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A rapid fenoprofen USP assay method

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Application benefits

- Five-fold increase in method throughput compared to original method (fifty samples/hour)
- Associated 10-fold reduction in cost per sample through reduced mobile phase consumption and waste generation
- Additional reduced method complexity from easy-to-prepare mobile phase

Goal

To demonstrate practical approaches that can be used to significantly improve throughput of the fenoprofen USP assay monograph keeping to the spirit of USP-NF Chapter <621> guidelines while maintaining USP quality acceptance criteria. To then take this optimized assay monograph and reduce analysis time even further.

Introduction

Most existing pharmacopeial methods were established prior to the turn of the century and are configured for large particle size (≥5 µm) and long columns (>200 mm). As a consequence, the method times are long and the mobile phase consumption is high compared to modern equivalents.

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Since 2014 the USP-NF Chapter <621> has allowed adjustments to these methods, within certain criteria, to benefit from the increased performance of smaller particle size products. For isocratic methods, the main changes relate to particle size, column length, and flow rate.

- Particle size and column length can be changed but must maintain a constant length to particle size ratio or in a -25% to +50% range.

- Flow rate can be adjusted using a defined formula to take into account changes to particle size and column diameter, or ±50%.

One of the key goals for the chromatographer is to achieve a consistent, reproducible separation. The selection of a highly reproducible HPLC column is essential if this goal is to be attained. Based on solid core technology, Accucore HPLC columns allow users of conventional HPLC methods to enjoy performance beyond that of columns packed with 5 µm or even 3 µm fully porous particles. High separation efficiencies provide increased peak resolution. An ultra-stable packed bed results in exceptionally robust columns that demonstrate excellent retention and response reproducibility. Accucore columns are available in a wide range of chemistries and particle sizes making them an ideal choice for this type of work.[1]

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

The Vanquish Flex Quaternary system also 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 fiber optics and total internal UV light reflection, and minimum peak dispersion due to small internal volume.

The fenoprofen method was selected due to the widespread use by generic pharmaceutical manufacturing and the potential for significant improvement. This will be demonstrated by direct comparison of legacy and modern column formats firstly within the USP guidelines for equivalence and then beyond those guidelines to demonstrate the savings that can be applied to reduce operating costs through mobile phase and waste reduction.

Experimental

Consumables and apparatus

- Thermo Scientific™ Hypersil GOLD™ C8, 150 × 4.6 mm, 5 µm column (P/N 25205-154630)
- Accucore C8 XL, 150 × 4.6 mm, 4 µm column (P/N 74204-154630)
- Accucore C8, 100 × 4.6 mm, 2.6 µm column (P/N 17226-104630)
- Accucore C8, 100 × 2.1 mm, 2.6 µm column (P/N 17226-104630)
- Accucore C8, 50 x 2.1 mm, 2.6 µm column (P/N 17226-054630)
- LC-MS grade 18 MΩ water from Thermo Scientific™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ LC-MS grade acetonitrile (P/N A955-212)
- Fisher Scientific™ Optima™ LC-MS grade formic acid (P/N A117-50)
- Fisher Scientific certified AR, orthophosphoric acid (P/N O/0500/PB08)
- Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit (P/N 60180-VT400)

Standards

The two compounds specified in the USP chromatographic purity method were fenoprofen (1) and gemfibrozil (2). The number relates to their elution order and peak labelling on subsequent chromatograms. These were purchased from a reputable supplier.

Instrumentation

Analyses were performed using a Vanquish Flex Quaternary UHPLC system consisting of:

- Quaternary Pump F (P/N VF-P20-A)
- System Base Vanquish Flex (P/N VF-S01-A)
- Split Sampler FT (P/N VF-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Active Pre-heater (P/N 6732.0110)
- Diode Array Detector HL (P/N VH-D10-A)
- LightPipe Flow Cell, 10 mm (P/N 6083.0100)

- Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 60180-VT-100)

Software

Thermo Scientific™ Chromeleon™ 7.2 SR4

Sample preparation

Solutions of the compounds were prepared by dissolving a known weighed amount in methanol/water (70:30 v/v) to produce 1 mg/mL primary solutions. A mixed standard solution and individual working standards were used to assess method development and were prepared in methanol/water (70:30 v/v) at a concentration of 0.1 mg/mL.

