Simultaneous Specific Determination of Six Antihistamine Drugs Using Isocratic HPLC-UV

Document No. 022920-02. Revision 01, 29 February 2022

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Description of the Method

Name of the Method

Simultaneous specific determination of six antihistamine drugs using isocratic HPLC-UV.

Scope of the Method

The method is intended for the highly selective determination of six antihistamine drugs (cetirizine, chloropyramine, chlorpheniramine, pheniramine, desloratadine, doxylamine) in various pharmaceutical and biological samples using simple isocratic 400 bar HPLC system with a conventional UV detector.

The method is capable to determine antihistamines in complex matrices that contain any neutral or acidic matrix compounds, as well as various basic compounds like drotaverine, dextromethorphan, phenylephrine, etc.

The method is not capable of determining loratadine.

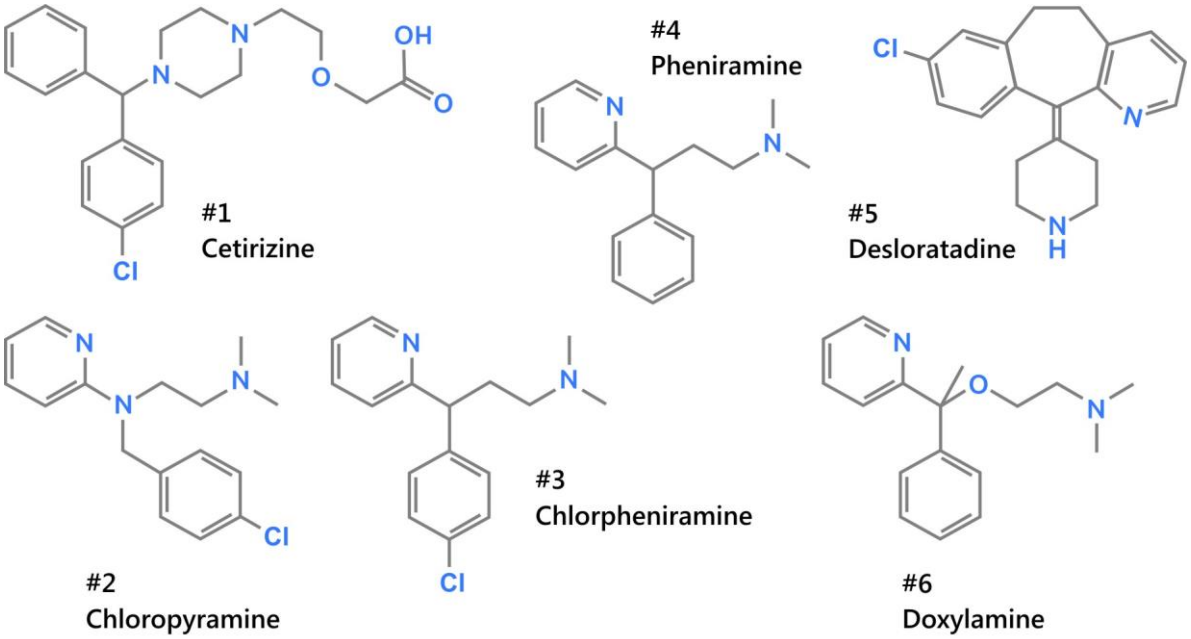
Main Method Characteristics

Elution Mode: Isocratic
Actual Analysis Time: 6.5 minutes
Actual Back-Pressure: Typically < 300 bar at 1.5 mL/min
Recommended Analysis Time: 6.5 minutes
Specificity: Highly specific

Minimum System Requirements

Solvent Delivery System: Isocratic HPLC pump with 400 bar upper back-pressure limit
Detector: Single wavelength UV detector
Column Oven: Preferable to ensure retention time stability

Analyte(s)



Standard HPLC Conditions

Mobile Phase: Acetonitrile-Buffer 80:20
Buffer: 20mM $NH_4H_2PO_4$ + 0.1% H_3PO_4
Flow Rate: 1.5 mL/min
Column Oven: 25 °C
Detection: UV 260 nm, or **UV 260 nm** + 280 nm

Typical Chromatogram(s)

