Specific Determination of Caffeine In Non-Alcoholic Beverages, Beverage Concentrates and Syrups Using Isocratic HPLC-UV

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Description of the Method

Name of The Method

Specific determination of caffeine in non-alcoholic beverages, beverage concentrates and syrups using isocratic HPLC-UV.

Scope of The Method

The method is intended for the highly selective determination of caffeine in various beverage, beverage concentrate and syrup samples using simple isocratic 400 bar HPLC system with a conventional UV detector.

The method is capable to determine caffeine in complex matrices that contain water-soluble vitamins (C, B1, B2, B3, B6, B9), preservatives (benzoic and sorbic acids), natural and synthetic dyes, UV-absorbing flavouring agents (vanillin, ethylvanillin, benzaldehyde, etc.), natural polyphenolic compounds including hydroxybenzoic and hydroxycinnamic acids and aldehydes.

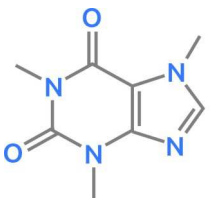
Main Characteristics of The Method

Elution Mode: Isocratic
Actual Analysis Time: 4 minutes/< 200 bar
Recommended Analysis Time: 7 minutes/< 200 bar
Specificity: 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)



#1
Caffeine

Standard HPLC Conditions

Mobile Phase: Acetonitrile-Buffer 12:88
Buffer: 25mM $NH_4H_2PO_4$
Flow Rate: 1.5 mL/min
Column Oven: 25 °C
Detection: UV 270 nm

Typical Chromatogram(s)

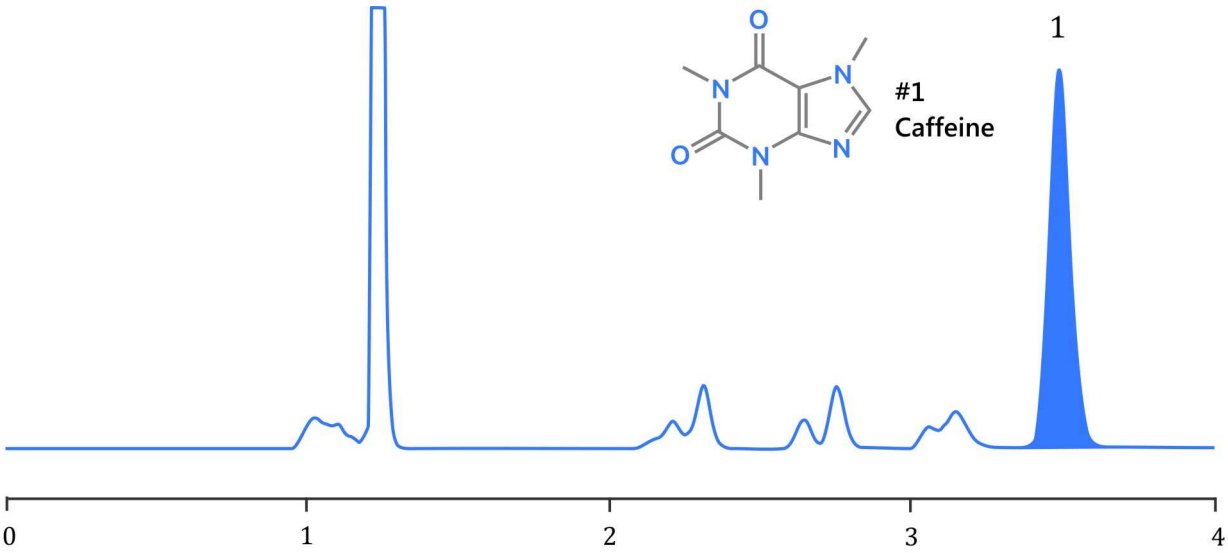


Figure 1. Specific determination of caffeine in the most complex matrix, beverage concentrate sample #1. Detection: UV 270 nm. HPLC column: Acclaim Mixed-Mode WCX-1, 250x4.6 5um.
1. *Caffeine.*

Suitable HPLC Column(s)

*Stationary phase **#1**:* Acclaim Mixed-Mode WCX-1
Column dimentions: **250x4.6 5um**, or 150x4.6 3um
Stationary phase manufacturer: Thermo Dionex, USA

