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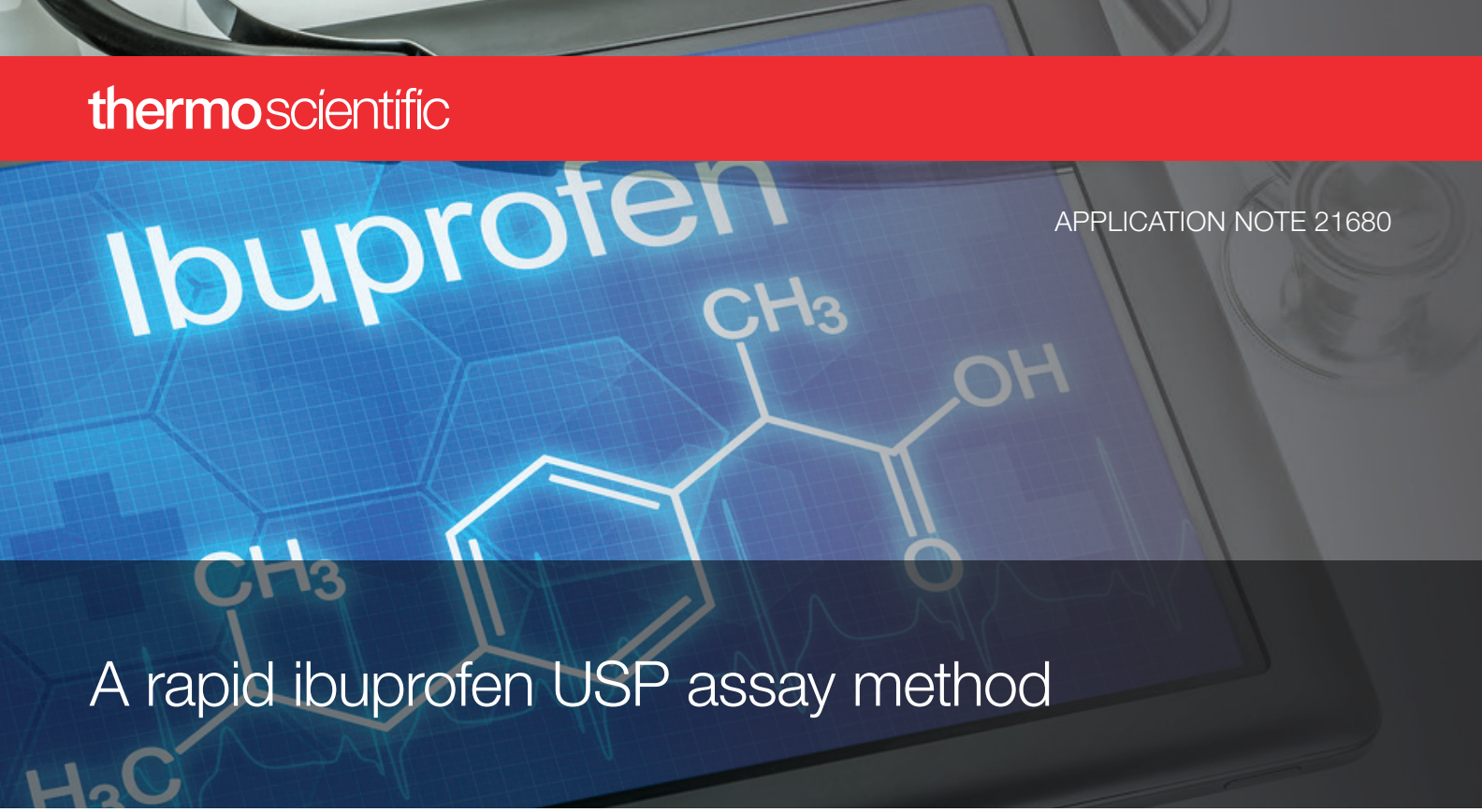

APPLICATION NOTE 21680

A rapid ibuprofen USP assay method

Author
Derek Hillbeck,
Thermo Fisher Scientific,
Runcorn, UK

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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 ibuprofen 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 usually >20 minutes 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, in order 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. Using solid core particles, the 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. The 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 UHPLC system offers intelligent SmartInject Technology to mitigate injection pressure drops 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 system also utilizes Thermo Scientific™ LightPipe™ flow cell technology, designed for the diode array detector (DAD). It 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 ibuprofen method was selected due to the widespread use by generic pharmaceutical manufacturing and the potential for method improvement. This will be demonstrated by direct comparison of legacy and modern column formats 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™, 150 × 4.0 mm, 5 µm column (P/N 25005-154030)
- Accucore C18 XL, 150 × 4.6 mm, 4 µm column (P/N 74104-154630)
- Accucore C18, 100 × 4.6 mm, 2.6 µm column (P/N 17126-104630)
- Accucore C18, 100 × 2.1 mm, 2.6 µm column (P/N 17126-102130)
- Accucore C18, 50 x 2.1 mm, 2.6 µm column (P/N 17126-052130)
- 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 A117-50º)
- 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 ibuprofen and valerophenone. 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™ software 7.2 SR4