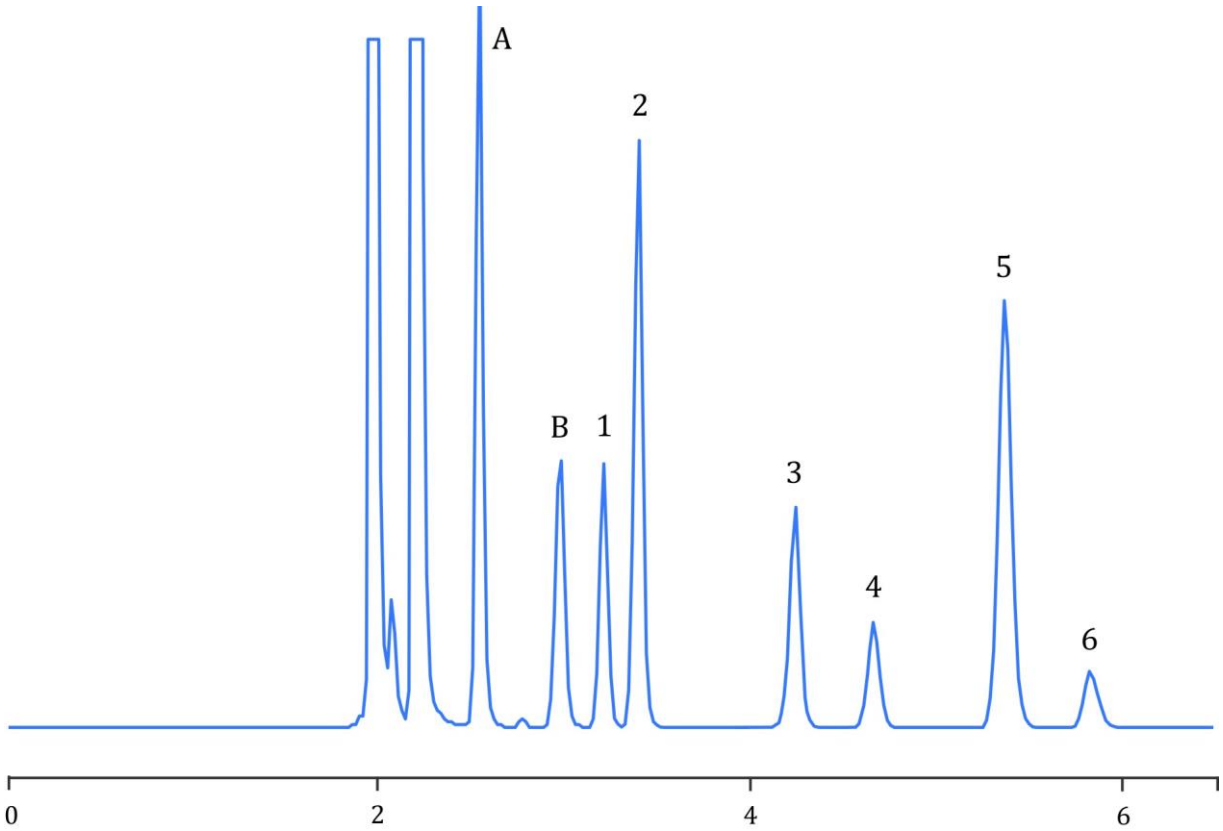


Figure 1. Specific determination of six antihistamines. Sample: mixture of six medications; each of them contains one of the analytes. Detection: UV 260 nm. HPLC column: Ace Excel HILIC-A, 250x4.6 3um.
1. *Cetirizine,* ***2.*** *Chloropyramine,* ***3.*** *Chlorpheniramine,* ***4.*** *Pheniramine,* ***5.*** *Desloratadine,* ***6.*** *Doxylamine. Identified matrix components:* ***A.*** *Drotaverine,* ***B.*** *Dextromethorphan.*

Suitable HPLC Column(s)

Stationary phase ***#1****:* *Ace Excel HILIC-A*
Column dimentions: 250x4.6 3um
Stationary phase manufacturer: ACT/WVR

