Application Note

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Development and Verification of a Generic Agilent 1260 Infinity II Binary HPLC Method

Simultaneous separation and quantification of seven OTC drugs using the Agilent InfinityLab Poroshell 120 column

Authors

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Abstract

A generic, reversed-phase, binary, high-performance liquid chromatography (RP-HPLC) method was developed and verified using an Agilent 1260 Infinity II LC system for the simultaneous determination of seven pharmaceutically active ingredients including acetaminophen, dextromethorphan, doxylamine, phenylephrine, guaifenesin, caffeine, and aspirin. The separation was achieved in a five-minute runtime on an Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm column using a simple linear gradient method with two mobile phases. Mobile phase A was 0.1% trifluoroacetic acid in purified water, and mobile phase B was a mixture of acetonitrile and methanol (750:250 v:v) with 0.1% trifluoroacetic acid. The flow rate was 0.35 mL/min and the injection volumes were 5 µL. Detection was performed at 280 nm using a diode array detector. As part of the method verification, specificity, linearity, and precision parameters were verified. The proposed RP-HPLC generic method is applicable for the routine analysis of cold and cough over-the-counter (OTC) products.

Introduction

OTC drugs, readily available to customers in pharmacies and general stores, do not require medical practitioners' authorization for use. These medications come in a range of formulations, including tablets, liquid-filled capsules, and syrups, and are generally considered safe for self-administration. Numerous pharmaceutical companies create OTC drugs by combining two or more active pharmaceutical ingredients in single-dosage forms such as tablets, soft gels, and liquid syrups for ease of administration (e.g., paracetamol, also known as acetaminophen (ACE), and aspirin (ASP), also known as acetylsalicylic acid).

A review of the literature shows that HPLC is the preferred method for numerous researchers when analyzing cold and cough medicines due to widespread availability and rapid analysis time. Various multicomponent tablets, soft gels, and liquid formulations containing active components such as ACE, dextromethorphan (DEX), and caffeine (CAF) are prevalent in the market and are commonly used to address cold and cough symptoms. Consequently, many researchers have dedicated their efforts to developing methods that can simultaneously identify and quantify one or more of these active ingredients in different formulations. Different protocols have been reported using various analytical facilities, including high-performance liquid chromatography.[1–5]

The primary objective of this study was to develop a rapid and straightforward generic HPLC method using the Agilent 1260 Infinity II LC system for the simultaneous quantification of seven specified compounds. To the best of our knowledge, only one single-assay HPLC method has been reported previously for the simultaneous estimation of these seven active ingredients, with a 20-minute runtime. In light of this, a simple, fast, and cost-effective HPLC protocol was developed and validated for the simultaneous estimation of these ingredients.

Agilent superficially porous particle columns generate high efficiency at lower pressure relative to their totally porous particle column counterparts. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column. The current trend with superficially porous particles is to reduce particle size for further efficiency improvements. Higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity. As such, the Agilent InfinityLab Poroshell 120 columns were selected for this method. During method verification, the impact of flow rate and column oven temperature variations on the separation of these compounds was examined.

