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Note

Simultaneous analysis of three antimicrobial agents in feed premixes by reversed-phase high-performance liquid chromatography

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The sulphonamides are a therapeutically important group of antimicrobial agents. They are often used in combination with antibiotics as feed supplements to promote growth and prevent disease in animals.

A number of analytical methods have been reported for the determination of sulphonamides and trimethoprim in pharmaceutical preparations and in biological fluids. The most widely used technique for the analysis of sulphonamides is a colorimetric assay based on the Bratton–Marshall reaction[1–3]. Other methods described for the individual determination of sulphonamides and trimethoprim employed thin-layer chromatography[4,5], gas–liquid chromatography[6,7] and more recently high-performance liquid chromatography (HPLC)[8–11]. Microbiological methods have also been employed[12]. The simultaneous determination of trimethoprim and sulphonamides present in mixtures has been investigated, especially by HPLC using a normal phase[13] or a reversed phase[14–18].

The purpose of this work is to describe a rapid method using reversed-phase HPLC for simultaneous determination of two sulphonamides: sulfamethazine and sulfamethoxypyridazine together with trimethoprim. These combinations were found particularly in feed premixes. The use of an eluent comprising a mixture of acetonitrile and 0.05 *N* sulphuric acid enabled low operating pressures and high peak resolution.

EXPERIMENTAL

Reagents and chemicals

Sulfamethazine sodium, sulfamethoxypyridazine sodium and trimethoprim were purchased from Sica-Deltavit (France), acetonitrile and sulphuric acid from Carlo-Erba.

Instrumentation

An LDC high-performance liquid chromatograph was purchased from So-

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pares (France) and equipped with a Constametric III pump, a Valco 7000-p.s.i. injector and a Spectromonitor III UV detector set at 254 nm and 0.5 a.u.f.s. Peak areas were measured and visualized with a LDC/Milton Roy CI/10 integrator.

Chromatography

Chromatography was carried out on a stainless-steel column (20 × 0.47 cm) of Spherisorb ODS Hichrom (particle size 5 μm) at room temperature. The separation of sulfamethazine sodium, sulfamethoxypyridazine sodium and trimethoprim was achieved by isocratic elution with acetonitrile–0.05 *N* sulphuric acid (22:78, v/v). The flow-rate was 1 ml/min.

Sample preparation

Standard solutions of sulfamethazine sodium, sulfamethoxypyridazine sodium and trimethoprim were prepared at concentrations of 150, 75 and 62.5 μg per ml of

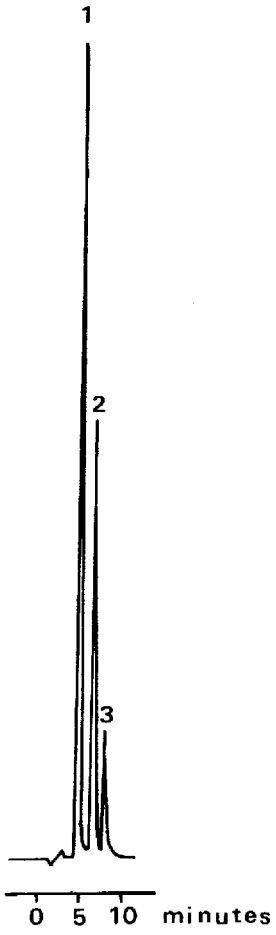

Fig. 1. HPLC separation and UV detection of sulfamethazine (1), sulfamethoxypyridazine (2) and trimethoprim (3) in feed premixes. Conditions: column, C_{18} ODS Hichrom; eluent, acetonitrile–0.05 *N* sulphuric acid (22:78, v/v); flow-rate, 1 ml/min; UV detection, 254 nm.

TABLE I

PEAK AREAS

Values of areas found after six injections of 25 μl containing respectively 3.750, 1.875 and 1.562 μg of sulfamethazine sodium, sulfamethoxypyridazine sodium and trimethoprim.

Sulfamethazine sodium	161 100	162 000	162 100	161 800	161 500	161 400
Sulfamethoxy-pyridazine sodium	91 300	92 000	92 100	91 700	91 400	91 300
Trimethoprim	31 900	31 800	32 000	32 200	31 850	32 100

the eluent, respectively. Mixtures of these three compounds were made up under the same conditions. A 25-μl aliquot of each sample was injected into the chromatograph.

RESULTS AND DISCUSSION

The retention times of sulfamethazine sodium, sulfamethoxypyridazine sodium and trimethoprim were respectively 5 min, 6 min 50 sec and 9 min 30 sec. Fig. 1 shows a chromatogram of a mixture of the three compounds.

The limits of detection were 20 ng for sulfamethazine sodium and sulfamethoxypyridazine sodium, and 80 ng for trimethoprim.

A linear relationship was found between the concentrations of sulfamethazine sodium, sulfamethoxypyridazine sodium and trimethoprim injected and their peak area ratios (from 100 to 30 000 ng injected: $r > 0.99$). The reproducibility of this method was tested by six consecutive injections of the same sample (Tables I and II).

CONCLUSION

The good reproducibility of this method allowed a direct analysis of three antibiotics without the addition of an internal standard. The compounds were rapidly eluted within 10 min and there was a complete separation of all compounds. This method was applied to the quantitative analysis of these compounds in 200 premixes and the results obtained confirmed the validity of the method. Furthermore, no interference was observed from other compounds present in the "support" (corn-flour).

TABLE II

MEAN AREAS (M.A.), STANDARD DEVIATIONS (S.D.) AND COEFFICIENTS OF VARIATION (C.V.)

	Sulfamethazine sodium	*Sulfamethoxypyridazine sodium*	*Trimethoprim*
M.A.	161 650	91 630	31 975
S.D.	384.406	355.90	154.11
C.V. (%)	0.24	0.39	0.48

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