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Fast methods for the determination of ibuprofen in drug products

Authors

Sylvia Grosse, Mauro De Pra, Frank Steiner, Thermo Fisher Scientific, Germering, Germany

Keywords

Pharmaceutical, drug, USP 40 monograph, ibuprofen, valerophenone, Chromeleon CDS, DII for Empower software

Goal

To demonstrate straightforward approaches to improve the throughput compared with the USP 40 method by using a 50 × 2.1 mm column, with a 2.6 µm particle size, operated in a UHPLC instrument (Thermo Scientific™ Vanquish™ Horizon UHPLC system), while maintaining USP acceptance criteria on relative retention time, resolution, and tailing factors.

Application benefits

- Thermo Scientific™ Vanquish™ UHPLC instruments can be controlled by Waters™ Empower™ 3 software
- Rapid chromatographic method determining ibuprofen in drug products
- Fast method with 94% reduced analysis time, along with 97% solvent reduction and 69% cost per sample savings compared to the USP method

Introduction

In the pharmaceutical industry, United States Pharmacopeia (USP) guidelines and methods are used to standardize analytical processes and give the capability to compare results between laboratories. Those methods are often developed based on columns packed with 5 µm particles. The run times are typically long compared with modern UHPLC standards. USP General Chapter <621> describes permitted modifications in terms of mobile phase composition and pH, column length, inner diameter and particle size, as well as flow rate settings;[1] however, the possibilities of increasing the method throughput by adhering to the permitted modifications remain limited. An example is the method for ibuprofen.[2] Much faster methods can be used that fulfill the quality requirements set by the USP methods.[3–5] However, the conditions were changed beyond those permitted by the USP monograph, and the methods would need full qualification prior to implementation.

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This application explores several options to improve existing USP methods[2] for the determination of ibuprofen in a reference standard and a tablet by using modern instrumentation, in combination with smaller column dimensions and smaller particle size. Furthermore, the influence of the organic content in the mobile phase composition will be discussed. For these studies a Vanquish Horizon UHPLC system was used. The Vanquish Horizon system was controlled by the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) version 7.2, or by Waters Empower 3 software.

Experimental

Chemicals	Part number
Deionized water, 18.2 MΩ·cm resistivity or higher	N/A
Fisher Scientific™ Optima™ Acetonitrile LC/MS grade	10001334
Fisher Scientific™ Optima™ Methanol LC/MS grade	10767665
Fisher Scientific™ Chloroacetic acid (99+%)	10216660
Fisher Scientific™ Ammonium hydroxide solution, for LC/MS, ≥25% in H_2O	15655540
Ortho-phosphoric acid, HPLC grade	10644732
Ibuprofen (purchased from a reputable vendor)	
Valerophenone (purchased from a reputable vendor)	
Equipment	**Part number**
Fisherbrand™ Isotemp™ Stirring Hotplate, Fisher Scientific	15353518
Fisherbrand™ Mini Centrifuge	10243043
Vials (amber, 2 mL), Fisher Scientific	11545884
Snap Cap with Septum (Silicone/PTFE), Fisher Scientific	10547445

Preparation of standards

Three stock solutions with 50 and 60 mg/mL of ibuprofen and 7 mg/mL of valerophenone were prepared in acetonitrile. The 50 mg/mL ibuprofen solution was used for spiking the recovery sample. Mixed working solutions and individual working standards were prepared in water at a concentration of 250 µg/mL each, and in 60% acetonitrile + 0.4% chloroacetic acid at a concentration of 12 mg/mL for ibuprofen and 0.35 mg/mL for valerophenone by diluting the 60 mg/mL ibuprofen and 7 mg/mL valerophenone stock solutions to the appropriate volume.

Five calibration standards of ibuprofen were prepared by diluting the 60 mg/mL stock solution with 60% acetonitrile + 0.4% chloroacetic acid to obtain concentrations of 1, 2.5, 5, 7.5, and 10 mg/mL.

Preparation of samples

The drug containing 400 mg ibuprofen per tablet was purchased in a local pharmacy. A placebo tablet (used for the determination of recovery) was provided by a local pharmacy for the study.

