A-29.3

Determination of Sulfonamides and Tetracyclines in Animal Feed with AMD

Key words

Instrumental TLC - quantitative analysis - densitometry by absorbance and fluorescence - multiwavelength scanning - spectra comparison veterinary drugs - sulfonamides - antibiotics.

Scope

Sulfonamides are administered to animals in the form of feed supplements. Their safe identification and quantification in such supplements is a task for regulatory and surveillance laboratories. Since such feed supplements often contain complex extraneous substances which are extracted simultaneously with the drugs, reliable chromatographic separation from matrix compounds is required as well as secure identification by in-situ spectra comparison.

Samples containing drugs are extracted, then lipids removed with n-hexane. The concentrated sample is separated by AMD (automated multiple development). The active substances are quantified by multiwavelength scanning, identification is ensured by computer assisted spectra comparison.

The AMD method achieves an improved resolution compared with conventional TLC procedures. For instance, the molecular difference between sulfadimidine and sulfamerazine is only one methyl group (-CH3). The shallow polarity gradient used in this AMD procedure, allows the safe separation of these substances.

What advantages does Instrumental TLC offer ... in this particular case?

* Determination limit: 10 ng absolute
* A large number of samples can be analyzed simultaneously.
* No derivatization necessary
* Reliable identification of the components by computer assisted spectra- and multiwavelength correlation.

Reagents

- n-Hexane
- Acetone
- Methanol
- Diisopropylether
- Acetonitrile
- Extraction solvent: methanol - acetonitrile - acetone 75:25:25

Standards: sulfadimidine (sulfamethazine) - furazolidone, dimetridazol, sulfamerazine, tylosinetartrate, chlortetracycline, oxytetracycline.

Sample preparation

- Extract 10 g of the animal feed supplement with 50 mL n-hexane by stirring for 30 min, then filter; discard the filtrate.
- Extract the dryed residue with 50 mL of extraction solvent by stirring for 1.5 h at 30 - 40 °C.
- Filter and rinse with extraction solvent heated at 30-40 °C.
- Let cool to room temperature, then dissolve residue in methanol at 100 mL.
- If the solution is turbid, clear it by centrifuging at 6-7000 RPM.
- Use this solution for analysis.

Calibration standards

Calibration standards are prepared by adding known quantities of substances to a blank matrix extract (solution A).

Solution A: Extract 25 g drug free animal feed as described and dissolve residue in 250 mL methanol.

Solution B: Weigh in 10 mg each of those pure substances which are expected in the supplement and dissolve in solution A to a volume of 100 mL.

Depending on the expected concentration range, mix different volumes of B and A to 10 mL, e.g.

solution B (mL)	+ A (mL)	= standard	10 µL corresp. mg/kg
1	9	S1	100
2	8	S2	200
3	7	S3	300
4	6	S4	400
5	5	S5	500

Layer

HPTLC precoated plates silica gel MERCK 60 F 254s (extra pure), 20x10 cm.

Prewash the layer by immersing it for one hour in isopropanol; then dry 2 h at 120°C.

Sample application

Bandwise with CAMAG Linomat, application speed 4 sec/µL, distance from lower edge 8 mm, distance from side edge 15 mm, bandlength 7 mm, space between samples 3 mm = 17 tracks. recommended pattern:

S1 U1 U2 U3 S2 U4 U5 U1 S3 U2 U3 U4 S4 U5 S5

S = standards, apply 10 µL ea.
U = unknowns, apply 20 µL ea.

Chromatography

Automated Multiple Development (AMD) with a shallow 25-step gradient based on diisopropylether, 2 mm migration distance increments, "empty mixer" function after step 3.

