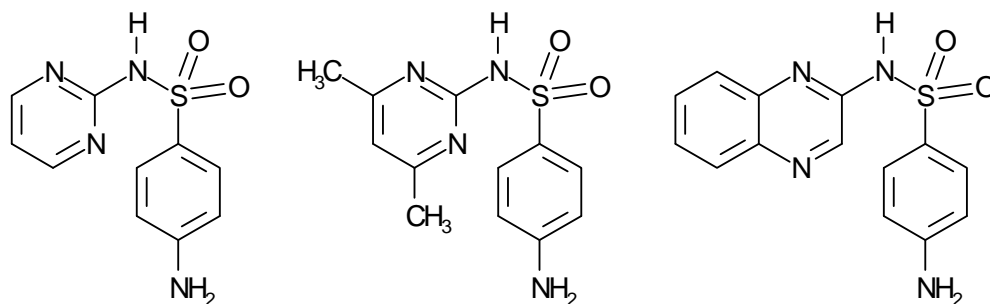


SULFONAMIDES



Sulfonamides are antibacterial drugs, which are used in human and veterinary medicine. Especially in veterinary medicine sulfonamides are well-established for treating diseases of the respiratory, gastrointestinal, and urinary tract. Furthermore they are very often the preferred choice for the medical treatment of rodents, since those in general have a broad intolerance against antibiotics (particularly penicillin). In the intensive poultry management sulfonamides were the treatment of choice for the disease control of coccidiosis. This can lead to residues in food derived from animal products that are detrimental to health.



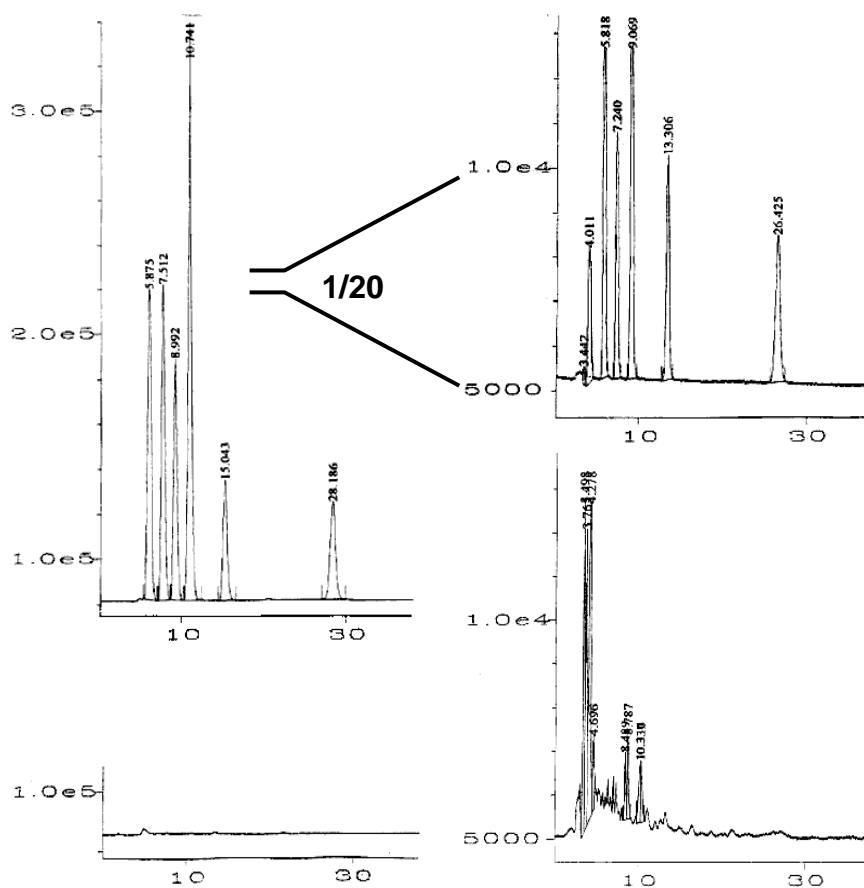
Structures of sulfadiazine, sulfadimidine, and sulfaquinoxaline

Method Description

The method is suitable for the determination of sulfonamides such as sulfanilamide, sulfadiazine, sulfamerazine, sulfadimidine, sulfamethoxypyridazine, sulfadoxine, and sulfadimethoxine. After a chromatographic separation via RP-HPLC the sulfonamides are subsequently derivatized with fluorescamin ("Fluram") in a single-step reaction, followed by fluorescence detection. In this reaction the fluorescamin reacts with the primary amino groups to a fluorescing pyrrolinone, while the excessive reagent is deactivated by water within a few seconds.

Chromatograms

Chromatograms of a standard and a meat sample (blank) after derivatization with fluorescamin (left) and with UV detection (right)



APPLICATION NOTE

HPLC Conditions and Derivatization Parameters

HPLC	
Operation Mode	Isocratic
Eluant	Na-acetate buffer, 5 mM, pH 4,65 156 mL Acetonitrile 34 mL Methanol 10 mL
Degassing	Helium- or vacuum-degassed
HPLC Column	RP C18; e. g. 50 x 4.6 mm, 3 µm with guard
Column Oven	40 °C
Flow Rate	0,8 mL/min
Injection Volume	100 µL
Post-Column Derivatization	
Pinnacle PCX	Single-pump
Reactor Volume	1.4 mL reactor
Reactor Temperature	60 °C
Reagent	50 mg fluorescamin; 150 mL 20 mM Na-hydrogen phosphate buffer pH 3,0; 50 mL acetonitrile; 0,4 mL mercaptoethanol
Reagent Flow	0.3 mL/min
Detection	
Detection Type	Fluorescence detection
Excitation Wavelength	420 nm
Emission Wavelength	480 nm
Flow Cell	Analytic; pressure stable up to 7 bar

APPLICATION NOTE

Literature

Review

1) D. Guggisberg, A. E. Mooser, H. Koch, *J. Chromatogr.* **1992**, 642, 425 – 437.

Derivatization with Flurescamin

2) A. Gehring, L. G. Rushing, H. C. Thompson, *J. AOAC International* **1995**, 78 (5), 1161 – 1164.

3) B. Pacciarelli, S. Reber, Ch. Douglas, S. Dietrich, R. Etter, *Mitt. Gebiete Lebensm. Hyg.* **1991**, 82, 45 – 55.

4) H. S. Sista, D. M. Dye, J. Leonard, *J. Chromatogr.* **1983**, 273, 464 – 468.

Order Information

Order Number	Description
1153-1032	PINNACLE PCX – Single-pump; 1.4 mL reactor