One ibuprofen tablet was weighed and ground with a mortar and pestle. The powder was transferred to a 100 mL volumetric flask and filled up to approximately 50% with solvent (60% acetonitrile + 0.4% chloroacetic acid) and subsequently stirred for 1 h. Afterwards it was filled up to volume with solvent (60% acetonitrile + 0.4% chloroacetic acid) and an aliquot centrifuged for 10 min. The supernatant was transferred into a HPLC vial for injection.

The placebo tablet was ground with a mortar and pestle. The powder was transferred to a 10 mL volumetric flask and 1 mL of 50 mg/mL ibuprofen solution added. Following the procedure of the ibuprofen tablet, it was filled up to approximately 50% with solvent (60% acetonitrile + 0.4% chloroacetic acid) and stirred for 1 h before filling up to volume. Subsequently, an aliquot was centrifuged for 10 min and the supernatant was transferred into a HPLC vial for injection.

Instrumentation	Part number
Vanquish Horizon UHPLC system consisting of:	
System Base Vanquish Horizon	VH-S01-A-02
Binary Pump H	VH-P10-A-01
Sampler HT	VH-A10-A-02
Column Compartment H	VH-C10-A-02
Diode Array Detector with Lightpipe™ Standard flow cell, 10 mm	VH-D10-A-01 6083.0100

Column and instrument settings used in the mobile phase screening and the USP compliant methods are shown in Tables 1 and 2, respectively.

Data processing and software

Chromeleon CDS version 7.2 SR5 was used for data acquisition and processing for the isocratic mobile phase screening.

For data acquisition and processing of method 1 and 2, Thermo Scientific™ Dionex™ Instrument Integration (DII) 1.15 for Empower software and Waters Empower 3 software (Build 3471) were used.

Table 1. Column and instrument settings used in the mobile phase screening

Column	Thermo Scientific™ Accucore™ XL C18, 100 × 3 mm, 4 µm (P/N 74104-103030)
Mobile phase	A: Water + 0.1% H_3PO_4 B: Acetonitrile + 0.1% H_3PO_4
Flow rate	1.125 mL/min
Isocratic mobile phase condition	Variable, 25% B to 50% B
Isocratic run time	Variable, 2–36 min
Mixer volume	10 + 25 µL
Column temperature	30 °C (forced air mode, fan speed 5)
Autosampler temperature	10 °C
UV wavelength	215 nm
UV data collection rate	20 Hz
UV response time	0.2 s
Injection volume	5 µL
Needle wash	10:90 water/methanol (v/v)

Table 2. Columns and instrument settings for USP compliant method (method 1) and fast method (method 2)

	Method 1	Method 2
Column	Thermo Scientific™ Acclaim™ C18, 250 × 4.6 mm, 5 µm (P/N 59149)	Thermo Scientific™ Accucore™ C18, 50 × 2.1 mm, 2.6 µm (P/N 17126-052130)
Mobile phase	40:60 water/ACN (v/v) + 0.4% chloroacetic acid, pH 3.0 ± 0.2	
Flow rate	2 mL/min	1.1 mL/min
Isocratic run time	8 min	0.5 min
Mixer volume	350 + 50 µL	
Column temperature	30 °C (forced air mode, fan speed 5)	
Autosampler temperature	10 °C	
UV wavelength	254 nm	
UV data collection rate	10 Hz	50 Hz
UV response time	0.5 s	0.1 s
Injection volume	10 µL	1 µL
Needle wash	10:90 water/methanol (v/v)	

Results and discussion

Several methods are described in the USP 40 monograph for the identification and quantification of ibuprofen and ibuprofen-related impurities either in reference standards, tablets, or oral suspensions.