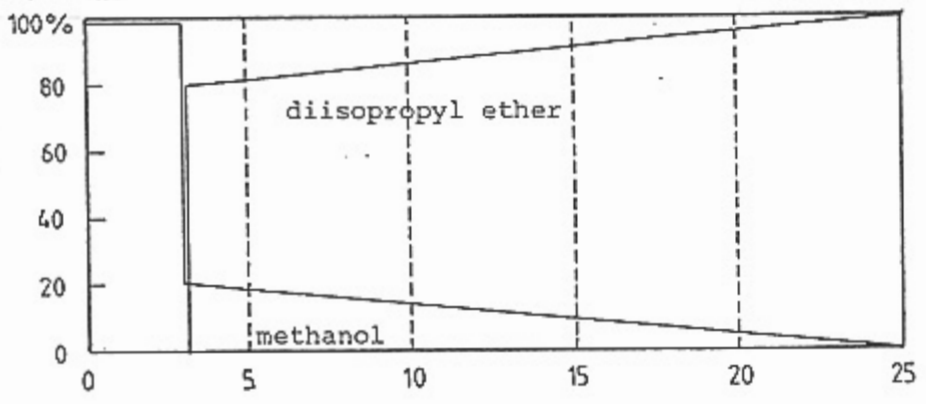

Fig. 1 AMD gradient over 25 steps, schematic

from step n=	1	4	6	11	16	21
bottle nr.	1	2	3	4	5	6
methanol	100	20	15	10	5	
diisopropyl ether		80	85	90	95	100
drying time (min)	3	3	3	3	3	3
wash bottle content	NH_3 0,75 n					

Densitometric evaluation

CAMAG TLC Scanner II computer controlled, TLC evaluation software 86 extended version or CATS. Multiwavelength scanning by absorbance at 254, 265, 302, 366 nm and by fluorescence at 366/>400 nm, Hg-lamp, monochromator bandwidth 10 nm, slit dimension 0.2x4 mm. Quantification via peak height.

Spectra recording 200-450 nm with deuterium lamp.

Substance	Absorption maximum	AMD migration distance
Tylosinetartrate	302 nm	15.9 mm
Furazolidone	356 nm	18.9 mm
Sulfamerazine	265 nm	24.2 mm
Sulfadimidine	265 nm	27.5 mm
Dimetridazole	313 nm	29.2 mm

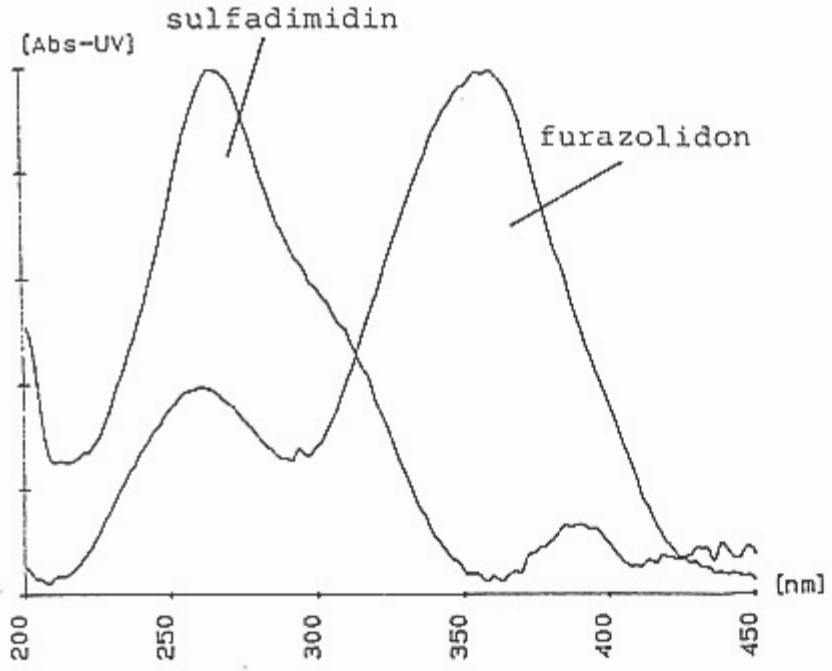

Fig. 2 In-situ absorption spectra of sulfadimidione and furazolidone plotted using scan & spectrum mode.

For identification, the absorption spectra of the fractions in the unknowns are correlated with those in the standards; the linear correlation is printed out.