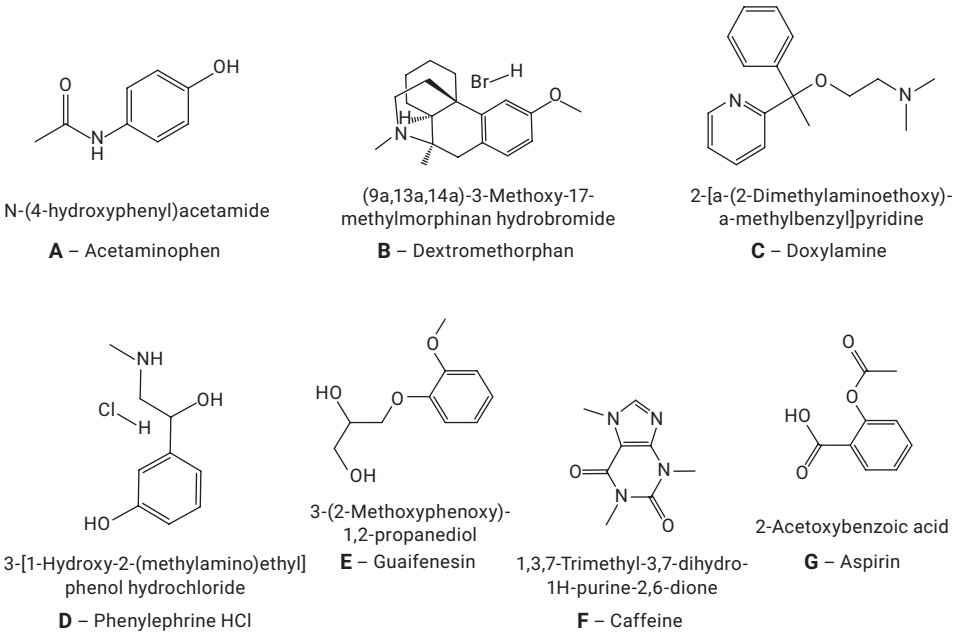


Figure 1. Chemical structures of (A) acetaminophen, (B) dextromethorphan, (C) doxylamine, (D) phenylephrine HCl, (E) guaifenesin, (F) caffeine, and (G) aspirin.

Experimental

An 1260 Infinity II LC system with Agilent OpenLab CDS software was used in this study. The system was used in the standard configuration, and details of the instrumentation are listed in Table 1. Paracetamol, caffeine, aspirin, dextromethorphan, doxylamine, phenylephrine, and guaifenesin were purchased from Sigma-Aldrich. The Poroshell 120 EC-C8 2.1 × 150 mm, 2.7 µm (part number 693775-906) column was evaluated. Trifluoracetic acid (TFA) was purchased from Sigma-Aldrich. Methanol and acetonitrile (HPLC-grade) were purchased from Honeywell (Burdick and Jackson). A Milli-Q system (Millipore) provided 18 MΩ-cm water, which was passed through a 0.2 µm filter.

Preparation of standard stock solutions

Initially, all stock solutions were prepared in methanol to obtain clear solutions, and were further diluted to a prepared standard mix solution with 0.1% of trifluoroacetic acid (mobile phase A) to achieve final concentrations of ACE (80 µg/mL), DEX (300 µg/mL), DOX (125 µg/mL), PHE (80 µg/mL), ASP (80 µg/mL), CAF (25 µg/mL) and GUA (50 µg/mL).

Results and discussion

Generally, cold and cough OTC drugs are available in different combinations and different types of formulations. The objective of this initial method development was to develop a rapid and simple analytical method to separate compounds with good peak symmetry and resolution. To optimize chromatographic conditions, method development started with a selection of buffer and mobile phase compositions.

Table 1. Instrument configuration details.

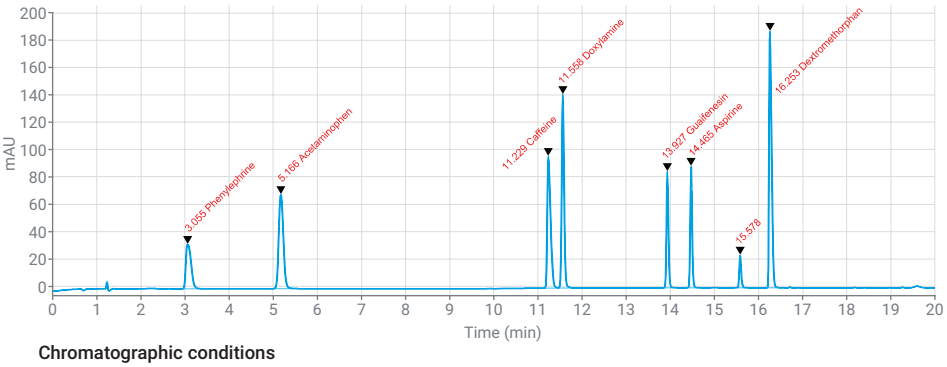
Agilent 1260 Infinity II LC System	
Agilent 1260 Infinity II Binary Pump	Agilent 4-position/10-port valve, 600 bar (p/n 5067-4279)
Agilent 1260 Infinity II Vial Sampler	– Agilent vial, screw top, amber with write-on spot, certified, 2 mL (p/n 5182-0716) – Agilent cap, screw, blue, PTFE/red silicone septa (p/n 5182-0717)
Agilent 1260 Infinity II Multicolumn Thermostat	– Agilent InfinityLab Quick Connect heat exchanger, standard – Heater and column: Agilent InfinityLab Quick Connect fitting assembly, 105 mm, 0.17 mm (p/n 5067-6166)
Agilent 1260 Infinity II Diode Array Detector	Agilent flow cell, 10 mm, 10 µL
Agilent OpenLab CDS, Version 2.6	

