

Thiamphenicol in Milk Powder

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

HPLC Column:	Purospher® Star RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
SPE Column	LiChrolut® RP-18 40-63µm 500mg 3mL	1.02023.0001
Injection:	20 µL	
Detection:	UV@ 225 nm	
Cell:	1µl/10mm	
Flow Rate:	1.0 mL/min	

Mobile Phase: Mix Acetonitrile and H₂O 20:80 (v/v)

Temperature: 30 °C

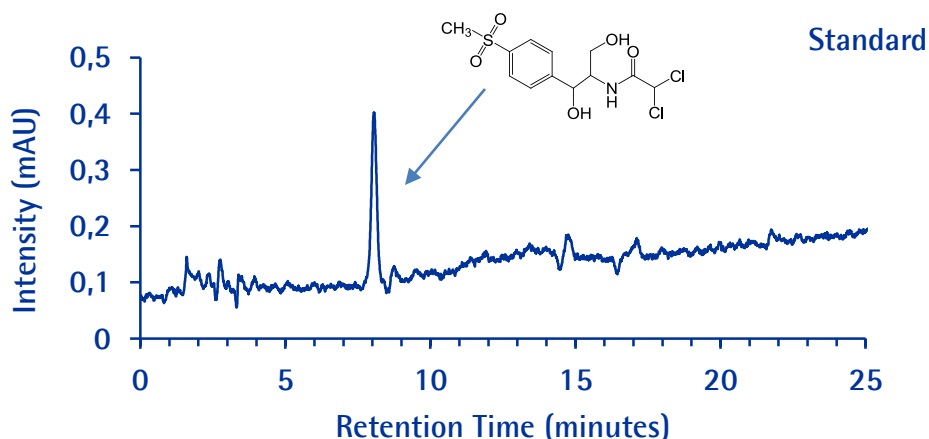
Diluent Mobile phase

Sample: Weigh 5.0g milk powder into a 50 mL centrifuge tube. Add 20 mL ethyl acetate. Vortex for 10min. Centrifuge at 4000 r/min for 5min. Take supernatant to a heart-shaped bottle. Repeat the extraction with another 20mL ethyl acetate, transfer the supernatant to the same bottle. Evaporate the solution to dryness with a rotary evaporator at 45°C. After a few minutes' standing, filtrate the solution. Dissolve the residue with total 10 mL H₂O by sonicating. Transfer the water solution to a 50 mL centrifuge tube, add 20 mL n-hexane, vortex and centrifuge, and finally take the supernatant for SPE extraction.

SPE procedure:

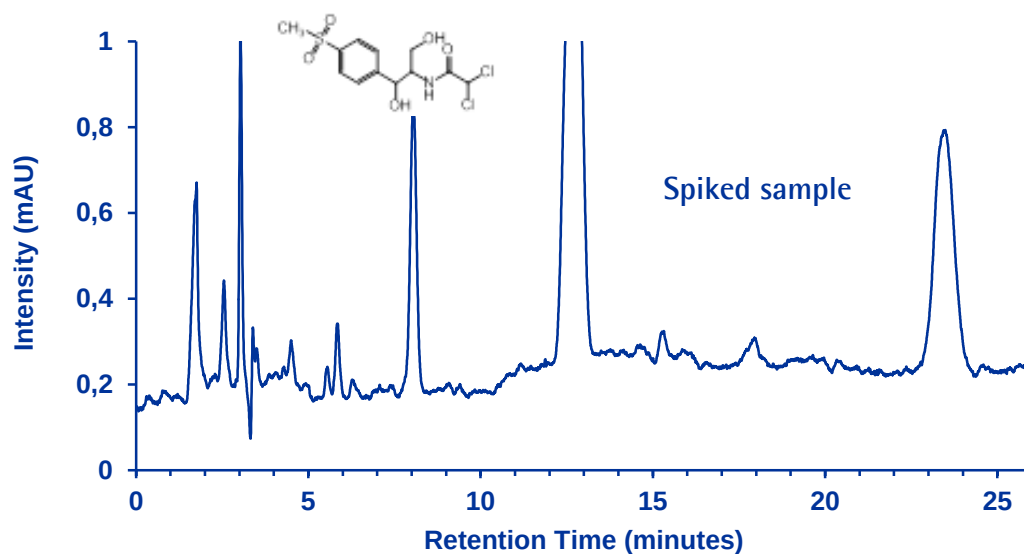
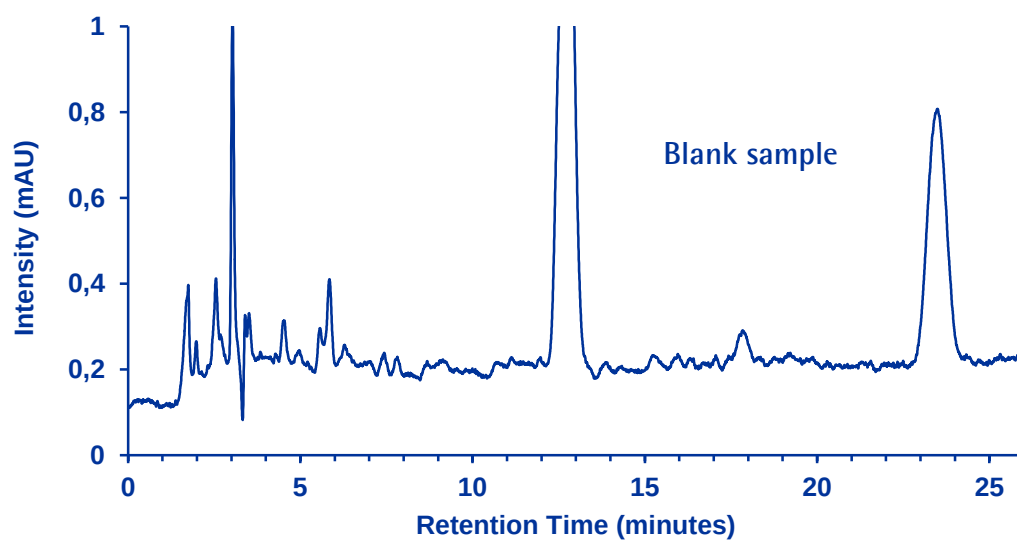
1. Condition LiChrolut® RP-18 SPE column - 5 mL acetonitrile and then 5 mL water
2. Load the sample,
3. Elute with 5mL ACN.
4. Dry the elution solution with N₂.
5. Dissolve the residue with 1.0 mL mobile phase, filter solution (0.45 µm filter) prior analysis.