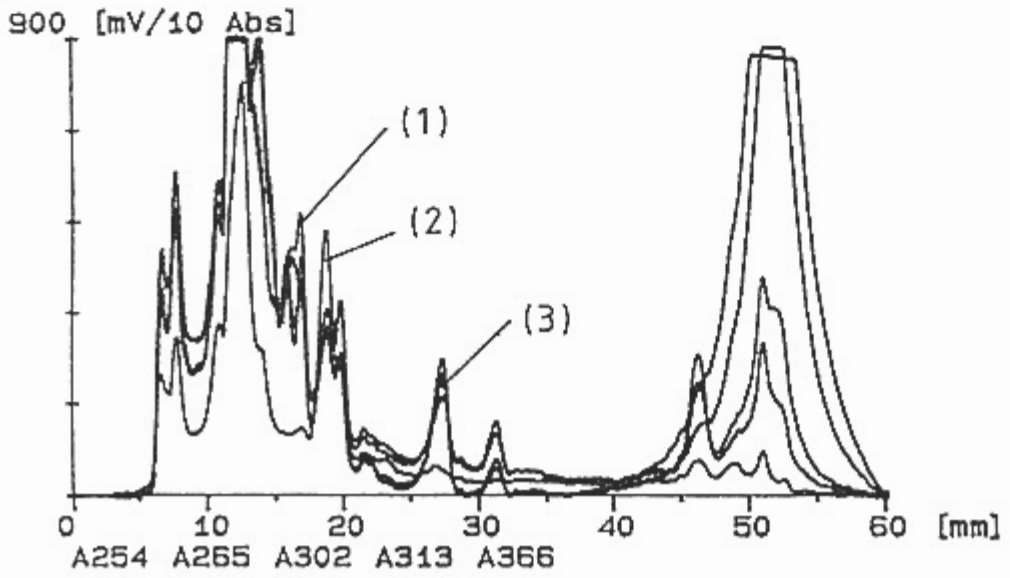

Fig. 3 Multiwavelength scan of a sample containing tylosine-tartrate (1), furazolidone (2) and sulfadimidine (3); concentration range 200-300 mg/kg.

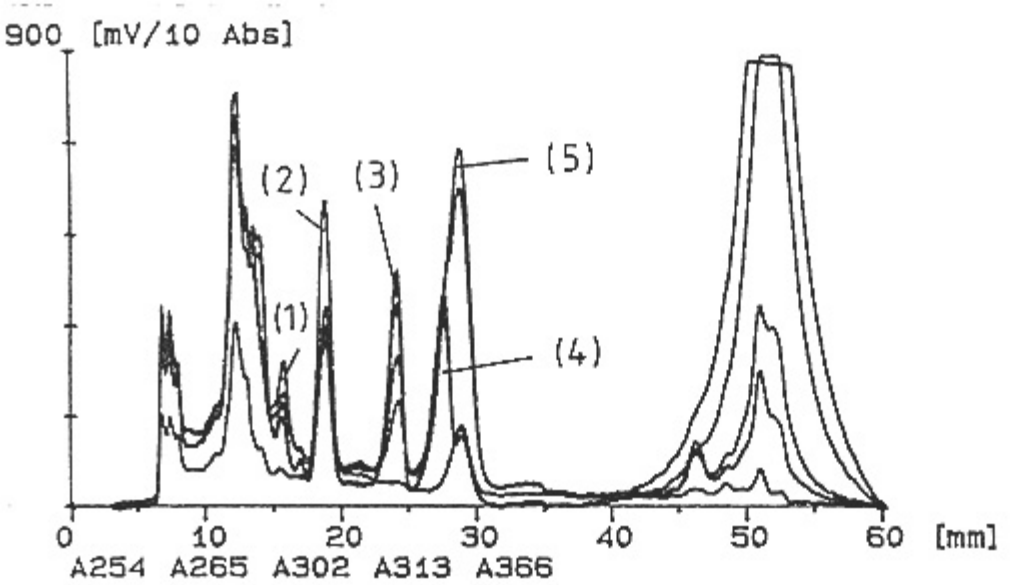

Fig. 4 Multiwavelength scan of a standard track containing tylosinetartrate (1), furazolidone (2), sulfamerazine (3), sulfadimidine (4) and dimetridazole (5), quantities corresponding to 300 mg/kg.