Experiment 1

Experiment 1 was conducted to select an LC/MS-compatible buffer with organic modifiers. For mobile phase A, 0.1% TFA in purified water was used as buffer and 100% methanol was used as mobile phase B. The Poroshell 120 EC-C8 (2.1 × 150 mm, 2.7 µm) column was selected for the separation of compounds. Figure 2 shows the chromatographic conditions. The standard mix solution was injected into the 1260 Infinity II LC system using 5 µL, and the detector response was monitored with OpenLab CDS software. All peaks (PHE, ACE, CAF, DOX, GUA, ASP, and DEX) eluted within 17 minutes of the runtime (Figure 2).

To select the wavelength, the mixed standard was injected into the diode array detector. The absorbance maxima were observed for PHE (271 nm), ACE (242 nm), CAF (203 and 271.0 nm), DOX (201 and 260.0 nm), GUA (222 and 272 nm), ASP (225 and 274 nm), and DEX (277 nm). Most of the components exhibited absorbance maxima in the range of 270 to 280 nm, and all peaks displayed a good response at 280 nm. The system suitability parameters (such as tailing factor and theoretical plates) were satisfactory, but the resolution between CAF and DOX was not adequate, and the runtime of the method was decreased.

Observation: All peaks eluted within 17 minutes. The resolution between caffeine and doxylamine is less, and the total run time of the method is more.



Chromatographic conditions

Mobile phase A:	0.1% TFA in water
Mobile phase B:	100% methanol
Column:	Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm
Injection Volume:	5 µL
Wavelength:	280 nm
Flow Rate:	0.3 mL/min
Column Temperature:	30 °C

Gradient:	Time (min)	%A	%B
	0.0	95	5
	8	85	15
	15	30	70
	18	20	80
	18.1	95	5
	20	95	5

Figure 2. Method optimization chromatogram and conditions for Experiment 1.

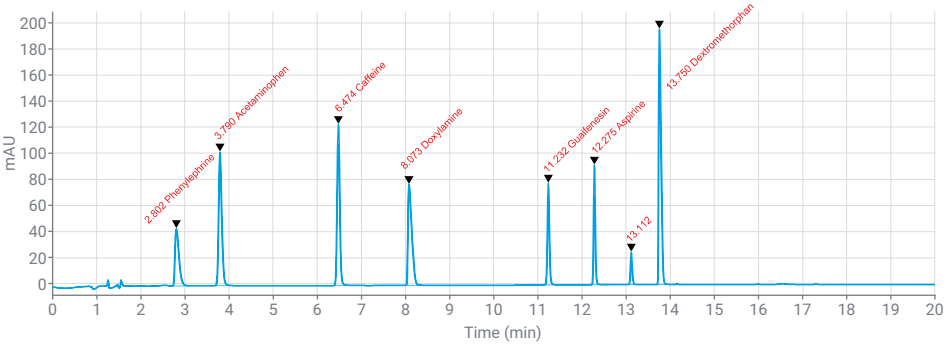
Experiment 2

Experiment 2 was conducted by exchanging mobile phase B with acetonitrile (100%), while all other chromatographic conditions remained the same. The standard mix solution was injected into the 1260 Infinity II LC system, and the chromatogram was processed in OpenLab CDS software. All peaks (PHE, ACE, CAF, DOX, GUA, ASP, and DEX) eluted within 14 minutes of the runtime (Figure 3). The system suitability parameters (tailing factor, theoretical plates, and resolution) between all peaks were satisfactory, but the runtime of the method needed to be decreased.

Experiment 3

To decrease the runtime of the method, Experiment 3 was conducted by changing the gradient program (see Figure 4) and flow rate (to 0.35 mL/min). Other chromatographic conditions remained the same as in Experiment 1. The standard mix solution was injected into the 1260 Infinity II system and the detector response was monitored with OpenLab CDS software. All peaks (PHE, ACE, CAF, DOX, GUA, ASP, and DEX) eluted within 5.2 minutes of runtime (Figure 4). System suitability parameters, like tailing factor and theoretical plates were satisfactory, but the resolution between PHE and ACE, and CAF and DOX were not adequate for the integration of peaks.