Demonstration of Specificity

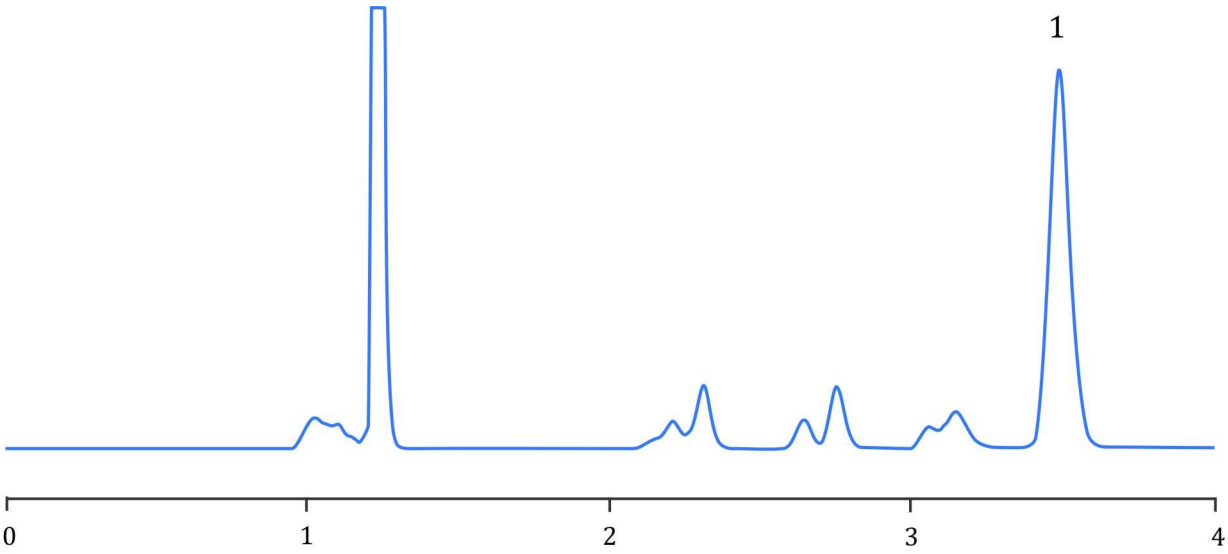


Figure 2. Specific determination of caffeine in beverage concentrate sample #1. HPLC column: Acclaim Mixed-Mode WCX-1, 250x4.6 5um.
1. *Caffeine.*

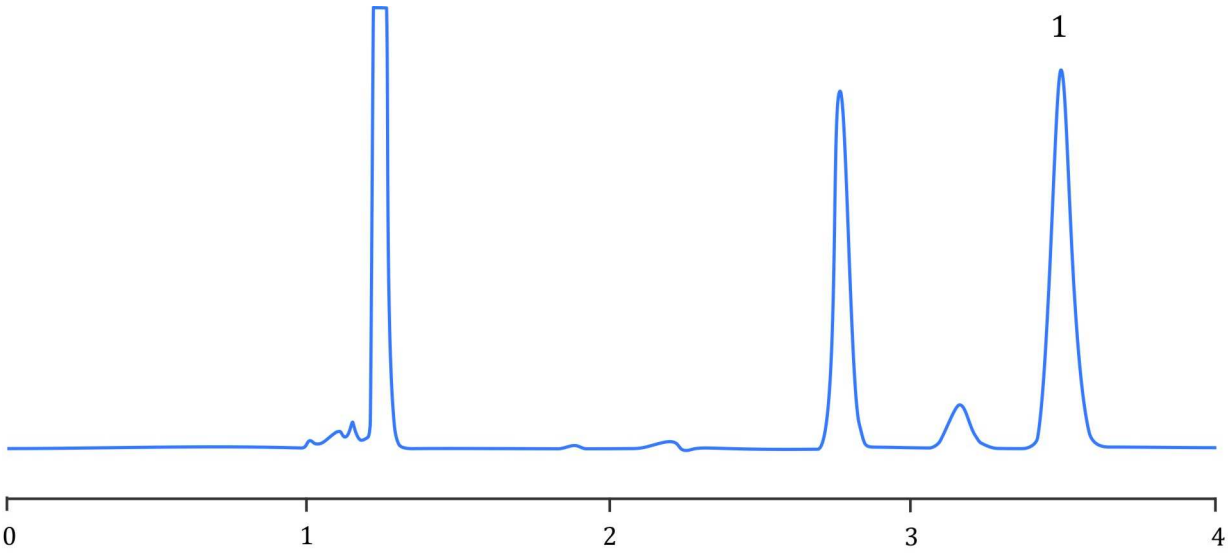


Figure 3. Specific determination of caffeine in beverage concentrate sample #2. HPLC column: Acclaim Mixed-Mode WCX-1, 250x4.6 5um.
1. *Caffeine.*