Specificity Verification

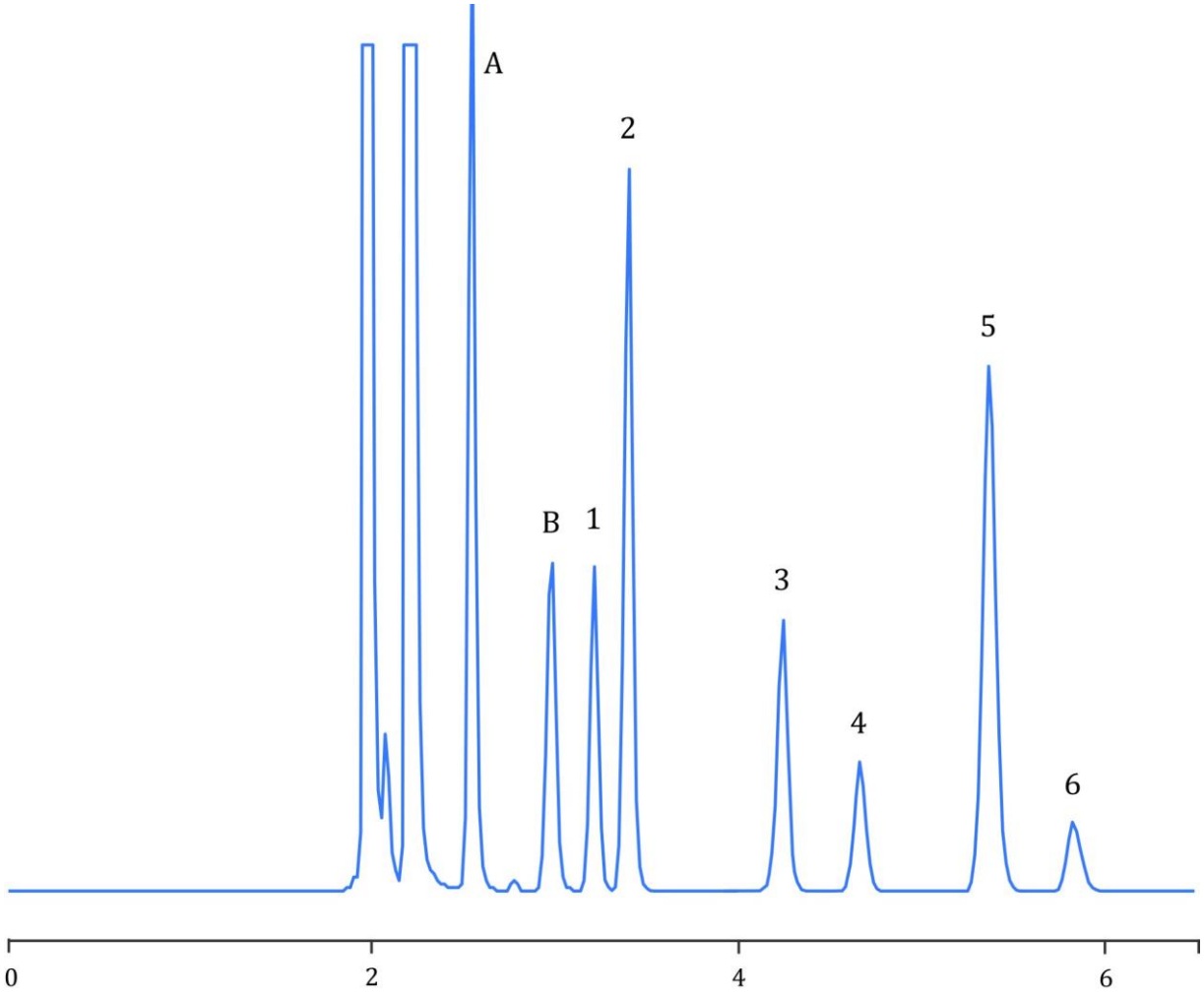


Figure 2. Specific determination of six antihistamines. Sample: mixture of six medications; each of them contains one of the analytes. Detection: UV 260 nm. HPLC column: Ace Excel HILIC-A, 250x4.6 3um.
1. *Cetirizine,* ***2.*** *Chloropyramine,* ***3.*** *Chlorpheniramine,* ***4.*** *Pheniramine,* ***5.*** *Desloratadine,* ***6.*** *Doxylamine. Identified matrix components:* ***A.*** *Drotaverine,* ***B.*** *Dextromethorphan.*

Quickstart Steps

Mobile Phase Preparation

Buffer preparation. Weight 2.3g $NH_4H_2PO_4$ in 1 L volumetric flask; transfer 1 mL phosphoric acid; transer 500 mL water and mix; bring to volume with water and mix.

Mobile Phase Preparation. Transfer 500 mL acetonitrile and 200 mL buffer in 1 L volumetric flask and mix, then bring to volume with acetonitrile, and mix.

Column Washing And Storage

The column can be washed with Acetonitrile-(20mM $NH_4H_2PO_4$) 70:30 and then with the mobile phase. The column can be stored in the mobile phase.