Observation: All peaks eluted within 14 minutes. The resolution between caffeine and doxylamine is improved with acetonitrile, but the total runtime of the method is more.

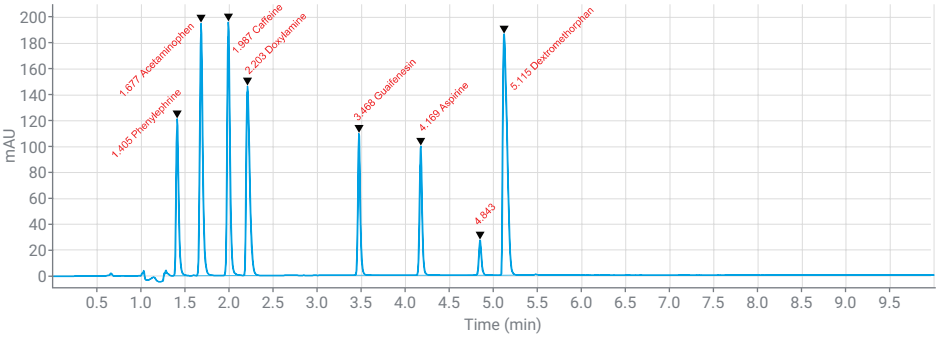


Chromatographic conditions

		Gradient:	Time (min)	%A	%B
Mobile phase A:	0.1% TFA in water		0.0	95	5
Mobile phase B:	100% acetonitrile		8	85	15
Column:	Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm		15	30	70
			18	20	80
Injection Volume:	5 µL		18.1	95	5
Wavelength:	280 nm		20	95	5
Flow Rate:	0.3 mL/min				
Column Temperature:	30 °C				

Figure 3. Method optimization chromatogram and conditions for Experiment 2.

Observation: All peaks eluted within 5.2 minutes. The resolution between phenylephrine, acetaminophen, caffeine, and doxylamine is decreased due to a more organic ratio, but the total runtime of the method is less.



Chromatographic conditions

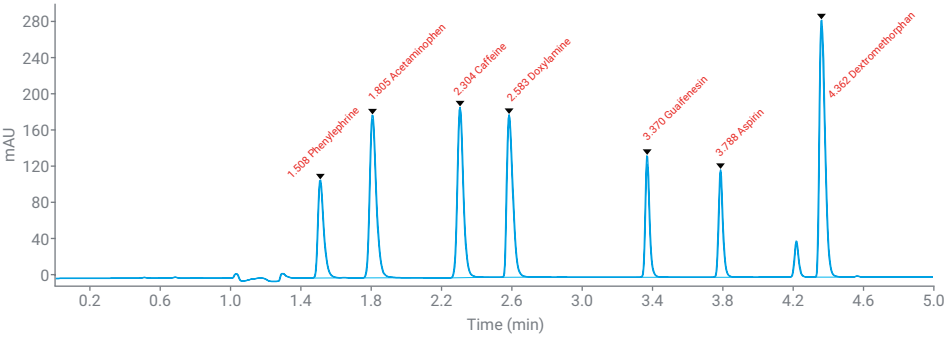
		Gradient:	Time (min)	%A	%B
Mobile phase A:	0.1% TFA in water		0.0	85	15
Mobile phase B:	100% acetonitrile		10	0	100
Column:	Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm				
Injection Volume:	5 µL				
Wavelength:	280 nm				
Flow Rate:	0.35 mL/min				
Column Temperature:	30 °C				

Figure 4. Method optimization chromatogram and conditions for Experiment 3.

Experiment 4

To increase resolution between PHE and ACE and CAF and DOX, mobile phase B was modified to include a mixture of 0.1% TFA in acetonitrile and methanol (75:25 v:v). The gradient program was also changed again (Figure 5). Other chromatographic conditions remained the same as in Experiment 1. The standard mix solution was injected into the 1260 Infinity II LC system and the detector response was monitored with OpenLab CDS software. All peaks (PHE, ACE, CAF, DOX, GUA, ASP, and DEX) eluted within five minutes of runtime (Figure 5). System suitability parameters (tailing factor, theoretical plates, and resolution between the peaks) were satisfactory. There was no interference from the diluent (mobile phase A) at the given wavelength. The proposed RP-HPLC method proved to be the superior and most rapid method for the determination of seven active ingredients in a single process. The overlay of method optimization chromatograms is shown in Figure 6, and the final LC conditions are listed in Table 2.