Sample preparation

Solutions of the compounds were prepared by dissolving a known amount in acetonitrile 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 water/acetonitrile (2:1, v/v) at a concentration of 0.2 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 adjusted to pH 2.5 with orthophosphoric acid
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:	66% A : 34% B
Mixer:	50 µL capillary + 350 µL static
UV detection:	214 nm

Final UHPLC method

UHPLC column:	Accucore, 2.6 µm HPLC column, 50 mm × 2.1 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	1.0 mL/min
Column temperature:	50 °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
UV detection:	214 nm

Results and discussion

A Hypersil GOLD column was configured on the Vanquish Flex 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 C18, 150 × 4.6 mm, 4 µm column as the direct equivalent and also on an Accucore C18, 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 columns to the Accucore column range due to the differences in stationary phase bonding (surface area and carbon load). However, the USP criteria of peak resolution R exceeding 2 is still attained and all the columns have the USP L1 designation. The solid core 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 20 to 15 minutes.

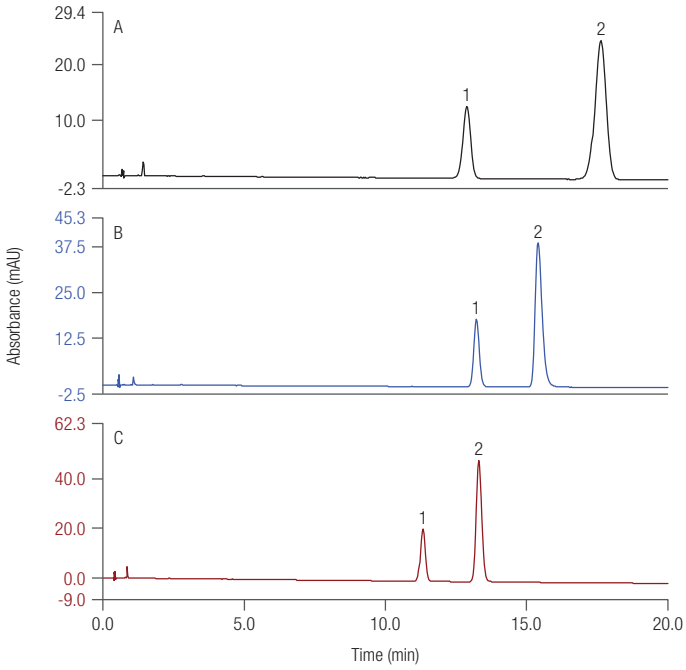

Figure 1. Separation of valerophenone (1) and ibuprofen (2) on three different columns.
(A) Hypersil GOLD, 150 mm × 4.0 mm, 5 µm
(B) Accucore XL, C18 150 × 4.6 mm, 4 µm
(C) Accucore C18, 100 × 4.6 mm, 2.6 µm

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.

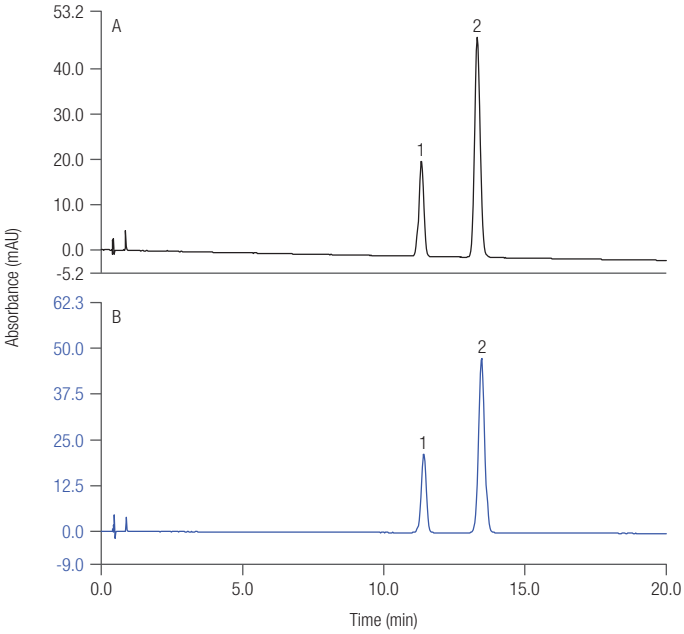

Figure 2. Separation achieved on Accucore 100 mm columns of two different diameters, flow rate scaled.
(A) Accucore C18, 100 × 4.6 mm, 2.6 µm, 2.0 mL/min
(B) Accucore C18, 100 × 2.1 mm, 2.6 µm, 0.417 mL/min

The separation is achieved within the same time frame, but with an eight-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. 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.

