

Levamisole in Milk Powder

Purospher® STAR RP-18 endcapped

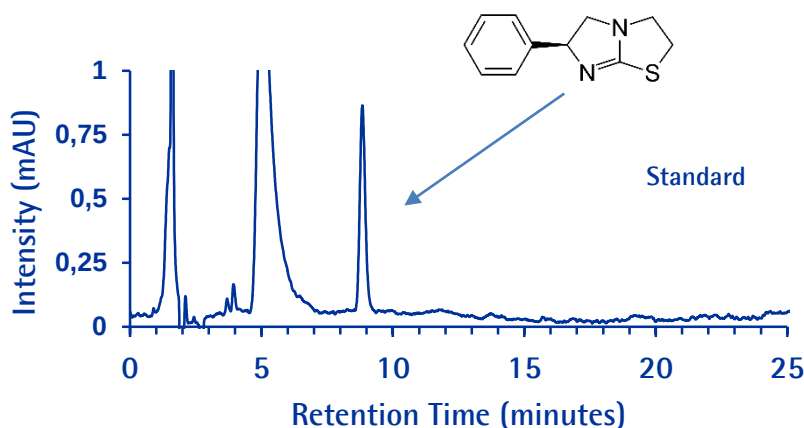
Chromatographic Conditions

HPLC Column:	Purospher® Star RP-18 endcapped (5 µm) 150x4.6 mm	1.51455.0001
SPE Column	LiChrolut® RP-18 40-63µm 500mg 3mL	1.02023.0001
Injection:	50 µL	
Detection:	UV@ 220 nm	
Cell:	1ul/10mm	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Weigh 2.44g NaH ₂ PO ₄ into a 1L volumetric flask, add 850mL H ₂ O and 3 mL diethyl amine. Adjust pH to 7.5 with 10% H ₃ PO ₄ solution, add H ₂ O to volume. Mix Methanol and NaH ₂ PO ₄ -Diethylamine aqueous solution 50:50 (v/v).	
Temperature:	30 °C	
Diluent	Mobile phase	

Sample: Weigh 5.0g milk powder into a 50 mL centrifuge tube. Add 5mL carbonate buffer solution (mix saturated NaHCO₃ aqueous solution and saturated Na₂CO₃ aqueous solution 900:100 (v/v) and 10 mL ethyl acetate into the tube. Vortex for 10min. Centrifuge at 6000 r/min for 10min. Take supernatant to a heart-shaped bottle. Repeat the extraction with another 10mL ethyl acetate, transfer the supernatant to the same bottle. Evaporate the solution to dryness with a rotary evaporator at 50°C. Dissolve the residue with carbonate buffer and follow SPE procedure.

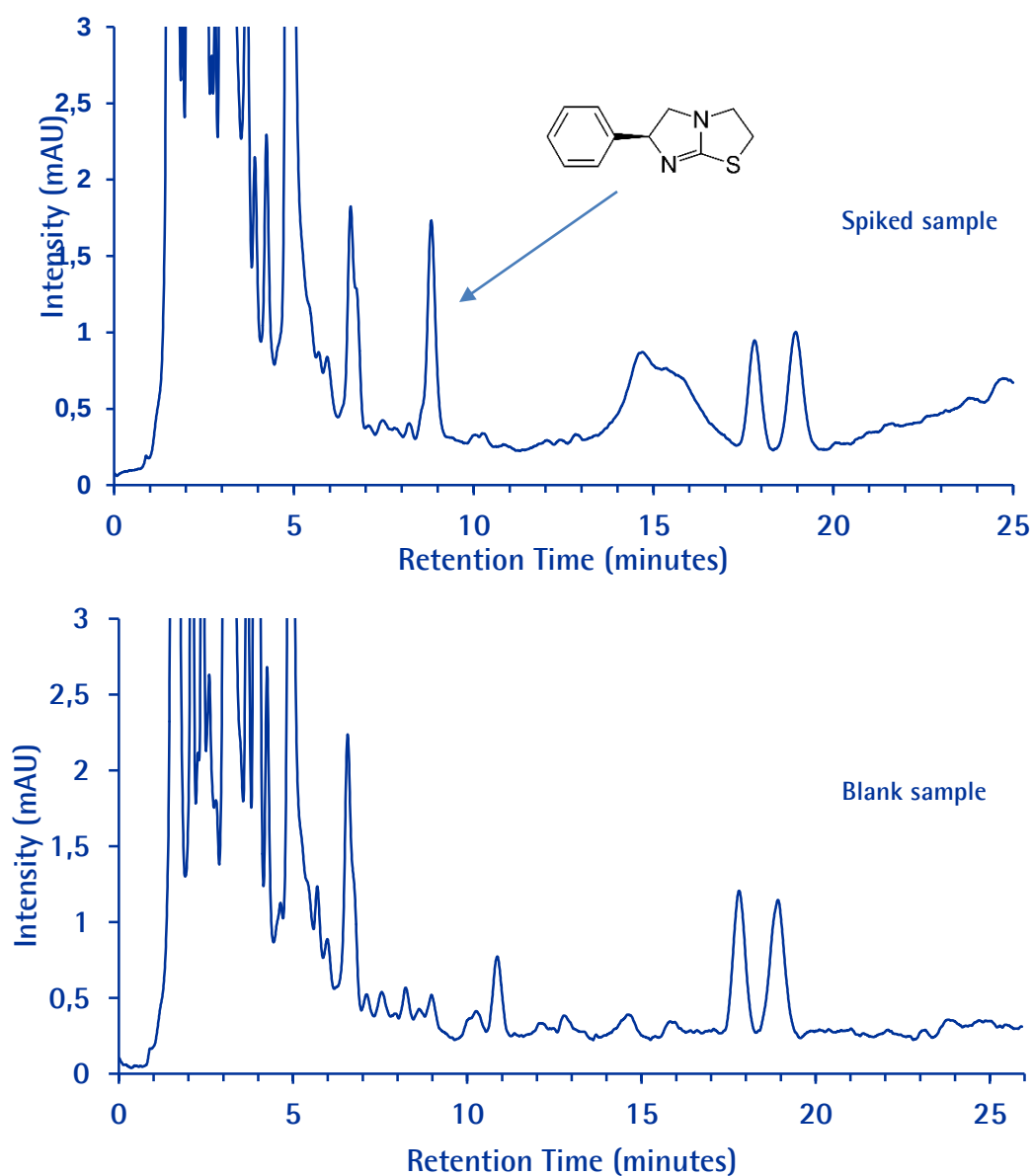
- SPE procedure:**
1. Condition LiChrolut® RP-18 SPE column - 3mL methanol and then 3mL carbonate buffer
 2. Load the carbonate buffer dissolved sample,
 3. Wash the SPE column with 3mL H₂O,
 4. Elute with 5mL ACN.
 5. Dry the elution solution with N₂.
 6. Dissolve the residue with 1.0mL mobile phase, filter solution (0.45 µm filter) prior analysis.