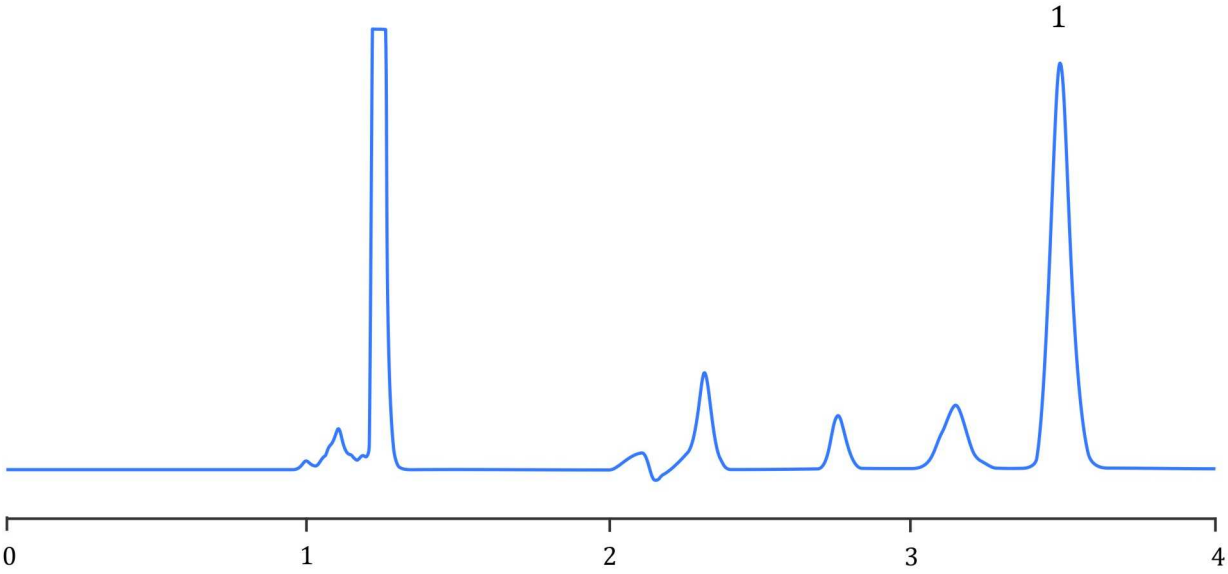


Figure 4. Specific determination of caffeine in beverage concentrate sample #3. HPLC column: Acclaim Mixed-Mode WCX-1, 250x4.6 5um.
1. *Caffeine.*

