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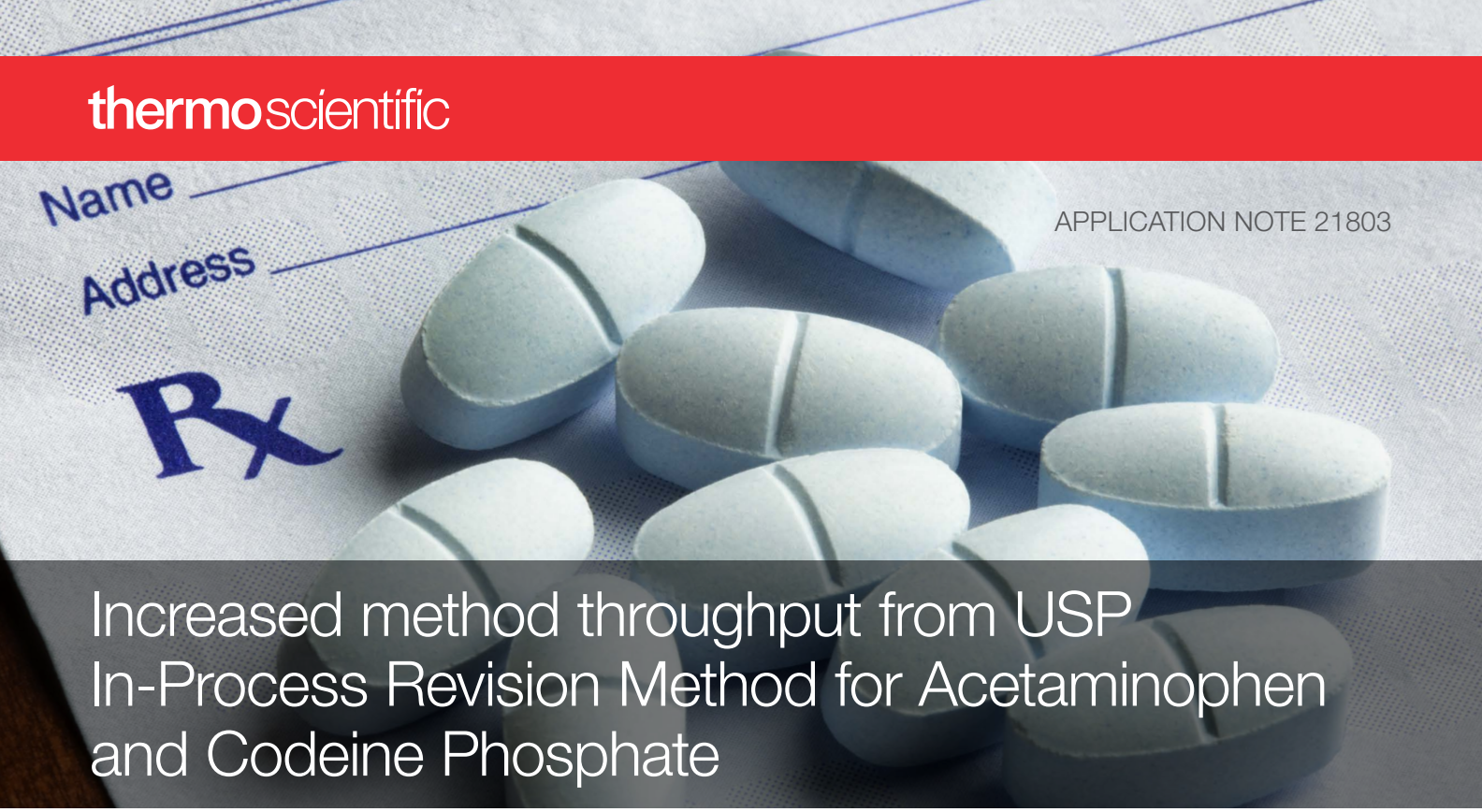

APPLICATION NOTE 21803

Increased method throughput from USP In-Process Revision Method for Acetaminophen and Codeine Phosphate

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Keywords
Pharmaceutical, drug substance, QA/QC, method speedup, Vanquish Horizon UHPLC, Accucore XR C18 column, Hypersil GOLD column, USP Acetaminophen, USP Codeine

Application benefits

- 88% reduction in analysis time resulting in a higher throughput workflow
- 95% reduction in mobile phase usage resulting in high cost savings
- Simple transfer from existing column formats to more modern formats
- High reproducibility in retention time for confidence in analytical results

Goal

To demonstrate the development of a rapid method for the assay of acetaminophen and codeine on a Thermo Scientific™ Accucore XR™ C18 column using a Thermo Scientific™ Vanquish™ Horizon UHPLC system. Examine the use of USP criteria to increase method throughput and reduce solvent use and cost per sample. Further explore these parameters beyond USP guidance to fully optimize methods while key method criteria such as resolution and precision are met.