Pressure Drop: 177 Bar (2567 psi)



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Chromatographic Data

No.	Compound	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Levamisole	8.8	7484	1.00

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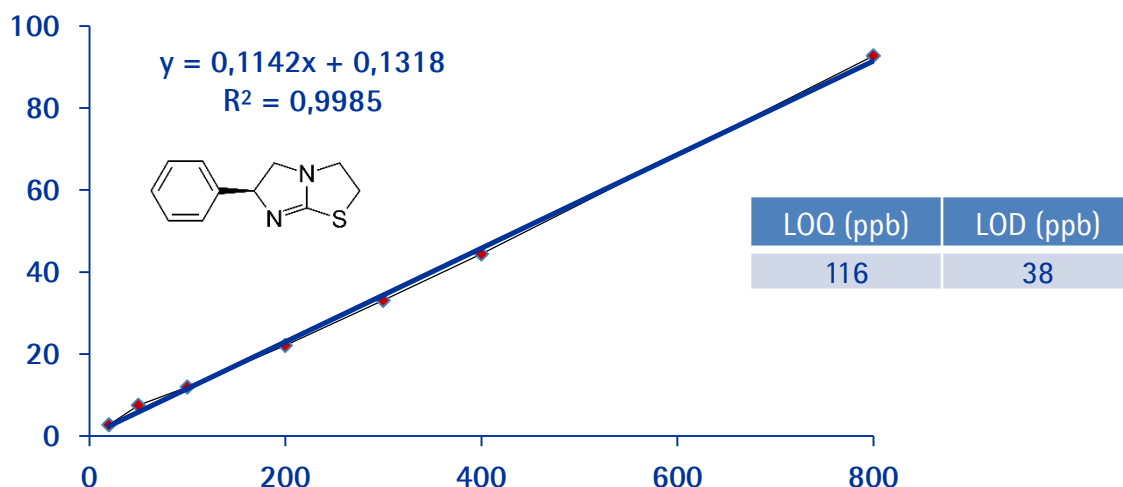
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Repeatability: Analysis of repeated injections of 100 ppb (ng/mL) standard solution (n=5).

Standard	Intensity (mV)
1	11.966
2	11.599
3	12.549
4	12.571
5	12.161
Average	12.169
STDEV	0.419
RSD (%)	3.4

Linearity: Analysis of repeated injections (n=5) of standard solutions in the calibration range 20–800 ppb (ng/mL).

Concentration (ppb)	Intensity (mV)
20	2.75
50	7.46
100	11.9
200	22.1
300	33.1
400	44.4
800	92.7



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SPE Recovery: Analysis of samples spiked with 100 ppb (µg/kg) of Levamisole in milk powder

Spiked sample	Recovery (%)
1	95.33
2	92.78
3	94.98
Average	94.36
STDEV	1.38
RSD (%)	1.5

SPE Recovery: Analysis of samples spiked with 50 ppb (µg/kg) of Levamisole in milk powder

Spiked sample	Recovery (%)
1	89.13
2	87.67
3	89.54
Average	88.78
STDEV	0.98
RSD (%)	1.1