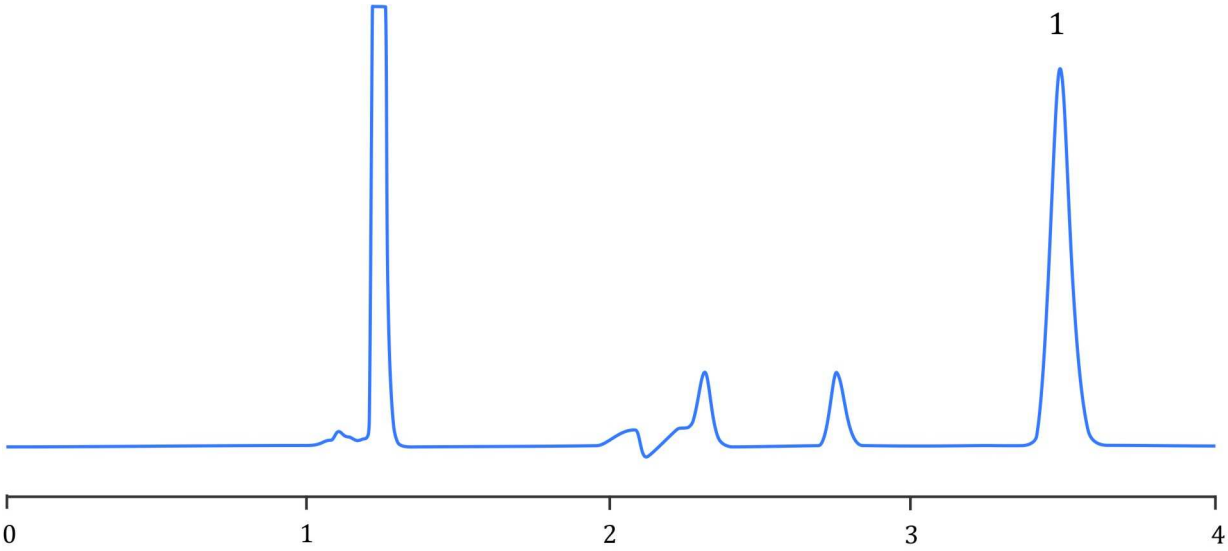


Figure 5. Specific determination of caffeine in beverage concentrate sample #4. HPLC column: Acclaim Mixed-Mode WCX-1, 250x4.6 5um.
1. *Caffeine.*

Quickstart Steps

Mobile Phase Preparation

Buffer preparation. Weight 2.875g $NH_4H_2PO_4$ in 1 L volumetric flask; transer 500 mL water and mix; bring to volume with water and mix.

Mobile Phase Preparation. Transfer 120 mL acetonitrile and 500 mL buffer in 1 L volumetric flask and mix, then bring to volume with buffer, mix, and degas.

Column Washing And Storage

The column can be washed with Acetonitrile-(25mM $NH_4H_2PO_4$) 50:50 and then with the mobile phase. The column can be stored in the mobile phase.

Column Conditioning

After washing the column should be 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 5um Acclaim Mixed-Mode WCX-1 at a flow rate 1.5 mL/min is more than 8’500, and the asymmetry factor is less than 1.2.

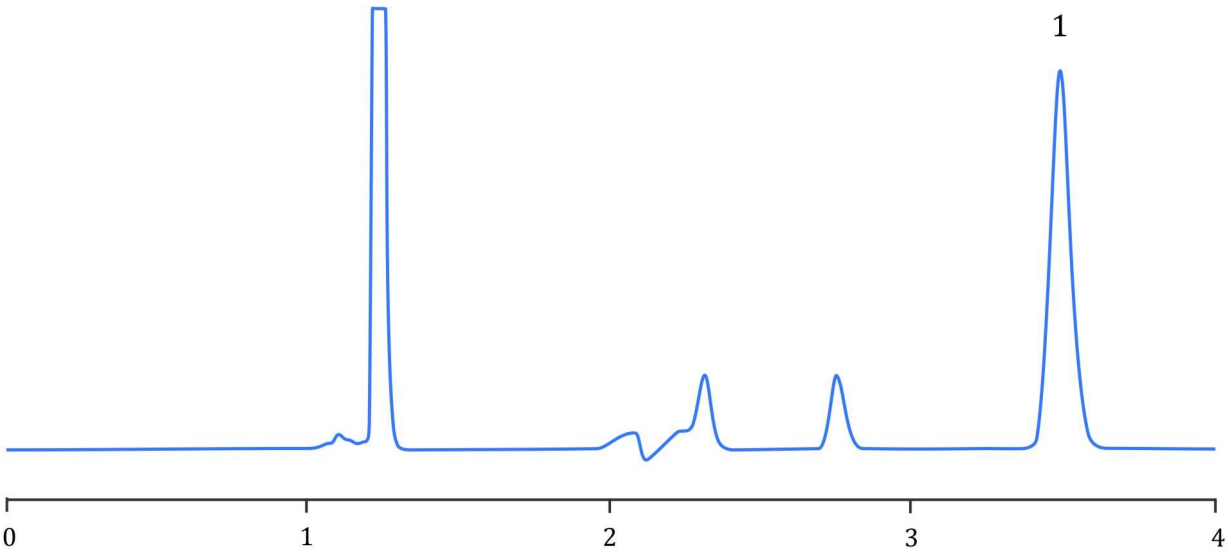


Figure 6. Specific determination of caffeine in beverage concentrate sample #4. HPLC column: Acclaim Mixed-Mode WCX-1, 250x4.6 5um.
1. *Caffeine.*

#	Name	N	A_f
1	*Caffeine*	9'600	1.1

Fine-Tuning Retention And Selectivity

The selectivity of the separation can be altered by varying the buffer concentration in the range 15-50 mM.

Retention of caffeine can be adjusted by changing acetonitrile content in the mobile phase in the range 10-15 v/v%.