Enhancement of Selectivity in Sample Cleanup for Tobramycin in Serum Using Derivatization before Solid Phase Extraction

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Introduction

Solid Phase Extraction (SPE) using reversed phase sorbent has proven very successful for the cleanup of nonpolar compounds from biological samples. Polar compounds, however, would have low selectivity and poor recovery.

Tobramycin, an aminoglycoside antibiotic, is a good example of a polar pharmaceuti- cal compound. To improve its cleanup from a biological matrix, a method has been developed using pre-SPE derivatization with orthophthalaldehyde (OPA). This method has a secondary advantage of enhanced detection sensitivity by means of fluorescence detection of the derivative formed.

Procedure

1. Prepare OPA reagent:
   OPA 5 mg/mL
   2-mercaptoethanol (2-ME) 1% v/v in borate buffer pH 10.4, 0.4M
2. Deproteinate serum sample using 10% (w/v) sulfosalicylic acid.
   Vortex and centrifuge.
3. Mix OPA reagent with Tobramycin sample (1:1 v/v) (Figure 1).

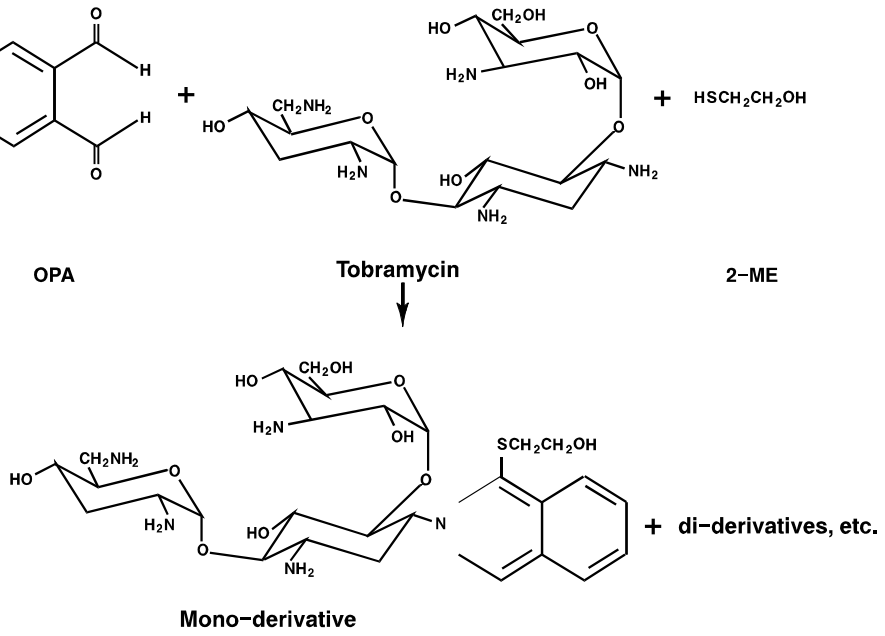

Figure 1. Analysis of Tobramycin Using Pre-column Derivatization (OPA)

Solid Phase Extraction

1. Condition Bond Elut C_{18} with acetonitrile, followed by acetonitrile/phosphate buffer pH 8 (10:90 v/v).
2. Load derivatized sample in acetonitrile/phosphate buffer.
3. Wash with acetonitrile/phosphate buffer.
4. Elute with acetonitrile/water (90:10 v/v).
5. Inject onto HPLC.

Results

1. Two derivatives are obtained. Due to multiple primary amino sites on the compound, multiple derivatives may be expected. Following SPE, Derivative 2 is the prominent peak, probably due to a stabilization effect of the SPE. There is no interference from the blank. The advantage of this method is shown by a comparison study where SPE was followed by derivatization. This latter method had a poor recovery of 2% (Figure 2).
2. Peak heights and standard concentrations from 13 to 128 μM (40-400 pmoles on column) were linearly related with a correlation coefficient of 0.998.
3. Reproducibility of 6 runs of a 96 μM standard (300 pmoles on column) had a relative standard deviation of 1.1% .
4. Peak heights and spiked serum concentrations from 32 to 128 μM were linearly related with a correlation coefficient of 0.995.
5. Recoveries of spiked serum at 128 μM averaged 98% with a relative standard deviation of 3.6%.

