containing 100 ng/ml OMP, (C) blank bovine serum fortified with 100 ng/ml TMP and 100 ng/ml OMP, and (D) incurred bovine serum (LM 202, Table IV) found to contain 708 ng/ml TMP after extraction/clean-up and HPLC analysis. Chromatographic conditions are described in detail in the text.

TABLE II

ESTIMATION OF THE INTRA-ASSAY PRECISION AND ACCURACY OF THE DEVELOPED METHODOLOGY FOR THE ANALYSIS OF TMP IN SERUM

n = 5 (number of replicate determinations conducted at each level of fortification).

Concentration added (ng/ml)	Recovery[a] (%)	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Error[b] (%)
10	89 ± 5	10.7 ± 0.7	6.5	+7
50	86 ± 3	49.5 ± 2.5	5.1	−1
100	82 ± 2	99.1 ± 2.8	2.8	−1
200	83 ± 5	200.3 ± 0.9	0.4	0

[a] (Mean peak height of TMP in blank serum/peak height of TMP in external standard) × 100.

[b] (True concentration − experimentally determined concentration)/100.

TABLE III

INTER-ASSAY PRECISION AND ACCURACY OF THE METHOD FOR THE DETERMINATION OF TMP IN SERUM

Concentration added (ng/ml)	Concentration found (ng/ml)					C.V. (%)	Error[a] (%)
	Day 1	Day 2	Day 3	Day 4	Mean ± S.D.		
10	11.5	10.0	11.2	11.0	11.2 ± 0.2	2.1	+12
50	47.5	48.4	49.7	47.9	48.4 ± 1.0	2.0	−3
100	100.9	100.5	98.1	101.3	100.2 ± 1.4	1.4	0
200	200.1	200.1	200.9	199.8	200.2 ± 0.5	0.2	0
QC_1[b]	29.2	28.5	29.5	31.2	29.6 ± 1.2	3.9	−1
QC_2[b]	149.7	146.5	148.0	151.4	148.9 ± 2.1	1.4	−1

[a] Error = (true concentration − experimentally determined concentration)/100.

[b] QC_1 and QC_2 (quality controls 1 and 2) are blind-fortified samples spiked at 30 ng/ml and 150 ng/ml concentrations of TMP.

sion equation: $\bar{y} = (0.00651 \pm 0.000099)\ x + (0.013891 \pm 0.009161)$. The detection limit of the method for the determination of TMP (signal-to-noise ratio of 3) was 5 ng/ml.

The mass spectrum of TMP obtained with the VG TRIO 2 after elution from the C_8 Inertsil column with a mobile phase compatible with the ion source is shown in Fig. 5. Under the ion source conditions used for the thermospray MS analysis, few fragment ions are formed and the M + 1 ion at m/z 291 contributes almost 90% of the total ion intensity. The protonated molecular ion at m/z 291 was, therefore, monitored to qualitatively confirm the presence of TMP in the sample extract.

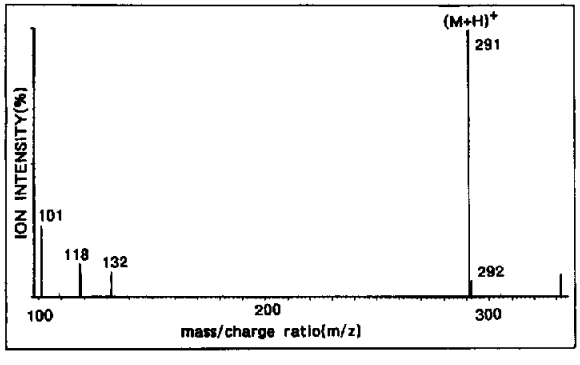

Fig. 5. Mass spectrum of TMP obtained on a VG TRIO 2 mass spectrometer coupled to a Waters multisolvent delivery system through a thermospray LC–MS interface.

