Determination of Sulphonamides in Milk by HPLC with Electrochemical Detection

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Bestimmung von Sulfonamidrückständen in Milch mit Hilfe der HPLC und dem elektrochemischen Detektor

Zusammenfassung. Es wird eine einfache Methode zum Nachweis von Sulfonamidrückständen in Milch am Beispiel von Sulfadiazin und Sulfadimidin beschrieben, mit der noch Rückstandsmengen von 10 ng/ml erfaßbar sind. Das Verfahren besteht aus einem einfachen Aufreinigungsschritt, der Trennung der Rückstände auf einer Umkehrphase (RP-C2) und der Detektion mit einem elektrochemischen Detektor. Die Wiederfindungsrationen liegen zwischen 94,4 % und 110 % für einen Konzentrationsbereich von 0,01 – 1,8 µg/ml Untersuchungsmaterial.

Summary. A simple quantitative method for the analysis of the residues of sulphadiazine and sulphadimidine in milk is described. The method is based on a simple extraction step and high-performance liquid chromatography (HPLC) with electrochemical detection. The chromatographic separation is performed on a reversed phase column (RP-C2) and an aqueous eluent. With this analytical system 10 ng/ml can be detected. Recoveries of sulphonamides from milk are between 94.4 % and 110 % in the concentration range of 0.01 – 1.8 µg/ml sample.

Key words: Best. von Sulfonamiden, Sulfadiazin, Sulfadimidin in Milch; Chromatographie, HPLC; elektrochem. Detektor

Introduction

In veterinary medicine and agriculture sulphonamides are used — in admixture with other antibiotics — in diet for cows to prevent udder diseases.

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After treatment with Sulphonamides the immediate excretion with the milk is unavoidable and may persist in subsequent milkings over a period of several days. This contaminated fluid is not suitable for nutrition. According to the food prescriptions in the Federal Republic of Germany drug residues are considered to be impurities. Because of the importance of milk for nutrition, it must be free of any contamination.

To detect low amounts of sulphonamides in milk, we worked out a sensitive, rapid and convenient method. Only few methods have been described in literature. These are based either on the condensation of the p-amino group with p-dimethylaminobenzaldehyde to Schiffbase [1] or on the diazotisation of the p-amino group and coupling of the diazonium salt with a suitable chromophor to azine dyes. These methods are not specific and determine only the overall quantity of the sulphonamides present.

Different methods have been described for the extraction of the sulphonamides from milk, such as the precipitation of albumin with trichloracetic acid, barium sulphate or with zinc oxide. Methods with such cleaning steps are not suitable for trace analysis of sulphonamides. Other authors as Houston and Umstead [2], Mooney and Paserela [3] and Selzer and Banes [4] concentrated and purified the sample extracts on chromatographic columns.

Although several HPLC procedures for the separation and determination of sulphonamides from biological materials have been published, none of them is suitable for milk.

High-performance liquid chromatography was the method of choice because of the simplicity of the sample preparation. This technique is suitable as a routine method for the determination of sulphonamides in milk.

We determined as much as 0.1 µg of sulphonamide/ml milk by combining a reversed phase column (RP-C2) with UV-detection (263 nm). The UV-detector registered other coextracted contaminations...
which interfered with the sulphonamide peaks. This interference was eliminated by application of an electrochemical detector. This works selectively and is more sensitive than the UV-detector, so that we were able to easily determine sulphonamides at the 10 ng/ml level.

**Experimental**

1. **Electrochemical Detector**

Chromatographic peaks were recorded with a Metrohm model E 611 electrochemical detector in conjunction with a three-electrode cell system having two glassy carbon electrodes as the working and auxiliary electrodes and Ag/AgCl (3 M KCl) as the reference electrode. The glassy carbon electrodes were polished daily with A1203 for 30 s.

The working electrode potential was maintained at +1.10 V and the sensitivity at 80 nA/cm.

2. **Chromatographic System**

2.1 The high-performance liquid chromograph consisted of the following parts:

- Tow-piston high-pressure pump model 52.00 (Knauer KG, Berlin);
- Injection system model 71.20 (Rheodyn-California) with a 20 μl loop;
- self-packed reversed-phase column (high viscosity method);
- a 100 mV compensation recorder model 41.00 (Knauer KG, Berlin). Paper: 5 mm/min.

2.2 Column: the stationary phase, RP-C2, 10 μm (Merck-Darmstadt) was packed in 250 x 4.6 mm VA-steel column (Knauer KG, Berlin).

2.3 Mobile phase: methanol/water 25:75 (v/v) and 40:60 (v/v) with 0.01 M LiClO4 as an electrolyte.

2.4 Flow rate: 2 ml/min.

2.5 Pressure: 130-140 bar.

3. **Procedure**

Ten milliliter of milk1 were mixed with 10 μg of sulphamerazine as internal standard and extracted twice with cooled chloroform. The chloroform phase was filtered through filter paper into a 250 ml round-bottomed flask. The filter paper was washed with 20 ml of chloroform. The solvent was evaporated at 30°C and under 20 mbar vacuum. The residue was dissolved in 1 ml of methanol and a 10 μl aliquot was analysed in the chromatographic system.

4. **Calibration Curve**

Ten microgram of sulphamerazine and known amounts of both sulphadiazine and sulphadimidine (25 μg - 250 ng) were added to seven separatory funnels containing 10 ml of milk. The samples were treated as described in the procedures. Ten microliter of the methanolic residue solution were injected onto the column and the peak heights were measured manually.

1 For concentrations below 10 ng/ml larger volumes of milk have to be extracted. In this case the ratio of chloroform to milk must be not smaller than 4.