Observation: All peaks eluted within 5 minutes. The resolution between all peaks is increased with the combination of acetonitrile and methanol mobile phase, and the total runtime of the method is less.



Chromatographic conditions

Mobile phase A:	0.1% TFA in water
Mobile phase B:	0.1% TFA in acetonitrile:methanol (75:25 v:v)
Column:	Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm
Injection Volume:	5 µL
Wavelength:	280 nm
Flow rate:	0.35 mL/min
Column Temperature:	30 °C

Gradient:	Time (min)	%A	%B
	0.0	85	15
	5	0	100

Figure 5. Method optimization chromatogram and conditions for Experiment 4.

Table 2. Final LC method conditions.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm (p/n 693775-906)
Mobile Phase A	0.1% TFA in water
Mobile Phase B	0.1% TFA in acetonitrile:methanol (75:25 v:v)
Gradient	Time (min) %B 0 15 5 100
Post Run Time	3.0 min
Flow Rate	0.35 mL/min
Column Temperature	30 °C
Injection Volume	5 µL
Needle Wash	Flush port 3 for seconds (mobile phase B)
Diode Array Detector	280 nm Bandwidth: 4 Peak width: 10 Hz

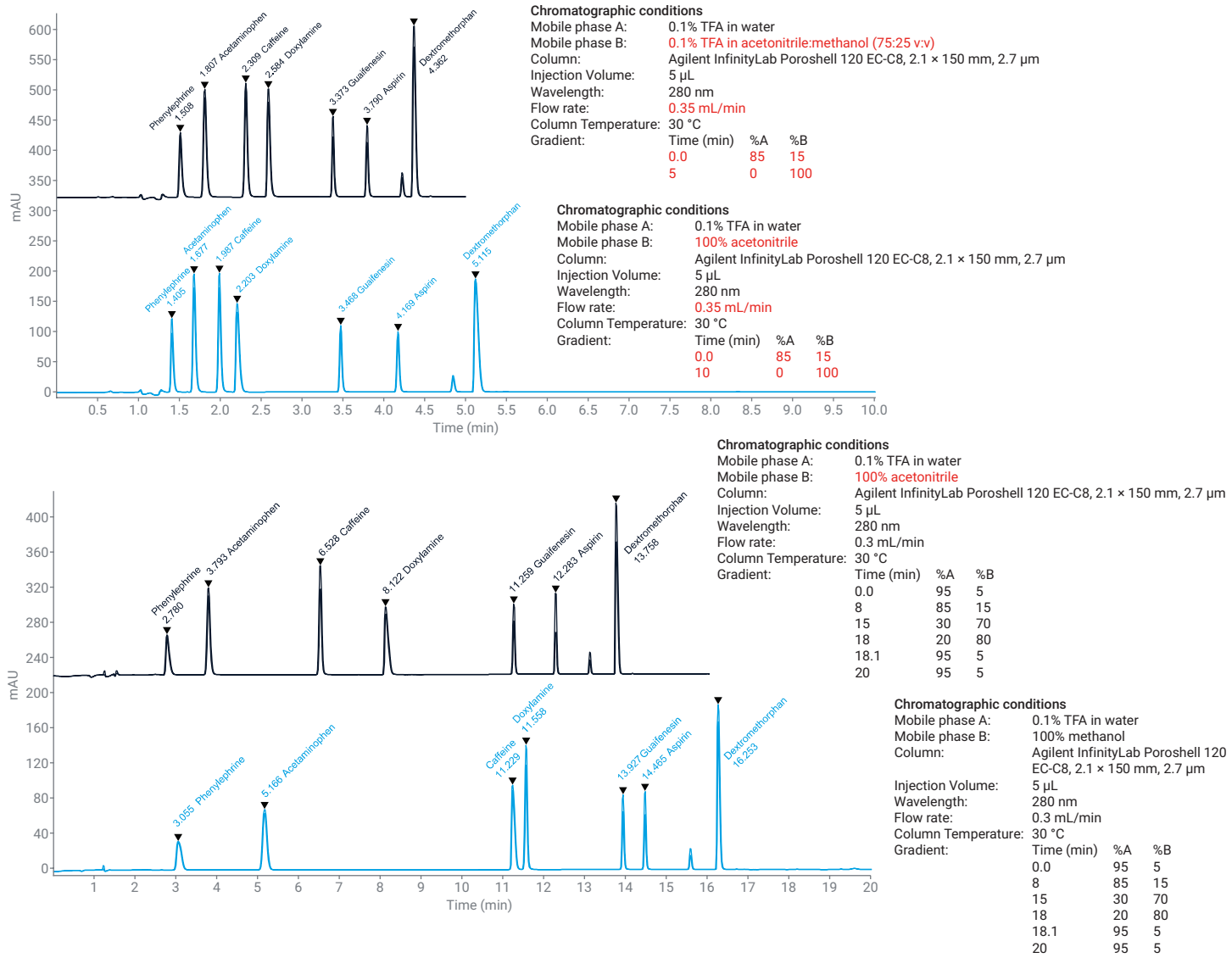


Figure 6. Method optimization overlay chromatograms.

