# Analysis of Methylene Blue Purity in Reversed-Phase Cation-Exchange and Reversed-Phase Cation-Exclusion Modes

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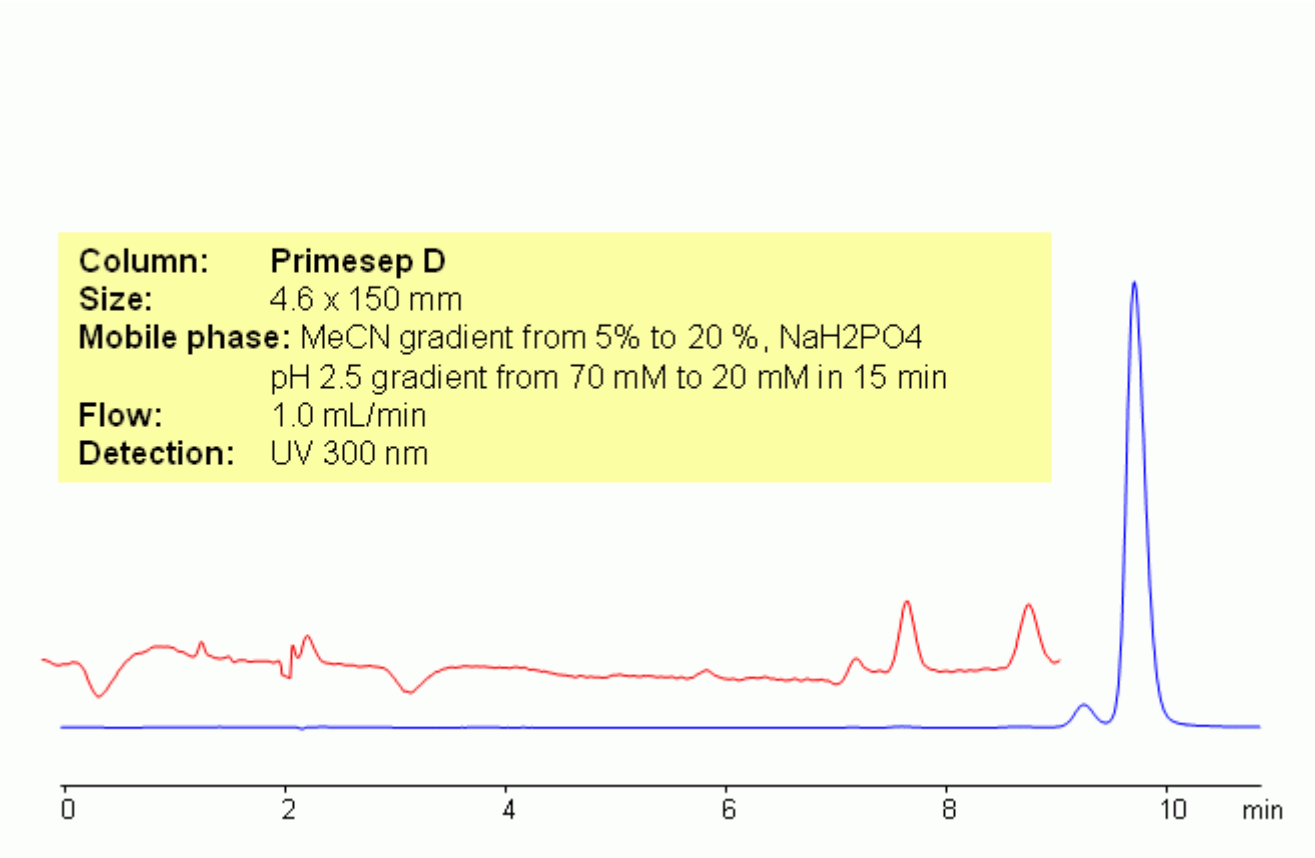
## Chromatogram




## Description

Methylene blue is a heterocyclic dye with a wide range of use in biology and chemistry. Purity of dyes rarely exceed 70% and separation of methylene blue and related impurities is required. Two alternative methods for purity analysis were developed on Primesep C and Primesep D mixed-mode HPLC columns. Primesep C is a reversed-phase cation-exchange (anion-exclusion column) column and Primesep D is reversed-phase anion-exchange (cation-exclusion) column. Various buffers can be used for the method. Depending on the buffer, method can be compatible with UV, LC/MS and prep chromatography. Method can be used for prep separation of methylene blue and related impurities.

## Method Parameters

| | |
|---|---|
| **Mobile Phase** | MeCN – 35% |
| **Buffer** | AmFm pH 3.5 – 35 mM |
| **Flow Rate** | 1.0 ml/min |
| **Detection** | UV, 300 nm |
| **Class of Compounds** | Duy, Hydrophobic, Ionizable |
| **Analyzing Compounds** | Methylene Blue |

## HPLC Column Used

### Primesep C, 4.6×150 mm, 5 µm, 100A

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