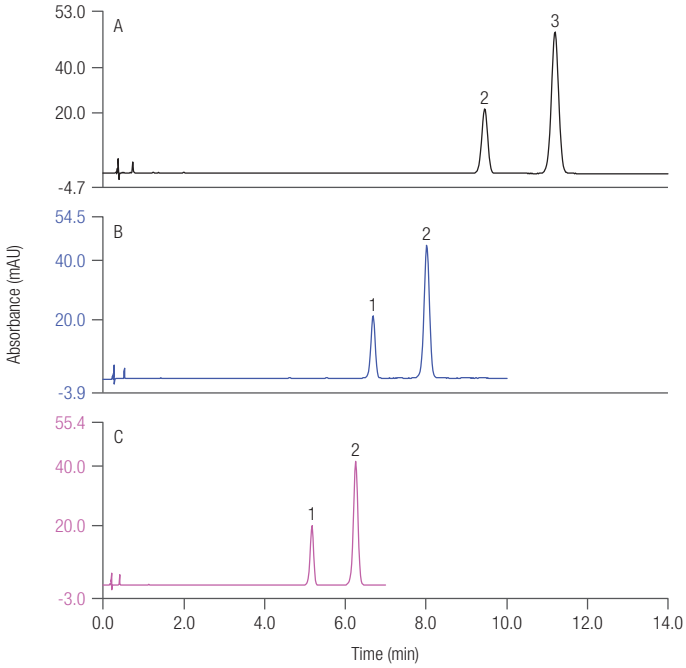

Figure 3. Representative chromatograms observed when increasing the method flow rate on an Accucore C18, 100 x 2.1 mm, 2.6 µm column.
(A) 0.5 mL/min (B) 0.7 mL/min (C) 0.9 mL/min

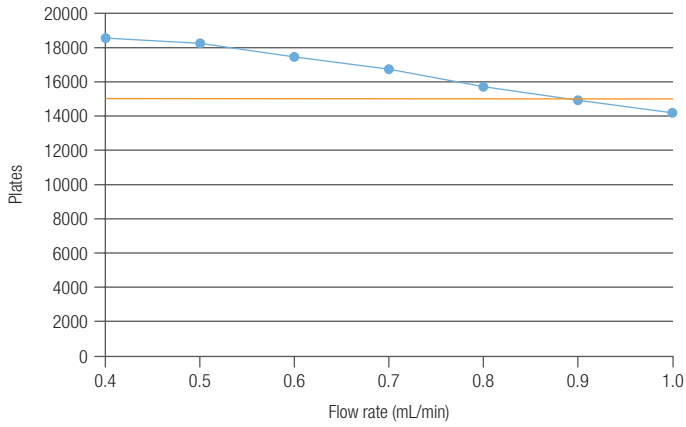

Figure 4. Plate count values when increasing the method flow rate on an Accucore C18, 100 x 2.1 mm, 2.6 µm column. Red line represents 80% value of the plate count at 0.4 mL/min.

Using the Accucore C18, 100 × 2.1 mm, 2.6 µm column at 0.9 mL/min represents the limit to which the USP approved adjustments can be applied. The speed of the assay has been reduced from 20 minutes to 7 minutes and the solvent consumption per assay reduced from 40 mL to 6.37 mL.

There is still opportunity for further assay improvement beyond the USP guidance, yet still meet the USP method guidance on resolution. The column length can be decreased further, column temperature can be increased, and mobile phase composition can be simplified.

Column length was reduced from 100 mm to 50 mm resulting in a decrease of both retention time and peak resolution. The latter shifted from 6.0 to 3.4 and was still above the usual USP limit of 2.0. This can be seen in Figure 5.