References

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2. Robert Cunico, Automatic Precolumn Derivatization of Amino Acids with Orthophthalaldehyde, Varian LC at Work #161, 1985.

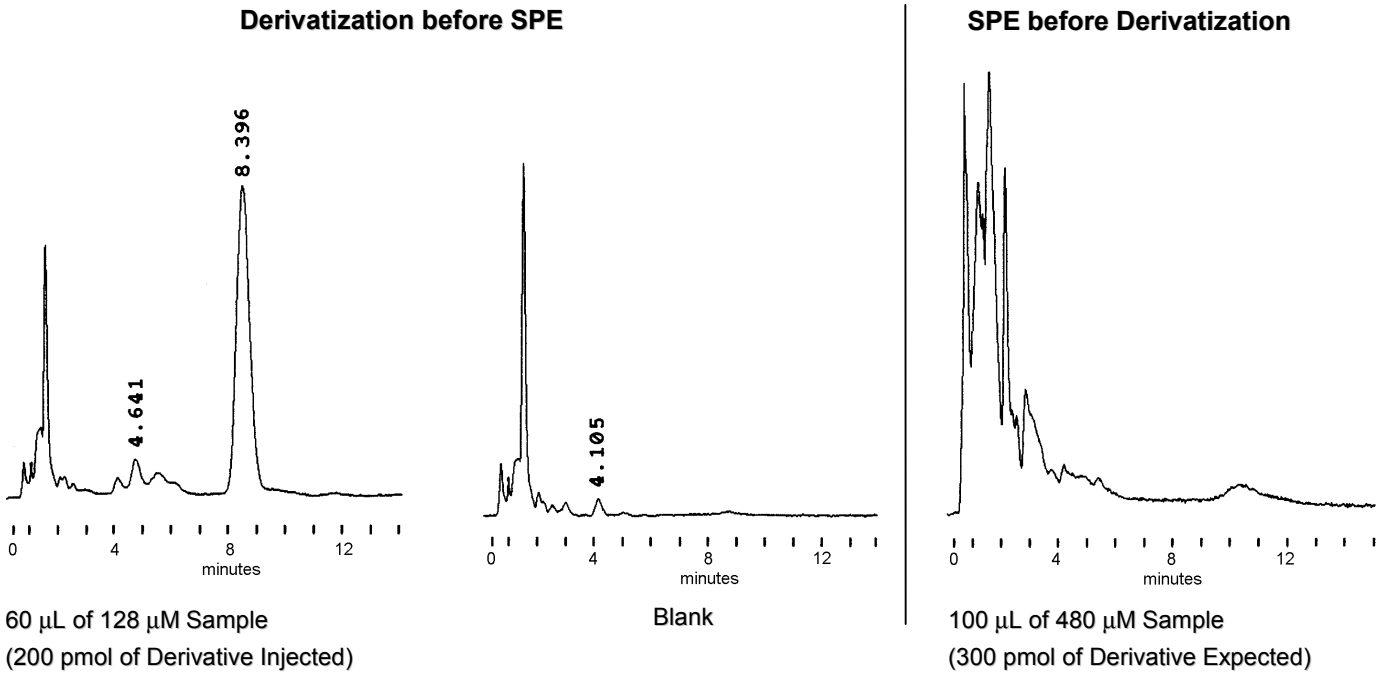

Figure 2. Chromatograms of Tobramycin-Spiked Serum

Column: MicroPak SP C_8, 4 mm x 15 cm
Mobile Phase: 0.02M Phosphate pH 6.5:Acetonitrile/50:50, 2 mL/min
Detection: Fluorescence
Ex. 340 nm (Bandpass filter CS-7-54, CS-7-60)
Em. 450 nm (Bandpass filter CS-4-76, Cutoff filter CS-3-73)

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These data represent typical results.
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