Sample handling

Vial labeling was supported by the Virtuoso Vial Identification System.

HPLC conditions

Various columns and conditions were explored as part of the method development described below. These values represent the initial and final method.

Initial USP HPLC method

HPLC column:	Hypersil GOLD, 5 µm HPLC column, 150 mm × 4.0 mm
Mobile phase A:	Water/phosphoric acid (92:8 v/v)
Mobile phase B:	Acetonitrile
Flow rate:	2.0 mL/min
Column temperature:	30 °C, still air, no eluent pre-heating
Injection volume:	5 µL
On-pump mixing:	50% A : 50% B
Mixer:	50 µL capillary + 350 µL static in combination UV detection at 272 nm

Final UHPLC method

UHPLC column:	Accucore, 2.6 µm HPLC column, 50 mm × 2.1 mm
Mobile phase A:	Water/phosphoric acid (92:8 v/v)
Mobile phase B:	Acetonitrile
Flow rate:	1.0 mL/min
Column temperature:	45 °C, still air with eluent pre-heating
Injection volume:	1 µL
On-pump mixing:	66% A : 34% B
Mixer:	50 µL capillary + 350 µL static in combination UV detection at 214 nm

Results and discussion

A Hypersil GOLD column was configured on the Vanquish Flex Quaternary system and data obtained, using the existing USP method, to provide a starting point for further method development.

Initial development focused on the column length and particle size. The initial analysis was repeated with an Accucore XL C8, 150 × 4.6 mm, 4 µm column as the direct equivalent and also on an Accucore C8, 100 × 4.6 mm, 2.6 µm column, selected to maintain the length to particle size ratio within the +50% / -25% limits as stated in the USP <621> guidance.

Figure 1 shows the chromatograms obtained with these three columns. There is a slight change in selectivity and hydrophobicity moving from the Hypersil GOLD column to the Accucore column families due to the differences in stationary phase bonding (surface area and carbon load). However, the USP criteria of peak resolution exceeding 2 is still attained and all the columns have the USP L1 designation. The core-shell particles provide narrow peaks with increased peak height due to their narrow particle size distribution and efficient packing. A reduced column length allows the method run time to be decreased from 6 to 4.5 minutes.

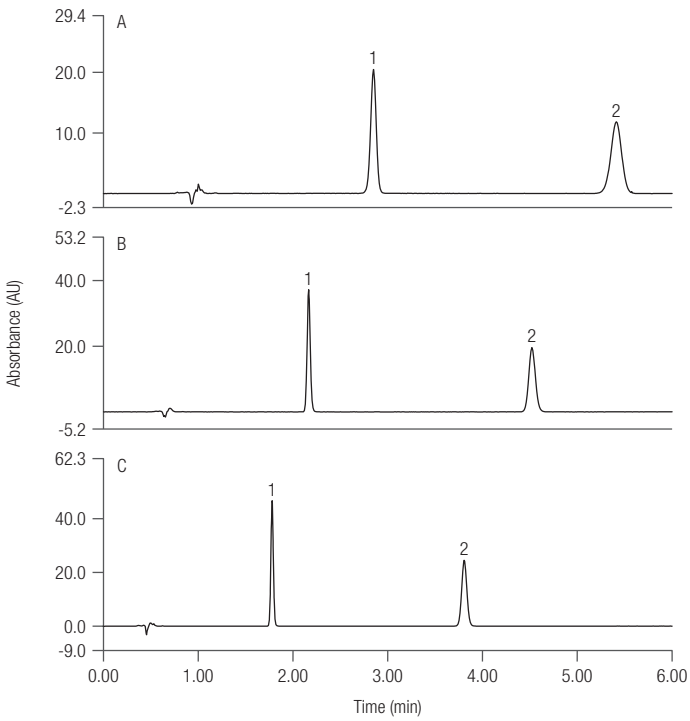

Figure 1. Separation of fenoprofen and gemfibrozil on three different columns A) Hypersil GOLD, 150 mm × 4.0 mm, 5 µm, B) Accucore XL, C8 150 × 4.6 mm, 4 µm, C) Accucore C8, 100 × 4.6 mm, 2.6 µm.