Introduction

The proposed monograph USP 43(5) In-Process Revision: Acetaminophen and Codeine Phosphate describes an isocratic HPLC method for the separation of a solution mixture of acetaminophen and codeine phosphate.

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The monograph uses a legacy 250 × 4.6 mm i.d., 5 µm L1 type column. For the column length to be scaled down, the ratio between particle size and new column length must be maintained.[1] The 100 × 2.1 mm i.d., 1.5 µm Accucore XR column satisfies these criteria, and can easily be substituted with appropriately scaled conditions. A shorter 50 mm length Accucore XR column was also assessed for additional time and cost savings. The system suitability requirements for the assay require the analyst to confirm a minimum resolution of 2.0 and reproducibility of less than 2% RSD are achieved prior to analysis.

Here we demonstrate how a routine assay can be improved to enhance sample throughput and reduce cost, based on shorter analysis time and lower solvent consumption.

Experimental

Consumables and apparatus

Based on Core Enhanced Technology™ using solid core particles, Accucore XR/Accucore UHPLC columns allow users of conventional HPLC methods to enjoy performance beyond that of columns packed with 5 µm, 3 µm or even sub-2 µm fully porous particles on a UHPLC system. High separation efficiencies provide increased peak resolution. An ultra-stable packed bed results in exceptionally robust columns that demonstrate excellent retention and response reproducibility.

- Thermo Scientific™ Hypersil GOLD™, 250 × 4.6 mm, 5 µm column (P/N 25005-254630)
- Thermo Scientific Accucore XR C18, 100 × 2.1 mm, 1.5 µm column (P/N 24101-102130)
- Thermo Scientific Accucore XR C18, 50 × 2.1 mm, 1.5 µm column (P/N 24101-052130)
- LC-MS grade 18 MΩ water from Thermo Scientific™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ Potassium phosphate monobasic (P/N 10035810)
- Fisher Scientific™ Trimethylamine (P/N O4885-1)
- Fisher Scientific™ HPLC grade methanol (P/N M/4056/17)
- Fisher Scientific™ Orthophosphoric acid (P/N O/0500/PB08)
- Thermo Scientific™ 2 mL screw thread vial and cap kit (P/N 60180-VT400)

Standards

USP grade standards were purchased from a reputable supplier.

Instrumentation

The Vanquish Horizon UHPLC system has the benefit of SmartInject technology and improvements in injection system hardware synchronization. This results in unrivalled retention time precision, providing the user with greater data confidence during method development.

The Vanquish Horizon system utilizes Thermo Scientific™ LightPipe™ flow cell technology designed for the diode array detector (DAD), which provides the user with increased sensitivity for analytes due to the long light path, and minimum peak dispersion due to small internal volume.

Vanquish Horizon UHPLC system consisting of the following:

- System Base Vanquish Horizon (P/N VH-S01-A)
- Binary Pump H (P/N VH-P10-A)
- Split Sampler HT (P/N VH-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Active Pre-heater (P/N 6732.0110)
- MS Connection Kit Vanquish (P/N 6720.0405)
- Thermo Scientific™ Virtuoso™ vial identification system (P/N 60180-VT-100)

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software version 7.2 SR5

Sample preparation

Acetaminophen was prepared at 0.3 mg/mL combined with 0.3*J* mg/mL (*J* being the ratio of the labeled amount, in mg) of codeine phosphate in initial mobile phase conditions.

Vial labeling was supported by the Virtuoso vial identification system.

HPLC conditions

A Hypersil GOLD column was configured on the Vanquish Horizon UHPLC system and data obtained, using the existing USP method,[2] to provide a starting point for further method development. HPLC conditions are described in Table 1.