Method verification

Linearity

As per the ICH Q2 (R1) guideline, the linearity of the method was assessed by preparing the six concentrations of the target analyte. The concentrations were as follows: 32 to 112 µg/mL ACE, 120 to 480 µg/mL DEX, 48 to 168 µg/mL DOX, 32 to 112 µg/mL PHE, 32 to 112 µg/mL ASP, 10 to 35 µg/mL CAF, and 20 to 70 µg/mL GUA. All solutions were prepared in the diluent (mobile phase A). Each concentration of standard mixture was injected into the HPLC system in triplicate, and the mean value was taken for the calculation of the calibration curve. Calibration graphs were drawn by plotting peak area versus concentration of standard drugs. The regression coefficient value was R^2 = 0.9999, obtained for all species. This indicates an acceptable degree of linearity. Linearity chromatograms are shown in Figure 7, and calibration curves are shown in Figure 8.

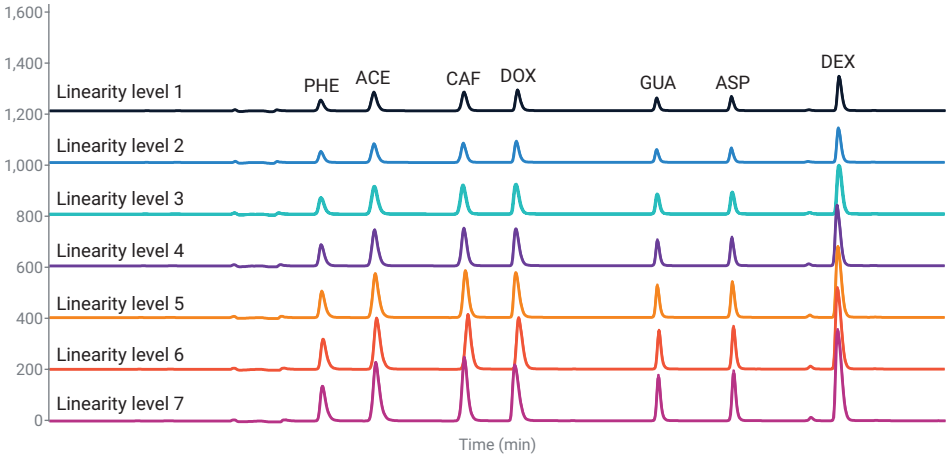


Figure 7. Linearity standard chromatograms. The linearity range is from 30 to 140% of the target concentration. The correlation coefficient for all compounds is > 0.999.

