Enhancement of Detection Sensitivity of Tobramycin Using Pre-column Derivatization

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Fran Lai
Varian Chromatography Systems

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Introduction

Reversed Phase HPLC and UV detection are very popular methods for the analysis of pharmaceutical compounds. This works well for nonpolar and UV-absorbent compounds, but for compounds that are rather polar and lack UV-absorptivity, the analysis becomes a major challenge.

Tobramycin, an aminoglycoside antibiotic, is a good example of a polar pharmaceutical compound with low UV-absorptivity. To overcome these problems, a method has been developed using pre-column derivatization with orthophthalaldehyde (OPA).

Procedure

The derivatization process is easily automated using the sample preparation features on the 9100 AutoSampler.

1. Prepare Tobramycin sample in acetonitrile:water/60:40.
2. Prepare OPA reagent:

   OPA 5 mg/mL

   2-mercaptoethanol (2-ME) 1% v/v in borate buffer pH 10.4, 0.4M

Derivatization Using Varian 9100 AutoSampler

1. Transfer and mix OPA reagent with Tobramycin sample (1:1 v/v) (Figure 1).
2. Wait for programmed reaction time.
3. Inject onto the HPLC.

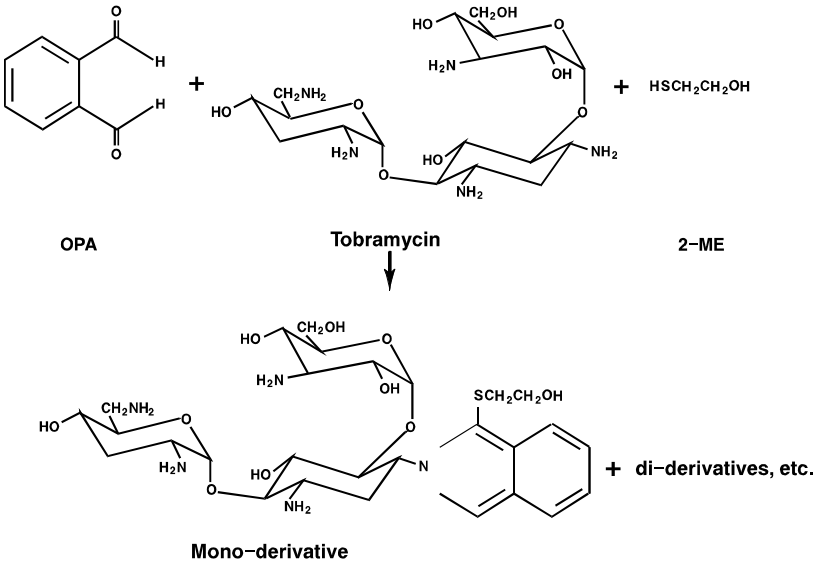

Figure 1. Analysis of Tobramycin Using Pre-column Derivatization (OPA)

Results

1. Two derivatives are obtained. Due to multiple primary amino sites on the compound, multiple derivatives may be expected. The ratio of Derivative 2/Derivative 1 increases with reaction time and with increase in acetonitrile in the sample solvent.
2. The optimum reaction time is 30 minutes, and the optimum solvent composition is 60% acetonitrile. There is no interference from the blank. (Figure 2)
3. Using fluorescence, the detection limit is about 400 times better than when using UV absorbance. (Figure 3)
4. Linearity was tested up to 1.2 nmoles and found to be linear with a correlation coefficient of 0.998.