Pressure Drop: 133 Bar (1928 psi)



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Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Thiamphenicol	8.1	8825	0.97

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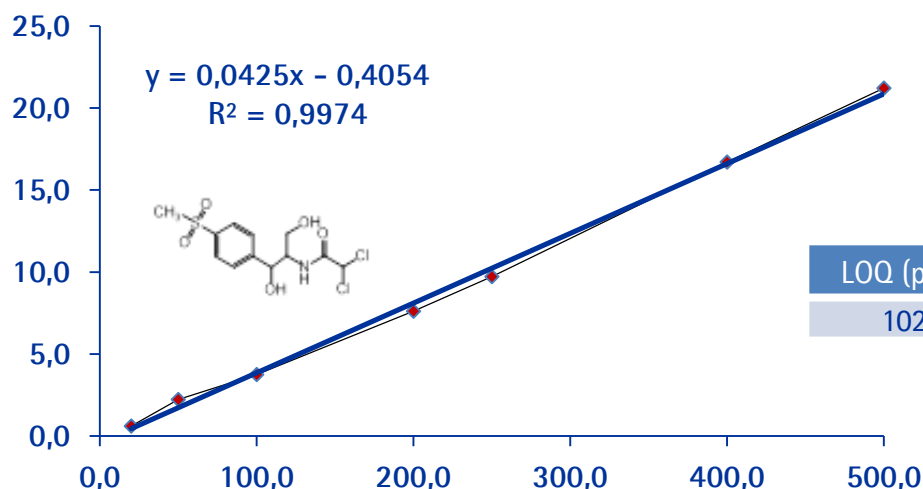
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Repeatability: Analysis of repeated injections of 100 ppb (ng/mL) standard solution (n=5).

Standard	Intensity (mV)
1	3.573
2	3.997
3	4.064
4	3.721
5	4.107
Average	3.892
STDEV	0.233
RSD (%)	6.0

Linearity: Analysis of repeated injections (n=5) of standard solutions in the calibration range 20–500 ppb (ng/mL).

Concentration (ppb)	Intensity (mV)
20	0.6146
50	2.2253
100	3.7523
200	7.6017
250	9.7091
400	16.703
500	21.205



LOQ (ppb)	LOD (ppb)
102	34

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SPE Recovery: Analysis of samples spiked with 100 ppb (µg/kg) of Thiamphenicol (n=3).

Spiked sample	Recovery (%)
1	85.01
2	89.32
3	88.10
Average	87.5
STDEV	2.22
RSD (%)	2.5

SPE Recovery: Analysis of samples spiked with 50 ppb (µg/kg) of Thiamphenicol (n=3).

Spiked sample	Recovery (%)
1	79.13
2	77.93
3	72.31
Average	76.5
STDEV	3.64
RSD (%)	4.8