Table 1. Method conditions for USP Monograph: Acetaminophen and Codeine

	Initial USP HPLC method	Final UHPLC method	Extra fast UHPLC method
Analytical column	Hypersil GOLD, 250 × 4.6 mm, 5 µm	Accucore XR C18, 100 × 2.1 mm, 1.5 µm	Accucore XR C18, 50 × 2.1 mm, 1.5 µm
Solution A	2.04 g of monobasic potassium phosphate in 950 mL, 2 mL of trimethylamine, adjust to pH 2.35 using phosphoric acid and dilute to water to 1 L		
Mobile phase	Methanol and Solution A (8:92)		
Flow rate (mL/min)	1.5	0.7	0.85
Column temperature	25 °C, still air, active pre-heating	30 °C, still air, active pre-heating	30 °C, still air, active pre-heating
Injection volume (µL)	30	2	2
Mixer	50 µL capillary + 350 µL static in combination	50 µL capillary + 350 µL static in combination	50 µL capillary + 350 µL static in combination
UV detection (nm)	214	214	214

A transfer column size of 100 × 2.1 mm was chosen to maintain length to particle size ratio of the original column. For isocratic methods, USP guidance allows changes in column internal diameter, providing that the flow rate is scaled.[3] Recent updates also take particle size into consideration.

The modified flow rate is calculated using the following formula.

$$F_2 = F_1 \times \left(\frac{dc_2^2 \times dp_1}{dc_1^2 \times dp_2} \right)$$

Equation 1. Where F is the flow rate (mL/min), dc is the column diameter, and dp is the particle diameter. Subscripts 1 and 2 relate to the original and modified methods, respectively.

Results and discussion

Method transfer to 100 mm column length

Converting from a 4.6 mm diameter fully porous column at 1.5 mL/min to a 2.1 mm diameter solid core column provides a scaled flow rate of 0.98 mL/min according to Equation 1; however, running sub-2 µm columns at high flow rates can result in high system pressure. Alternative flow rates may be used within ±50% and so far as not to change the efficiency (N) of the original separation by -25% or +50%. Equivalent efficiency was achieved at 0.7 mL/min on a shorter 100 mm length column (Figure 1 and Table 2). The total cycle time of the assay was reduced from 60 minutes to 7 minutes, while maintaining critical criteria with regards to resolution of the critical pair and repeatability (Table 2). Resolution of acetaminophen and codeine phosphate must be no less than 2.0, and the retention time reproducibility over a number of injections must not exceed 2% RSD (n=6) for acetaminophen and 3% RSD (n=6) for codeine.

While maintaining the efficiency of the original separation, a speed up method was developed to further reduce the time of this assay beyond USP guidance. The use of a 50 mm length column was assessed to match the chromatographic performance. Fast separation while maintaining the required resolution was achieved at 0.85 mL/min. The analysis time was reduced by 93% and the mobile phase usage by 96%. The separation time for both products was less than 1 minute; total cycle time can be reduced as appropriate.