For isocratic methods, USP guidance allows changes in column internal diameter, providing that the flow rate is scaled. Recent updates also take particle size into consideration.

$$F_2 = F_1 \times (dc_2^2 / dc_1^2) \times (dp_1 / dp_2)$$

Where F is the flow rate; dc relates to the diameter of the column and dp relates to the diameter of the particle. Subscripts 1 and 2 relate to the original and modified methods, respectively.

Converting from a 4.6 mm diameter column at 2 mL/min to a 2.1 mm diameter column provides a scaled flow rate of 0.417 mL/min. The resulting chromatogram is shown in Figure 2.

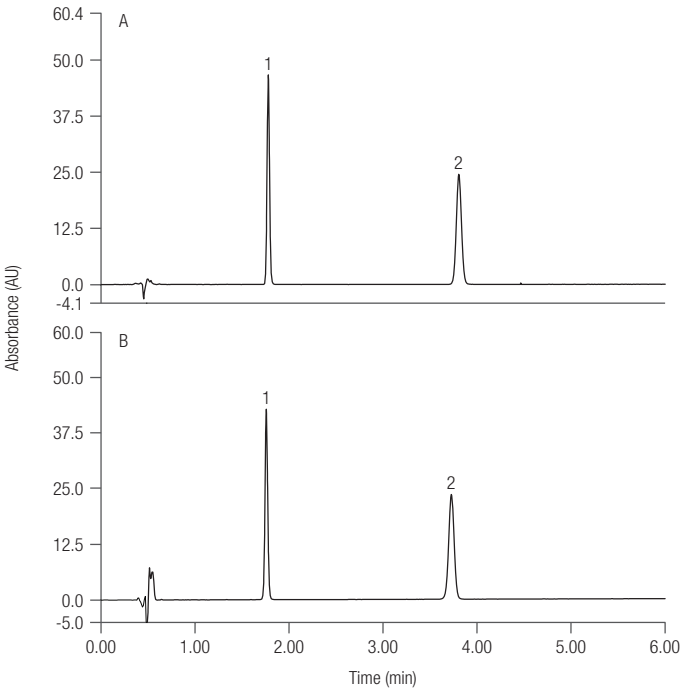

Figure 2. Separation achieved on Accucore 100 mm columns of two different diameters, flow rate scaled.
A) Accucore C8, 100 × 4.6 mm, 2.6 µm, 2.0 mL/min
B) Accucore C8, 100 × 2.1 mm, 2.6 µm, 0.417 mL/min

The separation is achieved within the same time frame but with a seven-fold reduction in mobile phase consumption when compared to the original method on the Hypersil GOLD column.

The final aspect of adjustment that lies within the USP guidance is to increase the flow rate. The guidance allows for an increase of ± 50% or until a 20% drop in column efficiency.

The 100 × 2.1 mm column was tested at flow rates from 400 to 1000 µL/min. Representative chromatograms can be seen in Figure 3 and the column plate values in Figure 4.

