

Quantification of Polysorbate 80 (Tween 80) by LC-MS

Background on Polysorbate 80

Polysorbate 80 (Tween 80) is a nonionic surfactant widely used in biopharmaceutical formulations as a stabilizing agent. It helps prevent protein aggregation, improves solubility, and enhances overall product robustness.

In downstream processing, however, it is equally important to demonstrate efficient removal of Tween 80 to meet regulatory expectations. Sensitive and accurate quantification is therefore essential, particularly at very low residual levels.

Objective

This application note summarizes our newly developed LC-MS method capable of quantifying polysorbate 80 from 100,000 ng/mL down to 40 ng/mL, with a limit of detection (LOD) of 8 ng/mL. The method supports both formulation development and downstream clearance studies where low-level quantification is critical.

Method Performance Summary

- Quantification range: 100,000 ng/mL to 40 ng/mL
- Accuracy of back-calculated calibration standards: 94–101%
- Limit of detection (LOD): 8 ng/mL
- Spike recoveries in complex matrices: 87–98%

Method overview

Sample preparation is performed using a liquid-liquid extraction (LLE) procedure that efficiently isolates polysorbate 80-derived analytes from complex matrices. Extracts are chromatographically separated on a reversed phase LC column, providing robust resolution of PS 80 oligomers and minimizing matrix interferences. Detection is carried out using electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode, enabling highly selective quantification. In this application note, we highlight the performance of one characteristic PS 80 MRM transition; however, additional transitions can be used to enhance specificity or monitor different polysorbate components depending on the analytical objective.

Figures and Tables

Sample Name	Signal / Noise	Calculated Concentration	Accuracy (%)
40 ng/mL PS-80	18.5	38	94
200 ng/mL PS-80	167.9	192	96
1000 ng/mL PS-80	621.2	945	95
10 000 ng/mL PS-80	1983.1	10072	101
100 000 ng/mL PS-80	6377.5	98620	99

Table 1: Calibration curve concentrations with back-calculated accuracies

Limit of detection (LOD)	Signal / Noise
8 ng/mL PS-80	3.4
8 ng/mL PS-80	5.4

Table 2: Limit of detection

	Polysorbate 80 concentration			Spike recovery
	ng/mL	PPM	%	
Sample 1	43	0.04	0.000	98%
Sample 1 + 7 PPM spike	6917	6.92	0.007	
Sample 2	6692	6.69	0.007	87%
Sample 2 + 7 PPM spike	12801	12.80	0.013	
Sample 3	5052	5.05	0.005	92%
Sample 3 + 7 PPM spike	11486	11.49	0.011	

Table 3: Spike recovery results across complex matrices

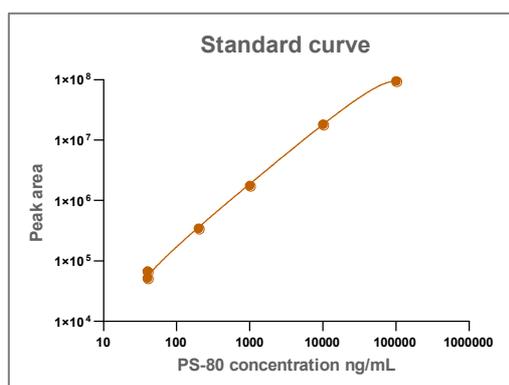


Figure 1: Calibration curve plot

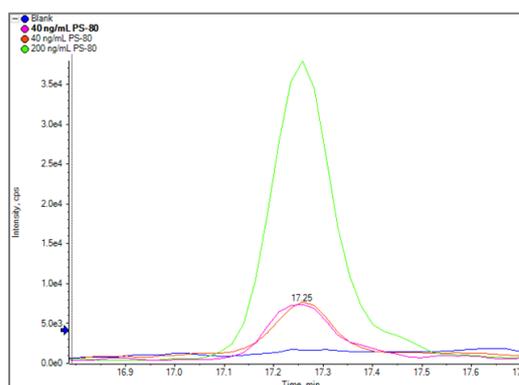


Figure 2: LC-MS chromatogram at LOQ (40 ng/mL)

Conclusion

The developed LC-MS method provides sensitive, accurate, and reproducible quantification of polysorbate 80 across a wide concentration range, supporting both formulation stability studies and downstream process clearance validation. The low LOQ of 40 ng/mL enables confident assessment of trace levels.

Advantages of this method

- very low LLOQ (40 ng/mL)
- method works in complex matrices
- great accuracy and spike recovery
- fast LC run time
- minimal sample prep

Applications

- residual polysorbate removal in downstream processing
- quantification in formulated drug products
- Confirmation of correct Polysorbate addition in final products
Monitoring of Tween degradation during stability studies
- Method can be upgraded to provide insight into Tween degradation mechanisms