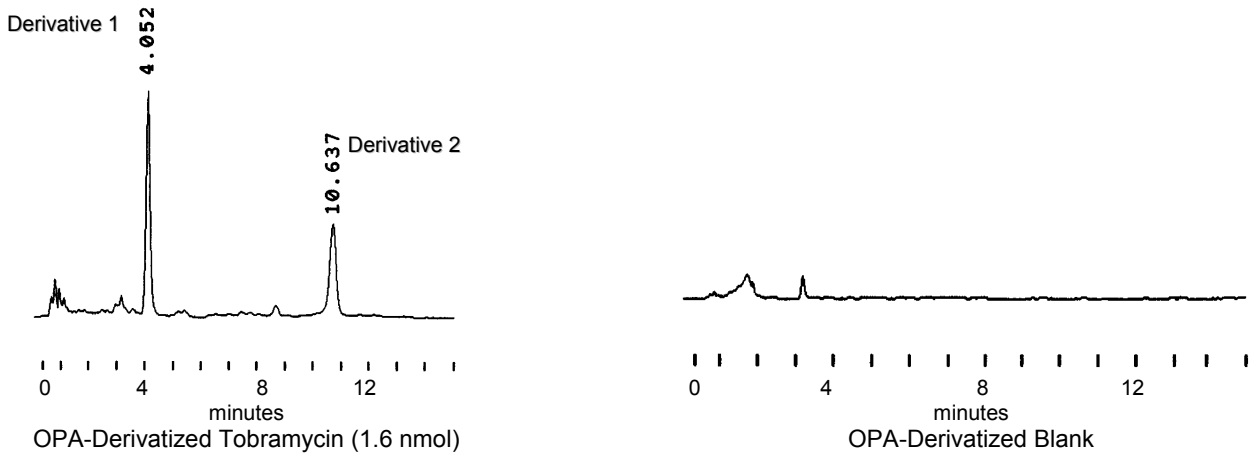

Figure 2. OPA-Derivatization of Tobramycin

Column:	MicroPak SP C_8 4 mm x 15 cm
Mobile Phase:	0.02M Phosphate pH 6.5:Acetonitrile/52:48, 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)

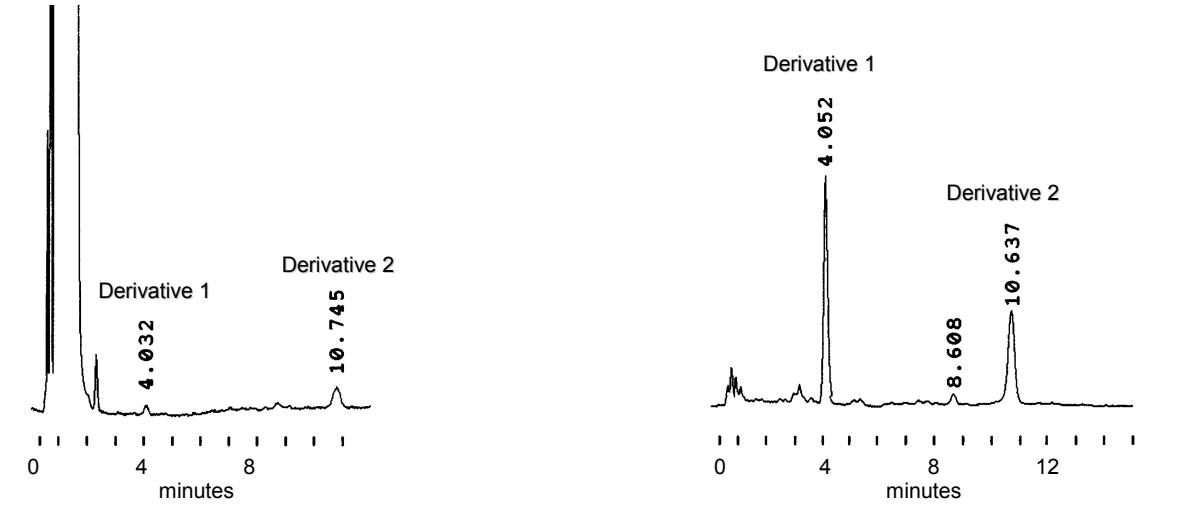

Figure 3. Detection Enhancement Using Fluorescence vs. UV Absorbance

References

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