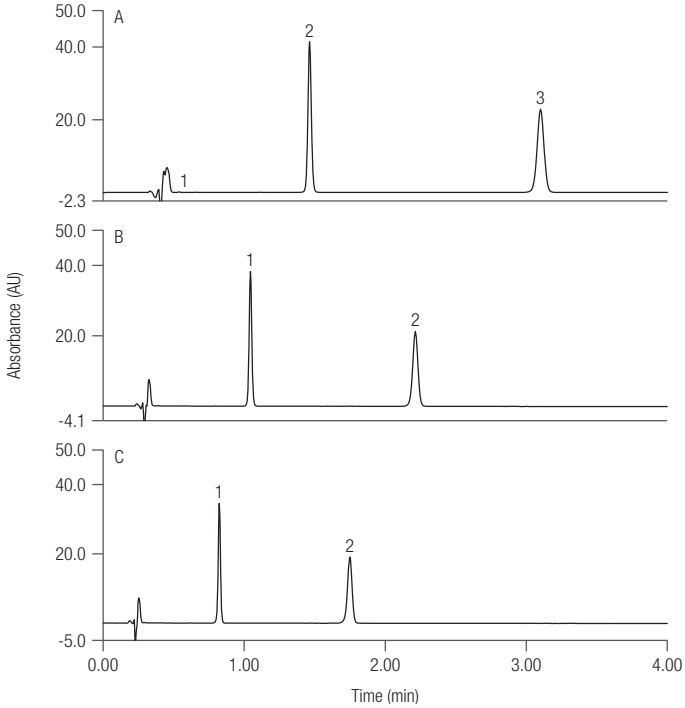

Figure 3. Representative chromatograms observed when increasing the method flow rate on an Accucore C8, 100 × 2.1 mm, 2.6 µm column.
A) 0.5 mL/min B) 0.7 mL/min C) 0.9 mL/min

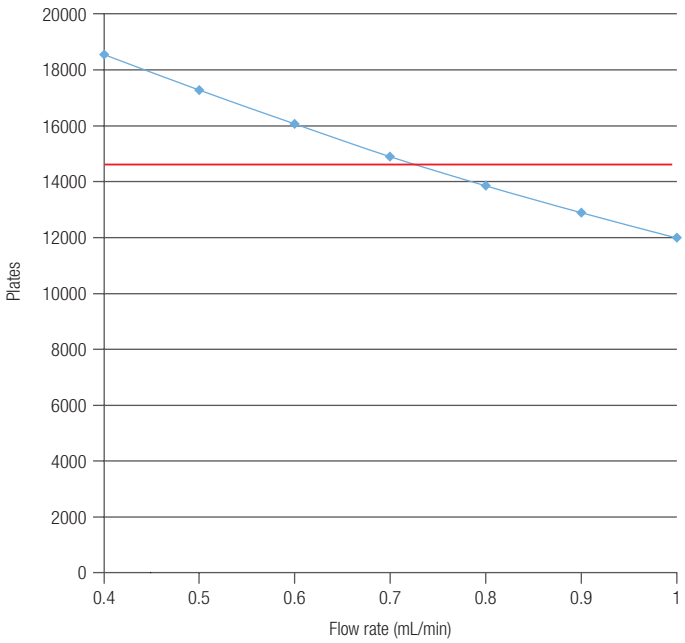

Figure 4. Plate count values when increasing the method flow rate on an Accucore C8, 100 × 2.1 mm, 2.6 µm column. The red line represents the 80% value of the plate count at 0.4 mL/min and the limit for USP equivalence.

Using the Accucore C8, 100 × 2.1 mm, 2.6 µm column at 0.7 mL/min represents the limit to which the USP approved adjustments can be applied. The speed of the assay has been reduced from 6 minutes to 2.5 minutes and the solvent consumption per assay reduced from 12 mL to 1.75 mL.

It is possible to extend the method beyond the conservative USP equivalence guidance and still produce an assay that meets the resolution requirements, and this was explored further using the flow rate of 1.0 mL/min and investigating the effect of column temperature. The mixed standard was analyzed at column temperatures of 25, 30, 35, 40, and 45 °C. The expected shift to earlier retention time with narrowing of the peak width was observed. The resolution value remained greater than 20 across all the experiments, again still above the USP limit (Figure 5).