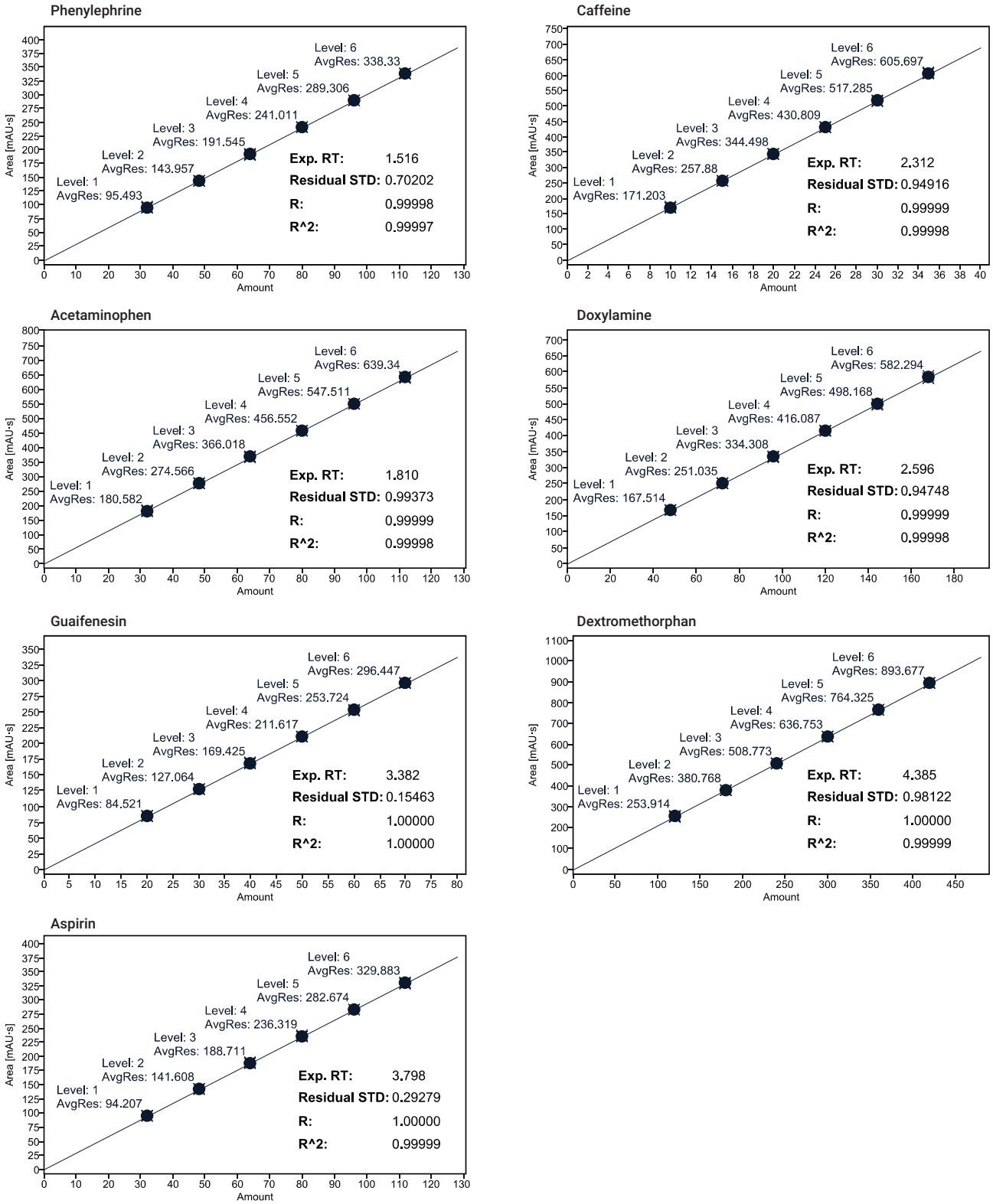


Figure 8. Linearity calibration curves. The linearity range is from 30 to 140% of the target concentration. The correlation coefficient for all compounds is > 0.999.

Specificity

The specificity of the method was proven by injecting with a blank (mobile phase A). There was no interference with the retention times of analyte peaks. The optimized method was able to separate the diluent peaks from the retention times of the analytes ACE, DEX, DOX, ASP, PHE, CAF, and GUA.

Precision

Method precision was performed by preparing six individual samples from the homogenous mixture of stock solution, including ACE (80 µg/mL), DEX (300 µg/mL), DOX (120 µg/mL), ASP (80 µg/mL), PHE (80 µg/mL), CAF (25 µg/mL) and GUA (50 µg/mL). Precision of the method was determined based on the %RSD of the six individual sample results. The %RSD values at the 100% concentration level were found to be below 2.0%. Precision overlay chromatograms are displayed in Figure 9.

Robustness

To assess the sturdiness of the optimized method, the chromatographic conditions were deliberately altered and injected with the standard mix solution into HPLC. The flow rate of the mobile phase was changed to 0.30 and 0.40 mL/min, and the column temperature was changed to 25 to 35 °C. One selected parameter was varied in each analysis, while the other conditions were kept constant. There were no significant changes in the system suitability results. The robustness overlay chromatogram is shown in Figure 10.

