

## Diphenhydramine HCL Analyzed with LCMS - AppNote

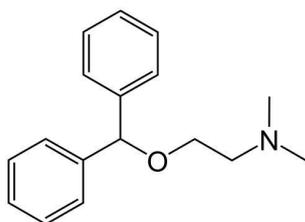
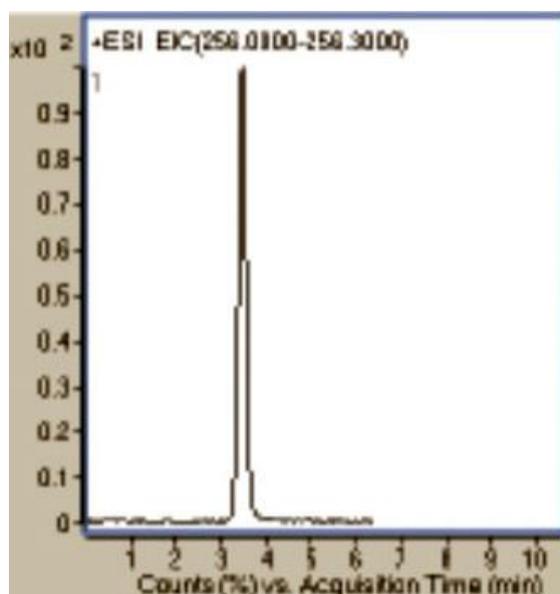
### Online SPE with a Simple Mobile Phase Step Gradient

When a Mobile Phase containing 80% Solvent B (with 10mM Pyridine in Solvent A and B) is used Diphenhydramine Hydrochloride (DPH) is fully retained on the Column, meaning it does not elute and continues to be retained until it would overload the Column if injections were continued.

When the Mobile Phase was changed to 25% Solvent B, the same DPH Peak elutes at 3.5 minutes.

This wide range of Retention Time can give analysts many possibilities of developing Methods for Quantitative Analysis of Drug Formulations. With this Method, you can perform On-Column Solid Phase Extraction (SPE) Methods for Isolation of DPH from complex matrices. Simply use 80% Solvent B to load the DPH on the Column and 25% Solvent B to elute the compound.

The Method below is very Reproducible and Sensitive with a Lower Limit of Quantitation (LLOQ) of 5ng / mL for DPH, with good Linearity in the range 1-500ng / mL ( $r^2 > 0.9990$ ).



### Peak:

Diphenhydramine 256 m/z (M-H)<sup>-</sup> RT = 3.49 min

## Method Conditions

**Column:** Cogent Diamond Hydride™, 4 µm, 100Å

**Catalog No.:** [70000-15P](#)

**Dimensions:** 4.6 x 150 mm

**Solvents:**

A: DI Water / 10 mM Pyridine

B: Acetonitrile / 10 mM Pyridine

**Mobile Phase:** *Isocratic* 25:75 Solvent B / Solvent A

**Flow rate:** 0.4 mL / minute

**Detection:** ESI – neg - Agilent 6210 MSD TOF Mass Spectrometer

**Sample Preparation:** 50 ng / mL prepared in 50:50 Solvent A / Solvent B

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**Notes:** *Diphenhydramine (DPH) is an antihistaminic drug mainly used as a sedative, hypnotic and antiemetic most know by the trade name, Benadryl®. It is available over-the-counter in many countries and is very common. Generally regarded as a harmless drug, there have been sixty-eight non-fatal and fifty five fatal poisonings with DPH or in combination with other drugs investigated in the past.*

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