Column Conditioning

The new column should be washed with Acetonitrile-(20mM $NH_4H_2PO_4$) 50:50 and then conditioned with the mobile phase until retention times become consistent.

Testing Column Performance

Column performance should be tested under the standard conditions. Typical plate count for 250x4.6 3um Ace Excel HILIC-A at a flow rate 1.5 mL/min is more than 20’000, and the asymmetry factor is less than 1.2.

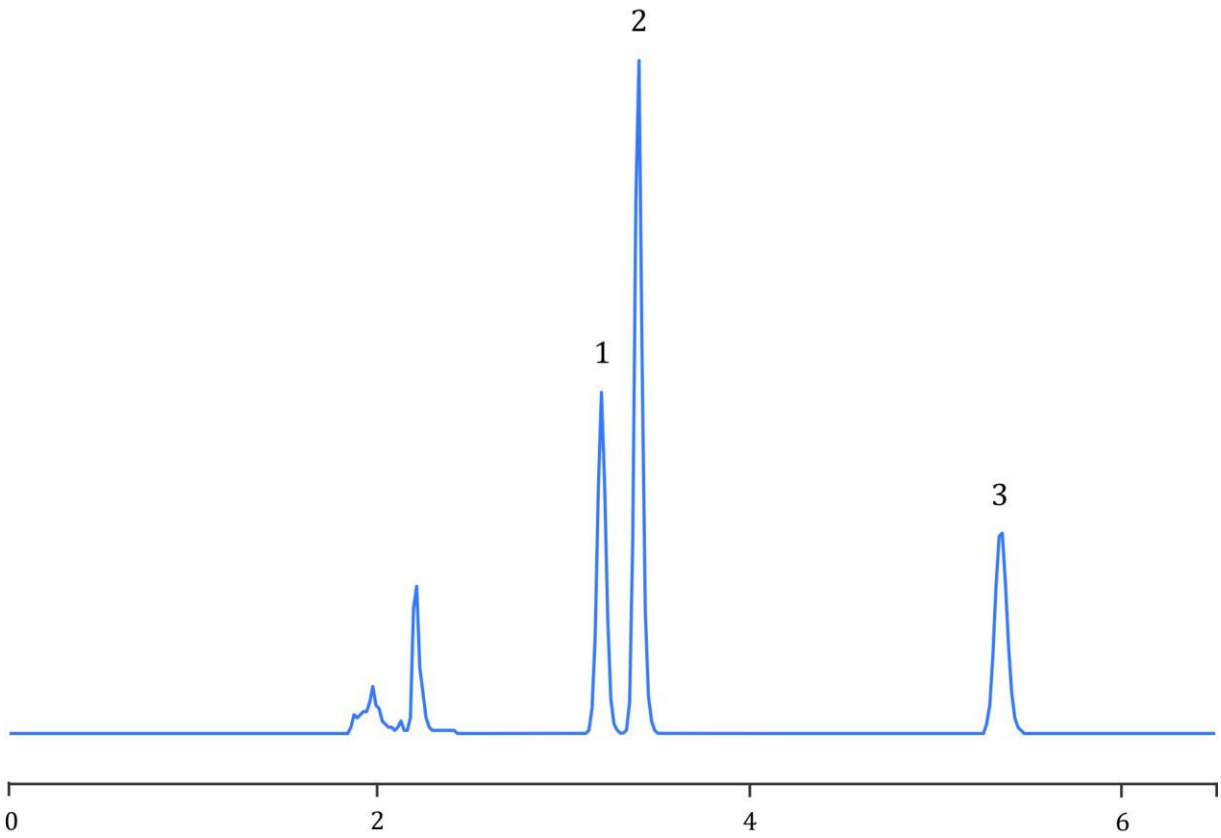


Figure 3. Test chromatogram. Detection: UV 260 nm. HPLC column: Ace Excel HILIC-A, 250x4.6 3um.
1. *Cetirizine,* ***2.*** *Chloropyramine,* ***3.*** *Desloratadine.*

#	Name	N	A_f
1	*Cetirizine*	24’000	1.0
2	*Chloropyramine*	31’800	1.0
3	*Desloratadine*	30’000	1.1

Fine-Tuning Retention and Selectivity

The selectivity of the separation can be fine-tuned by varying the buffer concentration in the range 5-20 mM, or by varying phosphoric acid concentration in the range 0.05-0.2 v/v%.

Retention can be adjusted by changing acetonitrile content in the mobile phase in the range 75-85 v/v%.

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