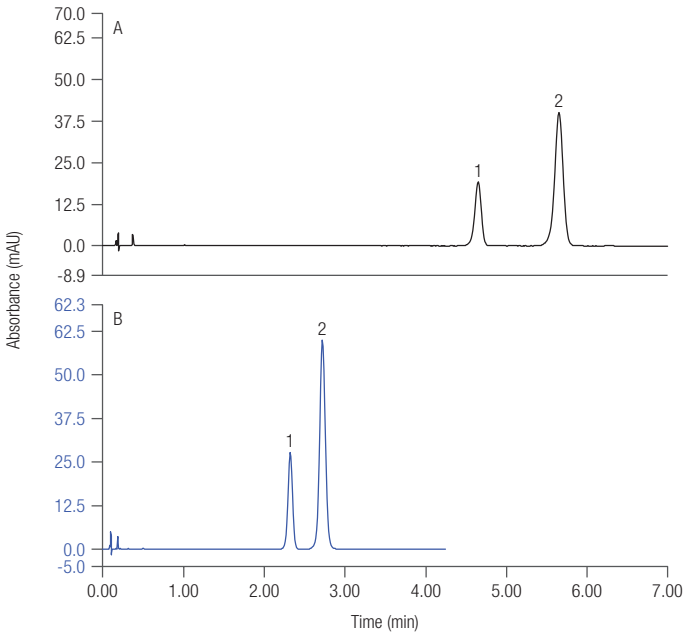

Figure 5. Standards mixture (A) on 100 mm × 2.1 mm 2.6 µm Accucore column, (B) standards mixture on 50 mm × 2.1 mm 2.6 µm Accucore column.

The mobile phase was simplified by substituting the original mobile phase with water and acetonitrile, both containing 0.1% formic acid. The 50 mm column was conditioned with the new mobile phase and the standards analyzed. There was no significant difference in the peak shape and retention.

Finally, the analysis was carried out at with column oven temperatures of 30, 40, and 50 °C.

The expected shift to earlier retention time with narrowing of the peak width was observed. There was a further slight decrease in resolution but again still above the USP limit (Figure 6.) The method with a temperature of 50 °C was selected for further development.

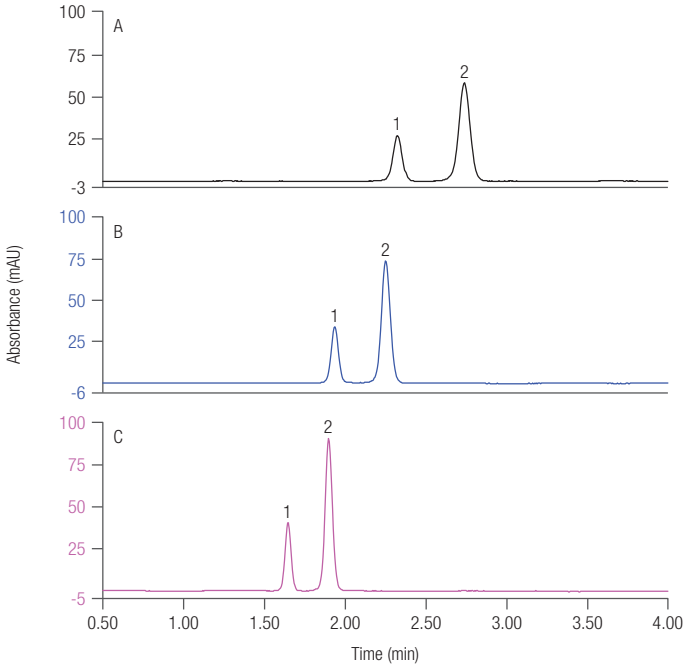

Figure 6. Chromatogram showing standards mixture analyzed on an Accucore 50 mm column at three different temperatures. (A) 30 °C, (B) 40 °C, and (C) 50 °C.

By applying the developed method, the method time has been decreased from 20 minutes to 4 minutes and the consumption of mobile phase (and waste) per assay has also been reduced from 40 mL to 4 mL, thus contributing a saving in both assay cost and an increase in throughput (Figure 7).

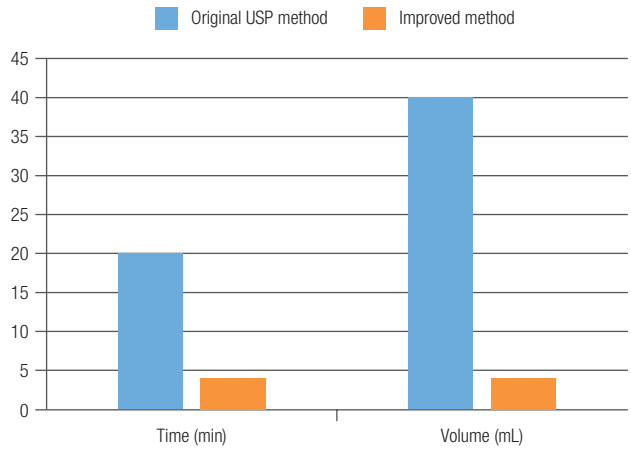

Figure 7. Indicative savings in time and mobile phase volume/waste between the original USP and the improved method.