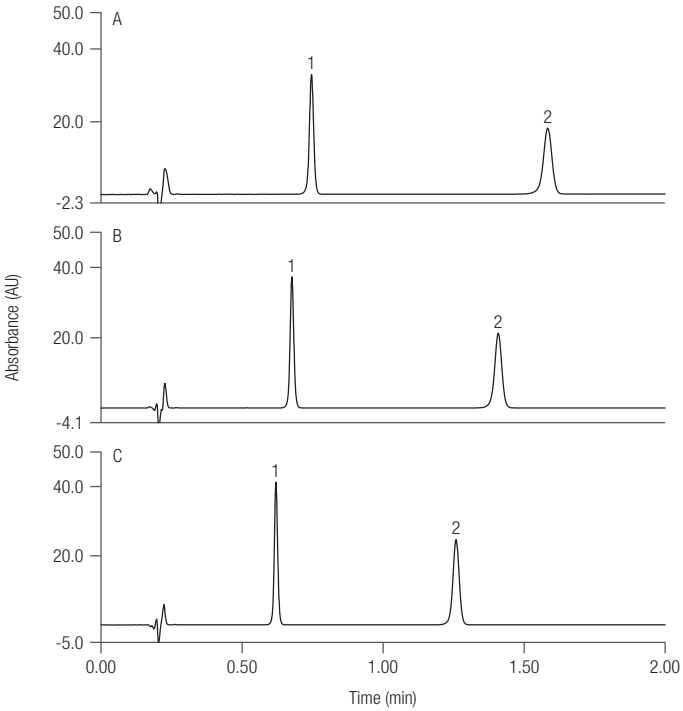

Figure 5. Chromatogram showing standards mixture analyzed on an Accucore C8, 50 × 2.1 mm column at three different temperatures A) 25 °C, B) 35 °C, and C) 45 °C.

By applying the developed method, the method time has been decreased further from 6 minutes to 1.5 minutes and the consumption of mobile phase (and waste) per assay has also been further reduced, from 12 mL to 1.5 mL, thus contributing a savings in both assay cost and an increase in throughput (Figure 6).

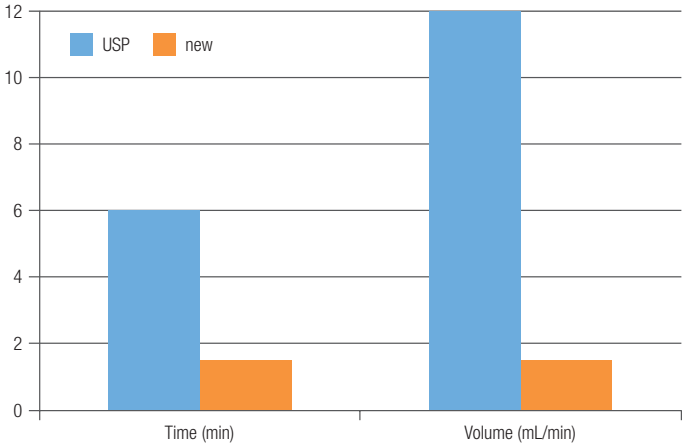

Figure 6. Indicative savings in time and mobile phase volume/waste between the original USP and the improved method.

A comparison of the key stages in this method development is shown in Figure 7.

