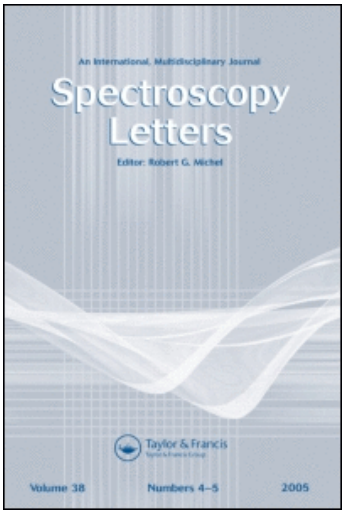

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SULPHONAMIDES DETERMINATION IN ANIMAL TISSUES BY ION-PAIR HPLC

Key words : Sulphonamides, determination, ion-pair, HPLC.

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Abstract

For the determination of four sulphonamides (diazine, thiazole, merazine and methazine) with sulphamonomethoxime as internal standard in animal tissues, ion-pair HPLC was studied. Optimum wavelength proved to be 270 nm and the composition of mobile phase acetonitrile : 0.025 mMol sodium laurilsulphate (22/78) at pH:3.5.

Regression analysis, precision and recovery data are presented in detail. The applicability of the method was tested in 161 animal tissue specimens. Almost 20% of them were positive for sulphonamides at concentrations between 0.01-1.28 ppm.

Introduction

Pharmaceutical compounds are used at Veterinary practice for precaution, treatment of animals and improvement of animal tissue production. Sulphonamides (SF) are between them since 1938, for their bacteriostatic action and low cost[1].

Besides these benefits, residual concentrations of SF in animal tissues affect unfavourly human beings either directly or indirectly, causing sensitization reactions, immunity and tolerance[2,3].

The European Community has established the value of 0.01 ppm as the tolerance limit for SF in animal foodstuff and FAO for milk. So, there is a need for SF detection and quantitation in consumable animal tissues[4,5,6] and this is the target of our work.

For SF detection several methods have been developed: micro-biological, enzyme-immunological and chemicals (spectrophotometric[7,8] or chromatographic[9,10]). The need for a sensitive and selective method of detection, led us to investigate ion-pair HPLC for routine use. Applicability of the method at aninal tissue specimens was, also, tested.

Table 1. Sulphonamides mean peak height values in mm^2 at several wavelengths

SF	254nm	270nm	280nm
SF_1	27.17	125.00	62.49
SF_2	28.57	66.00	37.14
SF_3	105.50	95.00	101.70
SF_4	59.43	63.00	56.40
SF_5	101.00	100.00	183.80

Table 2.Ratios of peak height to area at the ratios of wavelengths

SF	254/254	270/254	284/254
SF_1	1	4.60	2.30
SF_2	1	2.30	1.30
SF_3	1	0.90	0.96
SF_4	1	1.06	0.95
SF_5	1	0.99	1.82

Materials and method

Instrumentation : High Performance Liquid Chromatography (HPLC) was carried out on a JASCO system, model 870, with a variable wavelength UV-Vis spectrometer as the detector and equiped with a JASCO integrator, model 807.

Reagents : Five SF were tested among the most common in Veterinary use, with the last used as the internal standard : Sulphadiazine (SF_1), sulphathiazole (SF_2), sulphamerazine (SF_3), sulphamethazine (SF_4) and sulphamonomethoxime (SF_5).

Animal samples : Tissue specimens were derived from slaughtered animals intended for consumption.

Selection of HPLC conditions

For the selection of the optimum wavelength, three were tested close to the absorption maximum : 254, 270 and 284 nm. Five solutions of standard SF at the concentration of 1 ppm plus 2 ppm internal standard were injected 5 times each and gave mean peak height in mm^2 presented on table 1. The ratios of peak heights to areas and the ratios of wavelengths 254/254, 270/254, 284/254 nm, are presented on table 2.It seems that the optimum spectral area for almost all SF was 270 nm.