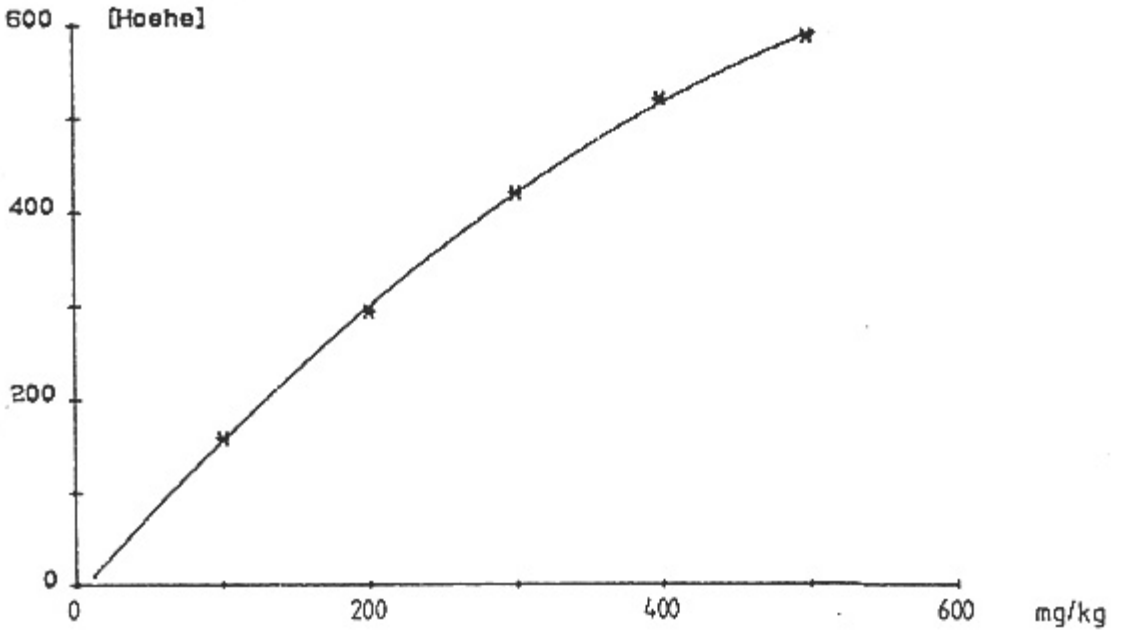

Fig. 5 Calibration curve for sulfadimidine via peak height using linear regression; regression quality CV = 1.4%. Calibration range 40-200 ng (corresponding to 40-200 mg/kg).

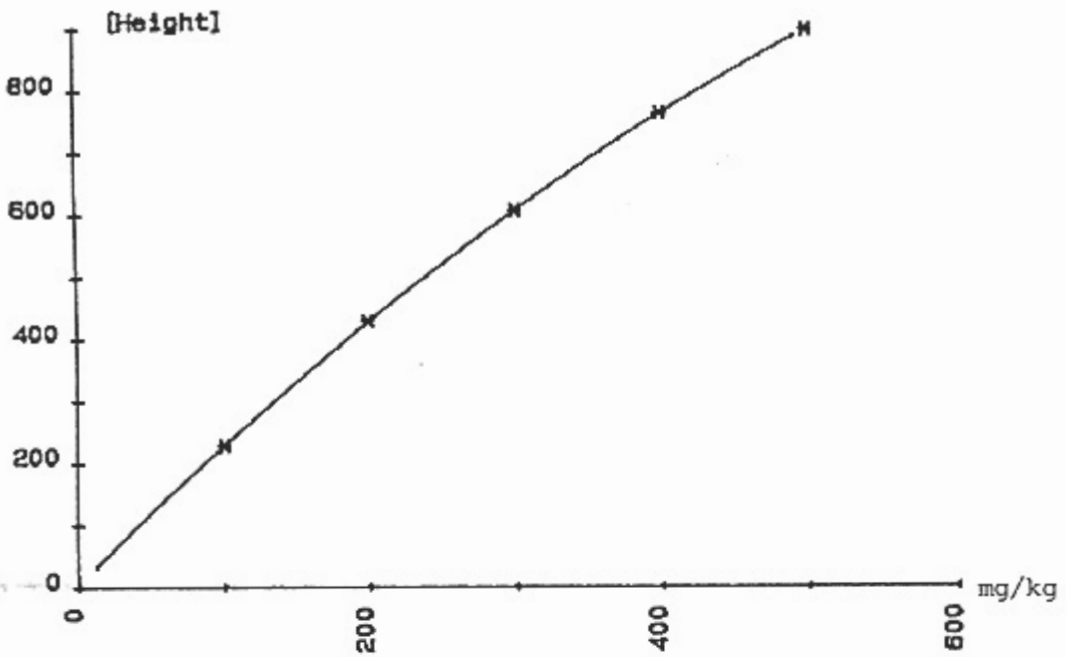

Fig. 6 Calibration curve for furazolidone via peak height using 2nd degree polynominal; regression quality CV = 0.2%. Calibration range corresponding to 100-500 mg/kg.

Literature

G. Knupp, H. Pollmann, D. Jonas, Chromatographia 22, 21-24 (1986).