HPLC SEPARATION GUIDE

Analysis of Antibacterial Residues in Cow Milk

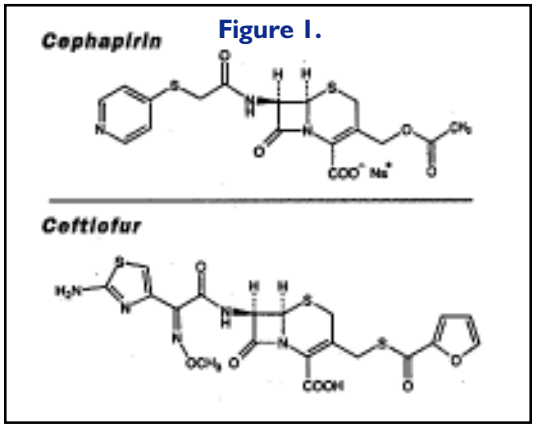
Antibacteria are often used by farmers to treat diseases in dairy cow. While the treatment has been very effective in prevention of various diseases in cows and other dairy products, the effect of residues of these drugs in humans has recently become a topic of concern for the U.S. Food and Drug Administration (USFDA).

Ceftiofur and Cephapirin (Figure 1.) Are commonly used antibacteria. In vivo and in vitro antibacterial activity and B-lactamase stability of ceftiofur has been recently studied by Yancey et al.[1] Cephamycins represent a family of B-lactam antibiotics produced by various Steptomyces species. Cephapirin has been shown to be quite effective against gram-pos, and gram-neg, bacteria including Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis[2]. Because of the significant human consumption of dairy products, especially among infants, it is very crucial to monitor the concentration level of residues of these antibiotics in milk.

We report here a reversed-phase HPLC method that results in a very high resolution of Ceftiofur and Cephapirin in cow milk*. Figure 2, shows the chromatogram of a: standard sample of 20 part per billion (ppb) of cephapirin and 50 ppb of cetiofur compared with b: a chromatogram of extract from a donated raw sample from the State of South Dakota, USA. The antibacteria in milk were extracted using a number of liquid-liquid extractions followed by solid phase extraction and pre-concentration.

This method allows chromatographers to effectively isolate, characterize, and quantitate residues of the antibacteria in cow milk and possibly other dairy products.

Figure 1.



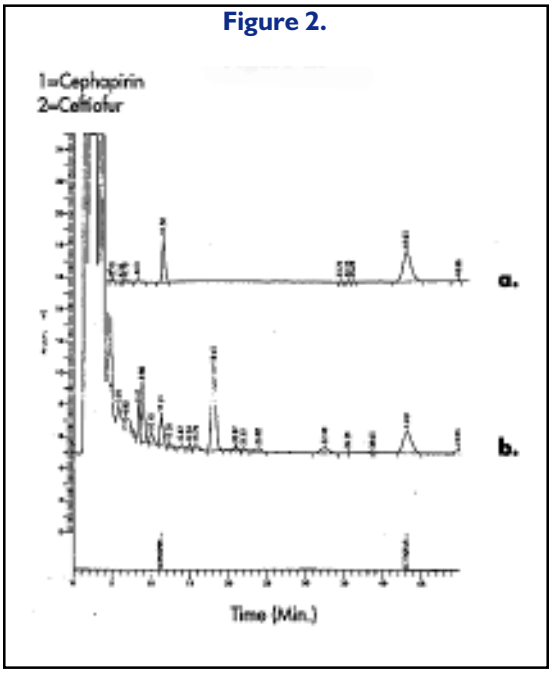
Chromatographic Conditions

Column: SMT **0-5-100**
Mobile Phase: 35 / 65 ACN / A
A=9mM Sodiumdodecylsulfonic acid/18mM H_3PO_4
Flow: 1mL/min
Inj: 200μL
Detector: UV, 290nm

Column Specifications:

Particle:	Spherical silica, 5 μm
Pore Size:	100Å
Surface Area:	340 m^2/g
Pore Volume:	1.0 ± 0.1
% Carbon:	22%
pH range:	1-12

Figure 2.



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1. Yancey et. al. Am. J. Vet. Res. 48, 1050, 1987.
2. Crast et. al. J. Med. Chem. 16, 1413, 1973.

SEPARATION METHODS TECHNOLOGIES
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