

# Application info 30

## FULLY AUTOMATED SAMPLE PREPARATION AND HPLC ANALYSIS OF TETRACYCLINE ANTIBIOTICS IN RAW MILKSAMPLES.

### INTRODUCTION

The Food Inspection Service in Leeuwarden is specialized in the analysis and control of raw milk and milkproducts. The described assay is developed to analyze tetracyclines as; chlorotetracycline (CTC), oxytetracycline (OTC), tetracycline (TC) and doxycycline (DC) in raw and homogenized milksamples. The automated sample preparation method and HPLC analysis is used to screen and control milksamples at the farmers collection side and to control traded milkproducts. Currently 300 samples have been analyzed with this method.

### EXPERIMENTAL

#### INSTRUMENTATION

For the chromatographic separation a Perkin Elmer gradient pump was used (410 series), detection was performed by an Applied Biosystems 1000S diode array detector (DAD). Automated on-line solid phase extraction (SPE) was performed by a Spark Holland PROSPEKT system which controlled the MARATHON autosampler and Solvent Delivery Unit (SDU). The milksamples are introduced by the MARATHON and the switching diagram shows how the samples are prepared prior to on-line desorption.

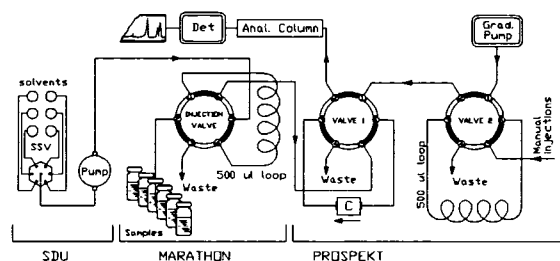


Figure 1: Switching diagram

#### CHROMATOGRAPHIC CONDITIONS

Anal.Column : Merck Lichrospher 100 RP 18, 5 µm, 125 × 4 mm I.D.

Mobile phase : gradient program, see diagram

Flow rate : 1.5 ml/min

Detection : diode array detection; 240 to 400 nm

Injection vol. : 500 µl (flushed loop)

#### SAMPLE PREPARATION

Raw milksamples are kept at 7°C during the night. The milkmatrix underneath the fat layer is transferred to the MARATHON autosampler vial. Homogenized milksamples do not need pretreatment, autosampler vials are filled with the milksamples and directly analyzed. On-line SPE was performed on PLRP-S cartridge (10 × 2.0 mm I.D., 15-25 µm) using the following sample preparation program:

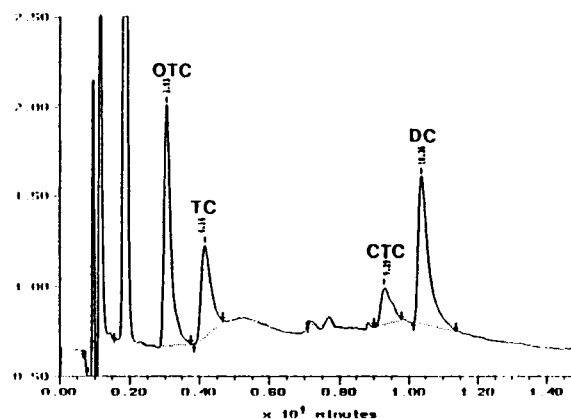
1. Activate the cartridge with 1.5 ml methanol.
2. Condition cartridge with 3.0 ml water.
3. Load and extract milksample on cartridge with 2.25 ml of water.
4. Elute clean sample from cartridge to analytical column with the gradient during 8 minutes. The final percentage of organic modifier is 50%.
5. MARATHON loop and connecting tubing flushed with 4.5 ml methanol and 4.5 ml water.

Clean up flow is 1.5 ml/min. While eluting the clean samples the auxiliaries 1 to 4 are switched on to start DAD, gradient and data system and to autozero the DAD.

Table 1: Gradient Program

Time	0.02 M Oxalic acid pH 2.5	MeOH	ACN
0:00	80%	5%	15%
1:30	80%	5%	15%
6:30	50%	17%	33%
12:30	50%	17%	33%
14:30	80%	5%	15%

### RESULTS



#### Chromatogram:

The chromatogram shows a milksample spiked with 100 ng/ml of oxytetracycline (OTC), tetracycline (TC), chlorotetracycline (CTC) and doxycycline (DC).

The PLRP-S solid phase extraction cartridge used has a recovery of 95% for the 500 µl milk sample. The recovery of a C8 Analytichem SPE cartridge was about 80% and Baker C18 or PRP-1 SPE cartridges showed a maximum recovery of 60% and 80% for the milk samples. Currently the C18 analytical column will be replaced by a PLRP-S column which has a better peak profile for CTC and is less sensitive to pH 2.5. The EEC norm to analyze tetracycline antibiotics in milk is set at 100 ng/ml. With the new assay developed OTC can be measured at 5-10 ng/ml, TC at 10 ng/ml, CTC at 50 ng/ml and DC at 10-50 ng/ml all far below the minimum level.

### REFERENCE

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