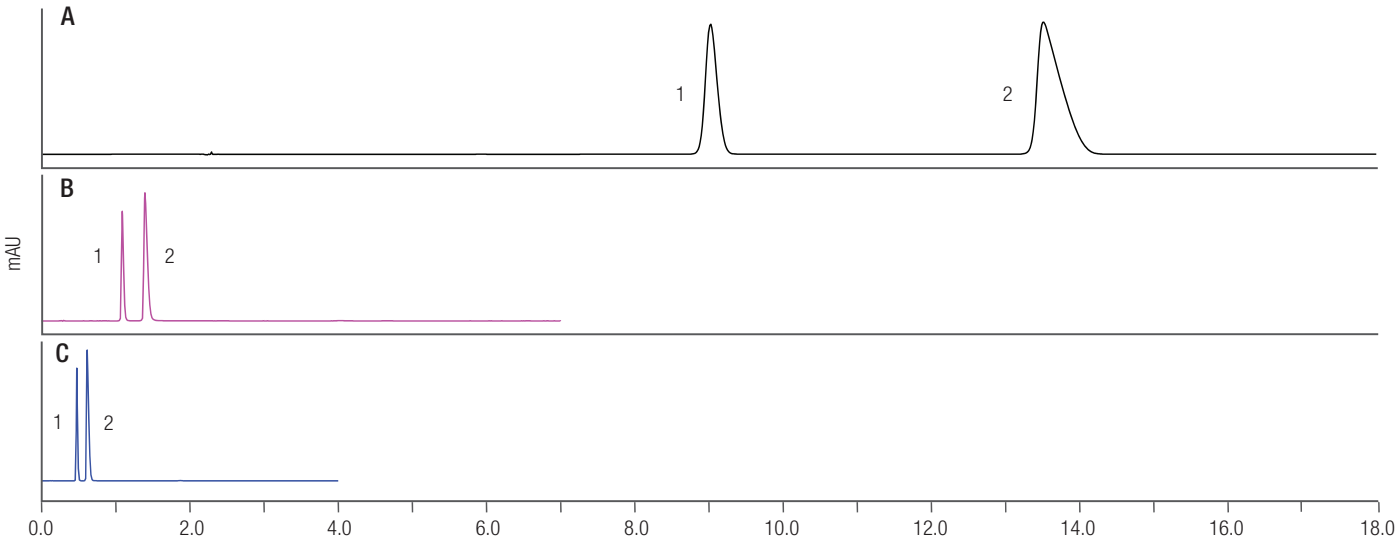

Figure 1. Main chromatogram: Comparison of run times for acetaminophen (1) peak and codeine phosphate (2) peak. A: Hypersil GOLD 250 × 4.6 mm, 5 µm, 1.5 mL/min, B: Accucore XR 100 × 2.1 mm, 1.5 µm, 0.7 mL/min, C: Accucore XR 50 × 2.1 mm, 1.5 µm, 0.85 mL/min.

Table 2. Effect of different columns on efficiency, retention time, and resolution of acetaminophen and codeine.

Column	RT Acetamino-phen (min)	RT Codeine (min)	System Pressure (bar)	Flow rate (mL/min)	Efficiency (N) Codeine peak	Change in N (%)	Resolution	Injection volume (µL)
Hypersil GOLD 250 × 4.6 mm 5 µm	9.80	14.90	297	1.5	30094	-	10.75	30
Accucore XR 100 × 2.1 mm 1.5 µm	1.09	1.40	1335	0.7	39250	+30%	4.55	2
Accucore XR 50 × 2.1 mm 1.5 µm	0.48	0.62	935	0.85	41080	+37%	3.49	2

Figure 1 compares the original (A) and modified method (B) and demonstrates:

- Faster retention time: 14.9 minutes to 1.4 minutes retention time
- Significant reduction in peak width 0.37 minutes to 0.05 minutes measured at 50% peak height (codeine)
- Maintains high resolution above 2.0

This in turn can also improve the response of the impurities; as peak widths narrow, the peak heights increase and smaller concentrations of impurities can be detected.The use of a shorter column outside USP criteria demonstrates quicker separation time (0.62 minutes retention time) and a narrow peak width (0.03).

Table 2. Assay criteria and mobile phase, sample, and time savings

	Criteria	Original column 250 × 4.6 mm, 5 µm	Transfer column 100 × 2.1 mm, 1.5 µm	Transfer column 50 x 2.1 mm, 1.5 µm (0.85 mL/min)
Mobile phase savings	-	-	95% reduction	96% reduction
Sample savings	-	-	93% reduction	93% reduction
Time savings	-	-	88% reduction	93% reduction
Resolution	NLT 2	10.74	4.55	3.49
%RSD of retention time acetaminophen	NMT 2	0.8	0.06	0.2
%RSD of retention time codeine	NMT 3	0.8	0.08	0.2

NLT – No less than
NMT – No more than

Conclusions

An application has been developed to improve the throughput and cost effectiveness of the assay of the In-process Revision for Acetaminophen and Codeine Phosphate.[2] The assay was assessed with the new Accucore XR 1.5 µm UHPLC (100 mm) column to improve speed and sensitivity, allowing a reduction in sample and mobile phase use along with reduction in analysis time, while maintaining USP scaling criteria.

When compared to the original USP method, this application demonstrates the following:

- Up to 88% reduction in analysis time resulting in a higher throughput workflow
- Up to 95% reduction in mobile phase usage resulting in high cost savings
- Simple transfer from existing column formats to more modern formats
- High reproducibility in retention time for confidence in analytical results

If the chromatographer wishes to enhance the method beyond USP guidance, the speed of the separation can be further improved by using the new Accucore XR 1.5 µm UHPLC (50 mm) column at 0.85 mL/min. This maintains the minimum resolution and reproducibility criteria while further improving analysis times.

References

1. USP General Information Chapter <621>
2. USP 43(5) In-Process Revision: Acetaminophen and Codeine Phosphate
3. USP General Information <1225> Validation of Compendial Procedures
4. Chromatography Accucore HPLC columns technical guide https://tools.thermofisher.com/content/sfs/brochures/TG-20666-Accucore-HPLC-Columns-TG20666-EN.pdf

Find out more at **thermofisher.com/accucore**

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