A comparison of the key stages in this method development is shown in Figure 8.

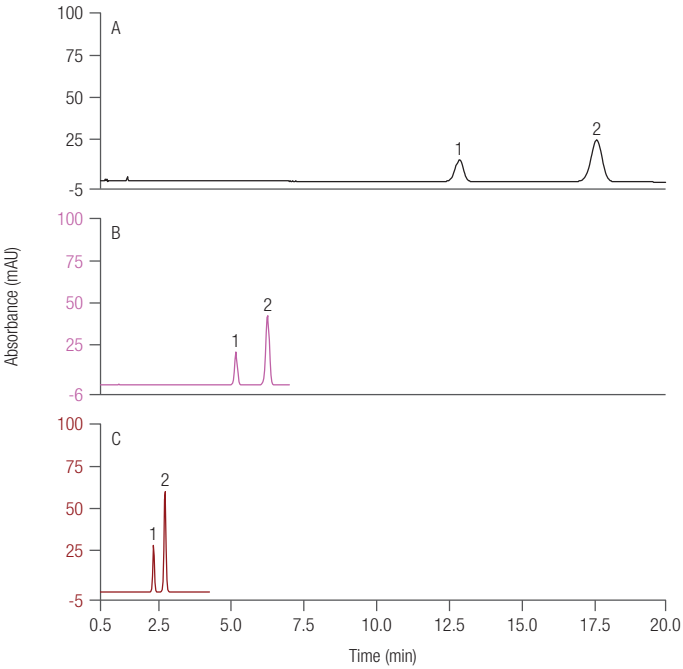

Figure 8. (A) Original USP method, Hypersil GOLD, 150 mm × 4.0 mm, 5 µm, 2 mL/min, 30 °C, (B) Scaled USP method, Accucore C18, 100 × 2.1 mm, 2.6 µm, 0.9 mL/min, 30 °C, (C) Beyond USP method, Accucore C18, 50 × 2.1 mm, 2.6 µm, 1.0 mL/min, 50 °C.

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

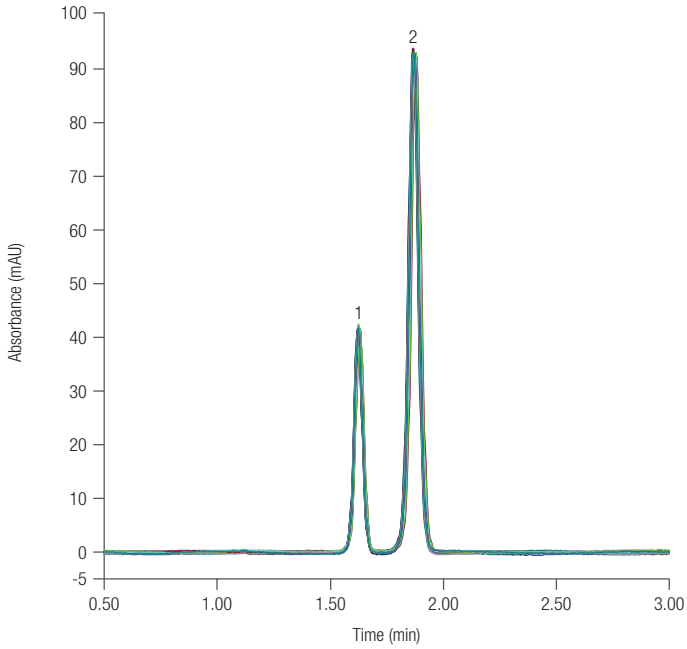

Figure 9. Overlay of 24 replicate injections of the standards mixture using the final developed method on an Accucore C18, 50 × 2.1 mm, 2.6 µm column, 1.0 mL/min, 50 °C.

Conclusions

A high-throughput assay for ibuprofen was developed keeping to the spirit of USP-NF Chapter <621> guidelines for method modernization that significantly increased throughput and maintained USP quality acceptance criteria. When compared to the original USP method, the updated method demonstrates the following:

- Significant increase in assay throughput (five-fold)
- Substantial associated cost reduction, through reduced mobile phase consumption and waste generation
- Associated reduced method complexity from simplified mobile phase preparation

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 **www.thermofisher.com/LC-columns**

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