1) Mobile phase screening in isocratic mode by varying the organic content

Within the USP 40 monograph for ibuprofen, under the section Chromatographic Purity, an L1 column with dimensions of 150 × 4 mm and 5 µm particles is reported. A complete list of columns belonging into the L1 category can be found in the USP40-NF35 S2 section under chromatographic columns and packings.[6] The method runs under isocratic conditions with a mobile phase composition of 66% water, adjusted to pH 2.5 with phosphoric acid, and 34% of acetonitrile at a flow rate of 2 mL/min. To study the effect of the organic content in the mobile phase with respect to selectivity, single standards and a mixture of ibuprofen and valerophenone at a concentration of 250 µg/mL of each were injected into the mobile phase. From run to run, the acetonitrile content was changed in steps of 5% starting at 25% acetonitrile and going up to 50%. As can be seen in Figure 1, valerophenone eluted before ibuprofen when mobile phases with acetonitrile content of 35% or lower were used. At 35% acetonitrile, the resolution was 2.56, which is above, albeit close to, the requirements of the USP method (Rs > 2). With 40% acetonitrile, the selectivity changed and ibuprofen eluted before valerophenone with a resolution of 1.29, hence below the acceptance criteria. When the acetonitrile content was further increased to 50%, the resolution increased again to 4.87. At the same time, the run-time strongly decreased at higher organic content, from over 35 minutes at 25% organic to less than 2 minutes at 50%. Based on these results, it can be shown that varying the organic content in the mobile phase significantly reduces analysis time. The obtained backpressure measured at 50% acetonitrile was 230 bar, which would allow the use of conventional HPLC instruments with a pressure specification limited to 400 bar.

2) Use of a fast method with reduced column dimensions and particle size

The USP 40 assay method for ibuprofen reports a column with dimensions of 250 × 4.6 mm and 5 µm particles, running with a mobile phase composition of 40% water and 60% acetonitrile containing 0.4% of chloroacetic acid, adjusted with ammonium hydroxide

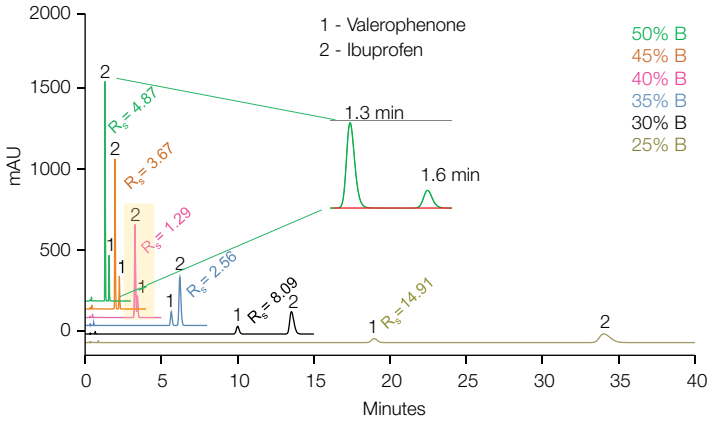

Figure 1. Overlaid chromatograms (1) valerophenone and (2) ibuprofen, obtained by the method shown in Table 1

to pH 3.0 (Table 2, method 1). Under these conditions, ibuprofen elutes before valerophenone. Figure 2 shows the comparison between the USP method and one developed with a 50 × 2.1 mm column packed with 2.6 µm particles (Table 2, method 2). The Plate Height (H) of the column packed with the 2.6 µm solid core particles is expected to be substantially lower than the one with the 5 µm particles. Consequently, a shorter column will be capable of delivering the required efficiency to resolve the ibuprofen and valerophenone peaks, with shorter run times. Moreover, the 2.6 µm solid core particles allow the use of a higher linear flow rate compared to the fully porous 5 µm particles without compromising in efficiency, thereby enabling even faster methods to be developed. The ibuprofen peak in the USP method (method 1) eluted at RT 5.69 min and the valerophenone peak at 7.40 min. By using a 50 mm column with an inner diameter of 2.1 mm packed with 2.6 µm particles, the retention times could be reduced to 0.26 min and 0.32 min, respectively. Even though the linear velocity in the short column was much higher than the one used for the original method, the combination of shorter run time and smaller diameter, resulted in a savings of organic solvent of 97%. The original method required more than 16 mL mobile phase per run, versus the 0.55 mL required for the fast method, as can be seen in Figure 3. One reference[5] describes more in depth the separation speed up of valerophenone and ibuprofene by several particle size and column length combinations, resulting in backpressures up to 1320 bar, while achieving the USP requirements of resolution and tailing factors. They concluded that for this application 50 mm columns packed with 2.6 µm particles delivered the fastest method.