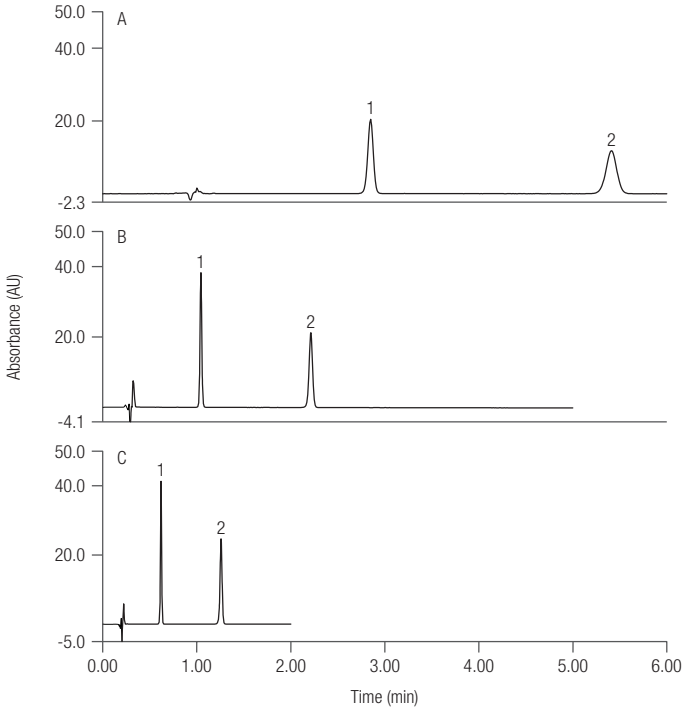

Figure 7. A comparison of the key stages of the method development. A) Original USP method, Hypersil GOLD, 150 mm × 4.6 mm, 5 µm column, 2 mL/min, 25 °C, B) Scaled USP method, Accucore C8, 100 × 2.1 mm, 2.6 µm column, 0.7 mL/min, 25 °C, C) Beyond USP method, Accucore C8, 100 × 2.1 mm, 2.6 µm column, 1.0 mL/min, 45 °C

To conclude this work, method repeatability was investigated by making 24 replicate injections using the final developed method. The results are shown in Figure 9 and Table 1.

Table 1. Data from 24 replicate injections of standards mixture using the final developed method on an Accucore C8, 100 × 2.1 mm, 2.6 µm column, 1.0 mL/min, 45 °C.

Compound	% RSD			
	RT	Area	PW (50%)	As
Fenoprofen	0.07	0.16	0.20	1.56
Gemfibrozil	0.06	0.17	0.25	1.00

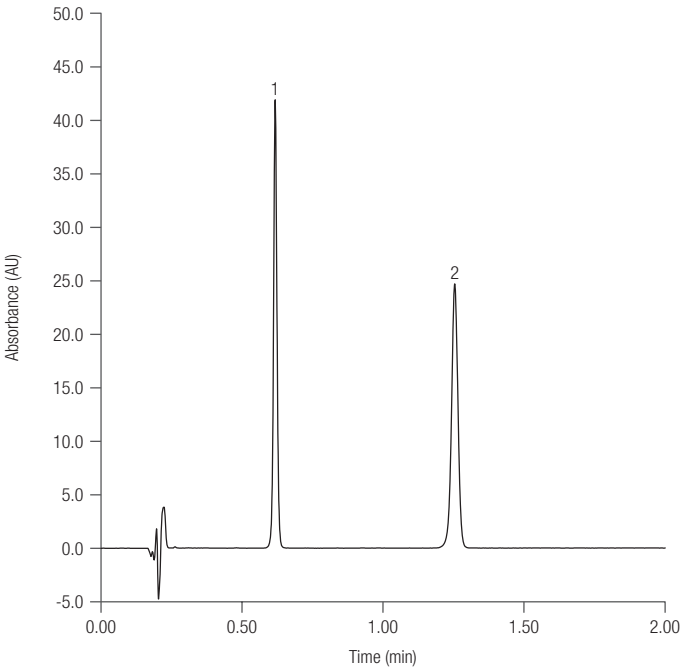

Figure 8. Overlay of 24 replicate injections of the standards mixture using the final developed method on an Accucore C8, 100 × 2.1 mm, 2.6 µm column, 1.0 mL/min, 45 °C.

Conclusions

A high-throughput assay for fenoprofen was developed keeping to the spirit of USP-NF Chapter <621> guidelines for method modernization that doubled throughput and maintained USP quality acceptance criteria. The method, when further optimized, demonstrated the following when compared to the original USP method:

- Four-fold increase in method throughput, allowing more samples to be analyzed in a given time
- Eight-fold reduction in cost per sample through reduced mobile phase consumption and waste generation

References

1. 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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