For the selection of the optimum organic solvent in the composition of the mobile phase, two reagents were tested :
a. Methanol : 1% acetic acid solution (20/80)
b. Acetonitrile : 1% acetic acid solution (25/75).
The advantage of a was satisfactory base line separation for all

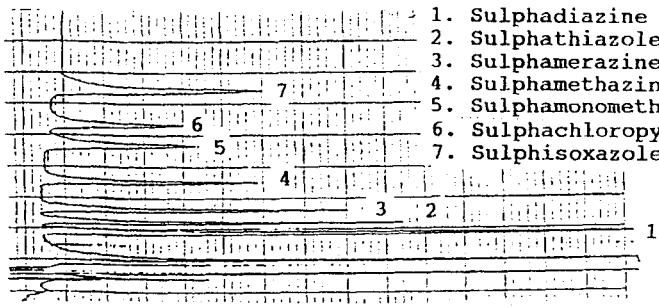

Fig.1.Typical chromatogram of sulphonamides

SF tested and that of b was decrease of compounds dispersion and of SF_5 retention time. The disadvantage of a was long retention time of SF_5 and wide compounds dispersion while that of b was the impotency of separation of SF_1 and SF_2. So, both mobile phases were improper. The problem was overcome by the addition of a surfactant, sodium laurilsulphate, as the counter ion.The new mobile phase was tested at different compositions.Finally, the optimum chromatogram was achieved with acetonitrile:0.025 mMol sodium laurilsulphate (22/78) at pH:3.5.

The rest optimum conditions were as follows: The chromatographic column was Spherisorb S10 ODS2, 10µm, 25cm in length and 0.46cm i.d. at ambient temperature. The pH=3.5, the flow rate was adjusted at 1.5ml/min and the chart speed at 5mm/min. The detector was UV at 270nm (0.01 AUFS), injection loop was 20µl, detection limit was 0.01ppm (0.2ng).Typical chromatogram of seven SF is presented in figure 1.

Results and discussion

Calibration curves were constructed by plotting peak area versus two series of concentrations a.0.1-1ppm (0.1 AUFS) and b.5-40ppm (16 AUFS). Regression analysis of the data obtained are presented at tables 3 and 4. The correlation coefficients obtained (0.99 at a=0.01) show a very strong correlation between peak area and concentrations. The detection limit was 0.01ppm and no interferences were noticed due to penicillin, tetracycline, tylozine and chloramphenicol.

Precision tests were performed by Two-way Analysis of Variance (ANOVA).From this statistical test the calculated F-values (Fa=the variation between concentration and Fb=the variation in time) were compared to the theoretical ones from the F-distribution. For significance level of 0.05, no statistically significant differende was observed. Precision data is presented on table 5.

Finally, relative (to internal standard) recovery data ranged between very satisfactory levels (96-103%). Concentrations of 0.05, 0.1 and 0.5 ppm of SF standard solutions were added to

Table 3. Regression data for SF concentrations 0.1-1 ppm (n=2, a=0.01, degrees of freedom=3)

SF	Regression equation y=a+bx	Correlation coefficient
SF_1	y=1.504+124.309x	0.999
SF_2	y=-0.439+74.634x	0.999
SF_3	y=1.243+83.536x	0.999
SF_4	y=1.032+59.471x	0.999
SF_5	y=4.459+49.154x	0.999

Table 4. Regression data for SF concentrations 5-40 ppm (n=2, a=0.01, degrees of freedom=3)

SF	Regression equation y=a+bx	Correlation coefficient
SF_1	y=-2.506+10.262x	0.999
SF_2	y=-1.890+6.280x	0.999
SF_3	y=-0.518+7.386x	0.999
SF_4	y=-3.018+5.286x	0.999
SF_5	y=0.329+4.041x	0.999

Table 5. Precision data for SF determination (a:0.05, theoretical Fa:2.53 and Fb:2.42)

SF	Calculated F-values Fa	Fb
SF_1	0.49	1.40
SF_2	0.76	0.05
SF_3	0.58	0.54
SF_4	1.73	1.30

Table 6. Recovery data for SF determination in animal tissues.