Application to biological samples

The method was applied to the analysis of ten samples of bovine serum obtained from feedlot steers being treated for BRD with 30 ml of Trivetrin per day. The BR test is a qualitative, non-specific microbial inhibition test and would therefore be unable to discriminate between detectable concentrations of TMP and SDX. To ensure that test responses obtained on the bovine samples with the BR test were indeed due to the presence or absence of the administered TMP and SDX instead of other natural inhibitors that may be present in the serum samples, it was decided to use the method developed for TMP analysis and the well characterized TLCD method [19] in routine use in our laboratory for the analysis of sulpha drugs for the analysis of SDX. This approach was adopted because it had been demonstrated from our interference studies that sulpha-drugs which are slightly acidic are not extracted by the TMP method. HPLC analysis for TMP, TLCD analysis for SDX and BR test of plasma samples obtained from all ten steers just prior to the administration of Trivetrin on the first day were found to be inhibitor-free and contained no detectable levels of TMP and SDX. For the non-specific, microbial inhibition BR test, this result indicated the absence of any microbial inhibitor (naturally occurring or otherwise) present in the blood of any of the animals prior to the administration of the drug. Table IV shows that with the

TABLE IV

RESULTS OF HPLC AND TLCD [15] ANALYSES AND BR TEST ON BOVINE SERUM OBTAINED FROM FEEDLOT STEERS TREATED FOR BRD WITH 30 ml OF TRIVETRIN PER DAY

Treatment days	Animal ID	HPLC[a] results for TMP (ng/ml)		TLCD results for SDX (ng/ml)		BR test results[b]	
		5 days	14 days	5 days	14 days	5 days	14 days
2	LH 268	535	ND[c]	31 400	NA[d]	+	−
3	LM 101	331	ND	29 400	NA	+	−
3	LR 153	220	ND	35 800	NA	+	−
3	LR 163	534	ND	32 600	NA	+	−
3	LM 169	529	ND	48 000	NA	+	−
3	LM 202	708	ND	51 000	NA	+	−
3	LR 208	306	ND	36 400	NA	+	−
3	LR 224	860	ND	42 400	NA	+	−
3	LM 254	350	ND	34 400	NA	+	−
4	LR 148	515	ND	43 600	NA	+	−

[a] 1 ml serum samples were used for analysis instead of 5 ml.

[b] The BR test was found to have a detection limit of 150 ng/ml for TMP alone and 50 ng/ml for SDX alone. + = positive BR test result; − = negative BR test result.

[c] ND, Analyte was undetectable.

[d] NA, sample was not analyzed for SDX.

BR test all the samples from the first blood collection (*i.e.*, samples collected five days after the first treatment) tested positive while those from the fourteen-day sample collection tested negative. The chromatographic results, which confirmed the presence of TMP and SDX and provided quantitative estimates of the concentrations of drugs present in the collected serum samples, clearly supported the qualitative results obtained with the BR test. As would be expected, the concentration of TMP in all ten animals was lower than that of SDX since the initial dose contained a 1:5 ratio of TMP to SDX. The ratio of TMP to SDX found in the serum of each steer, however, was much lower than might have been expected. Nevertheless, we believe that the much lower TMP concentrations found in these animals adequately reflect the higher rate of elimination of TMP compared to SDX from serum; such differences in the rates of elimination have been observed by previous investigators who used a spectrophotometric method to study the pharmacokinetics of TMP and SDX together in goats and cows [20,21].

CONCLUSION

The HPLC method described is simple, accurate, reproducible and selective for the determination of TMP in serum. It combines the judicious selection of an extraction–back-extraction and C_{18} column clean-up procedure with detection at the maximum wavelength of absorption. Consequently, detection of TMP in serum samples is possible down to concentrations of 5 ng/ml. It was also observed that there was no significant deterioration in column performance after more than 500 injections of serum extracts. The analytical parameters determined for this method demonstrate that the method can be used for the routine analysis of TMP in serum of food-producing animals. The procedure is currently being used in our laboratory to measure the pharmacokinetic parameters of gilts administered Borgal.

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