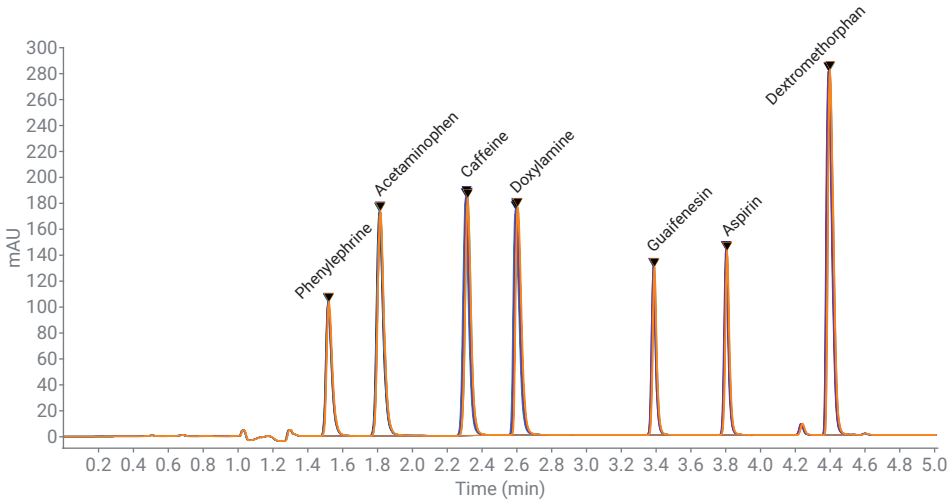
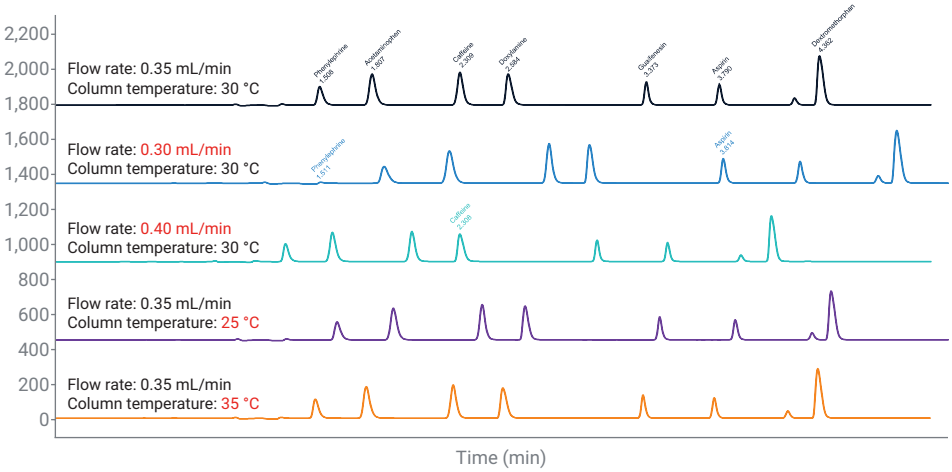


Figure 9. Precision overlay of mixed standard chromatograms. The five replicate injections of standard mix sample retention time and peak area %RSD is less than 0.2%.



Chromatographic conditions

Mobile Phase A:	0.1% TFA in water		
Mobile Phase B:	0.1% TFA in acetonitrile:methanol (75:25 v:v)		
Column:	Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm		
Injection Volume:	5 µL		
Wavelength:	280 nm		
Gradient:	Time (min)	%A	%B
	0.0	85	15
	5	0	100

Figure 10. Robustness chromatograms. With the flow rate of 0.30 mL/minute and a 25 °C column temperature, the resolution between the peaks is not affected, but the runtime of the method is increased. At a flow rate of 0.40 mL per minute and a 30 °C column temperature, the resolution between the peaks is not affected, but the runtime of the method is decreased.

Conclusion

A fast and low-cost gradient method for the analysis of seven OTC active ingredients is presented. The method was demonstrated on an Agilent 1260 Infinity II LC system and run at approximately 350 bar (5,365 psi) at 35 °C. The chromatographic run time was approximately five minutes. The flow rate of the method was 0.35 mL/min, and the consumption of mobile phase to run the sequence was very little (1.75 mL per injection).

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