SF	Concentration added µg/g	Mean concentr. found µg/g muscle	liver	kidney	serum	Recovery %
SF_1	0.05	0.050	0.050	0.049	0.049	99.0
	0.10	0.096	0.101	0.099	0.099	98.7
	0.50	0.499	0.520	0.498	0.477	99.7
SF_2	0.05	0.047	0.051	0.049	0.049	98.0
	0.10	0.087	0.099	0.100	0.100	96.5
	0.50	0.477	0.507	0.510	0.507	100.0
SF_3	0.05	0.049	0.050	0.050	0.049	99.0
	0.10	0.097	0.101	0.100	0.095	98.2
	0.50	0.470	0.500	0.620	0.470	103.0
SF_4	0.05	0.050	0.049	0.050	0.049	99.0
	0.10	0.097	0.099	0.100	0.100	99.0
	0.50	0.480	0.470	0.510	0.460	96.0

Table 7. Sulphonamides distribution in animal tissue

Specimens	Positive	SF percentage in positives			
		SF_1	SF_2	SF_3	SF_4
161	32	18.25	3.12	34.37	0.60

muscle, liver, kidney and serum tissue specimens. Concentrations found (measured) and percentage of recovery are presented on table 6.

To test the applicability of the method, a trial was undertaken with 161 animal (bovine and pig) tissue specimens to quantitate SF residues after isolation according to Manuell-Steller[11]. Thirty two specimens (nearly 20%) were found positive for SF at concentrations between 0.01-1.28 ppm with distribution presented on table 7. It has to be noted here that in some specimens more than one SF was identified.

REFERENCES

1.Review of Analytical Methods for Sulphonamides, J. Assoc. Off. Anal. Chem., 64 (1981) 104.
2.Bevill, R.F. et al : Disposition of Sulphonamides in Food-producing Animals, Am. J. Vet. Res., 38 (1977) 973.
3.Righter, H.F. et al : Tissue-residue Depletion of Sulphomethazine in Calves and Chickens, Am. J. Vet. Res., 31 (1971) 1051.
4.Spreux, O., Varoquaux, et al : Determination of Trimethoprim, Sulphamethoxazole and its N4-acetyl Metabolites in Biological Fluids by HPLC, J.of Chromatography, 274 (1983) 187-199.
5.Irwin, W.J. and Slack, J.A. : Detection of Sulphonamides in Urine by Pyrolysis-Gas Chromatography-Mass Spectrometry, J. of Chromatography, 153 (1978) 526-529.
6.Rader, B.R. : Application of Ion-Pairing to Separation of Selected Sulphonamides by Partition, J.of Pharmac. Sciences, Vol.62, No 7 (1973) 1148-1150.
7.Bratton, A.C., Marshall, E.K. : A new Coupling Component of Sulphonamide Determination, J. Biol. Chem., 128 (1939) 537-550.
8.Tishler, F. et al : Improved Method for Determination of Sulphonamides in Milk and Tissues, J. Agric. Food Chem., 16 (1968) 60.
9.Cobb, P.H. and Thill, G.T. : HPLC of some sulphonamides, J.of Chromatography, 123 (1976) 444-447.
10.Gochin, R.J. and Haigh, J.M. : Simultaneous Determination of Trimethoprim Sulphamethoxazole and N4-acetyl-sulphamethoxazole in Serum and Urine by HPLC, J.of Chromatography, 223(1981) 139-145.
11.Manuel, A.J., Steller, W. : Gas-liquid chromatographic deter mination of sulphamethazine in swine and cattle tissues, J. Assoc. Off. Anal. Chem., 64:794-799, 1981.

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