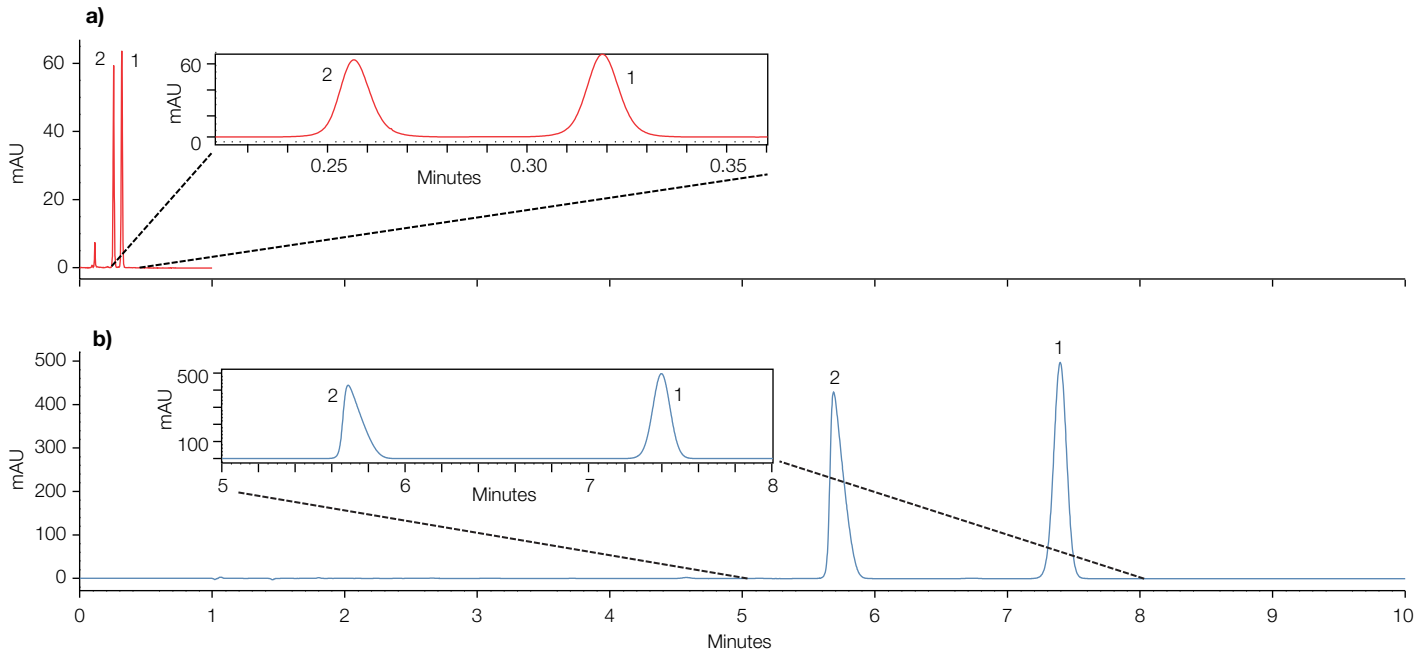

Figure 2. a) Chromatogram of the fast method (method 2) with zoomed view on (2) valerophenone and (1) ibuprofen; b) chromatogram of USP method (method 1) with zoomed view on (2) valerophenone and (1) ibuprofen

Reducing the particle size from 5 µm to 2.6 µm does not entail a large increase in pressure if the column length is reduced from 250 mm to 50 mm. The backpressure of the fast method (method 2) increased just to 330 bar compared to 305 bar for the USP method (method 1).

