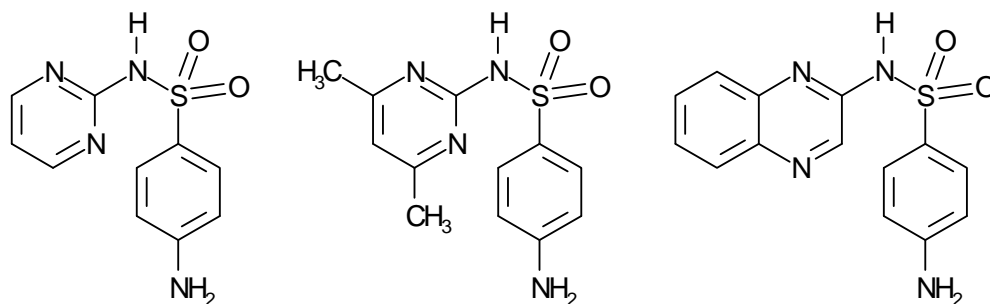


SULFONAMIDES



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

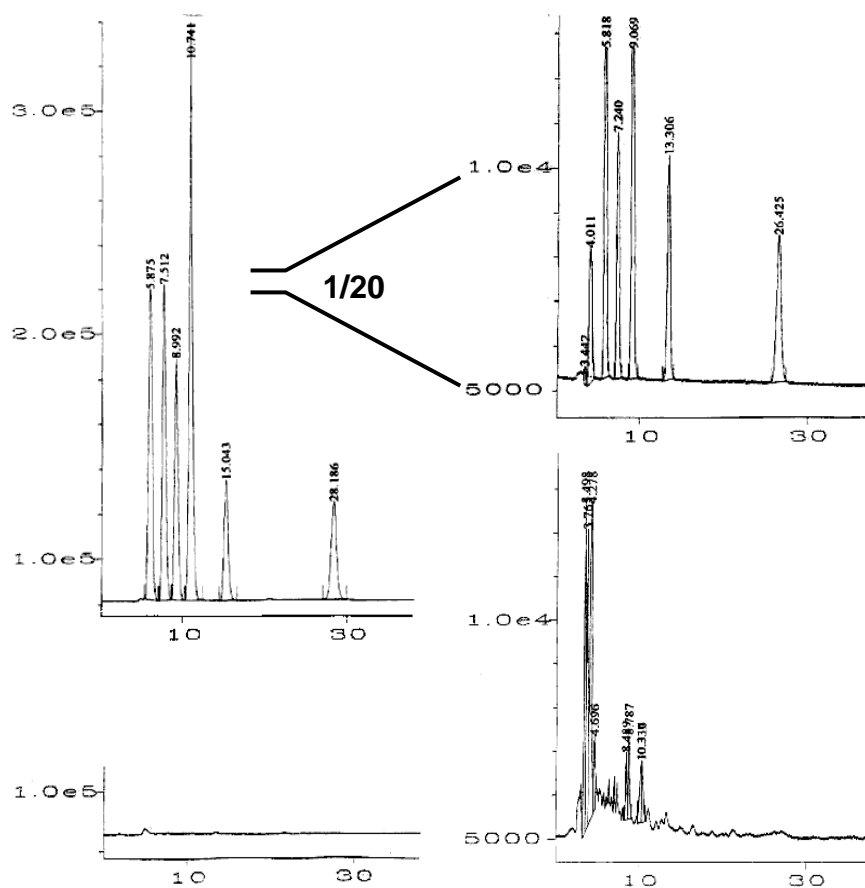
APPLICATION NOTE

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 |