The acceptance criteria described in the USP 40 monograph regarding relative retention times (RRT) and resolution (Rs) of ibuprofen and valerophenone is 1.4 for valerophenone and a minimum resolution of 2.5. As can be seen in Table 3, a RRT of 1.3 for valerophenone was observed when the USP method was applied, and a RRT of 1.2 with the fast method indicating excellent chromatographic consistency. The resolution decreased in the fast method to 4.2 compared to 9.1 in the USP method. This is more than adequate to meet the requirements of the USP monograph. Additionally, Figure 3 shows that the analysis time could be reduced by a factor of 16 and the associated acetonitrile consumption is clearly decreased, which also contributes significantly to the reduction of cost per sample (considering prices of organic solvent and columns, assuming 1000 injections per column).

Table 3. Comparison of RRT and Rs between the original USP method (method 1) and the fast method (method 2)

Analyte	RRT		R_s	
	USP method	Fast method	USP method	Fast method
Ibuprofen	1.0	1.0	—	—
Valerophenone	1.3	1.2	9.1	4.2

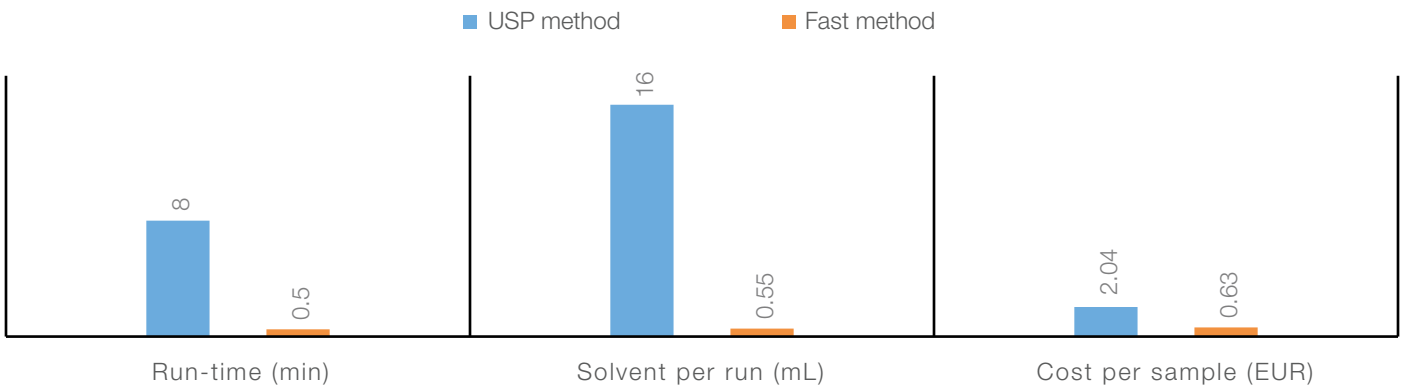

Figure 3. Comparison between USP method and fast method with respect to run time, organic solvent consumption, and cost per sample

3) Quantitation of ibuprofen in tablets

Quantitation was performed using the methods in Table 2. Both calibration standards and recovery samples were injected once, while the tablet sample was injected three times to obtain method-reproducibility data.

The USP monograph reports acceptance criteria with a tailing factor < 2.5 and the relative standard deviation (% RSD) of the area not more than 2.0%. The tailing factor was determined to be 1.2 for the USP method (method 1) and 1.1 for the fast method (method 2).

As can be seen in Table 4, the % RSD Area is slightly higher (0.25%) for the fast method compared with the USP method (0.04%) but is still below the given acceptance criterion of a maximum of 2.0%. For both the USP method and the fast method, the % RSD of retention time (RT) is excellent.

Both methods were run using the combination of Empower 3 software and DII 1.15 for Empower software. Figure 4 shows an overview of the data analysis part with the sample set and instrument controller on the left side and the data processing part on the right side within the software. The sample set contains the calibration standards, recovery sample, and ibuprofen tablet sample. The calibration curve and calculation of sample amounts were done within the software by using an appropriate processing method.

Table 4. Comparison of % RSD RT and Area of ibuprofen peak between USP method and fast method for three consecutive injections of sample

USP method		Fast method	
% RSD		% RSD	
RT	Area	RT	Area
0.01	0.04	0.02	0.25

Calibration standards of ibuprofen were prepared in the concentration range of 1 mg/mL to 10 mg/L. Linearity was found to be 0.9999 for the USP method and 0.9986 for the fast method.

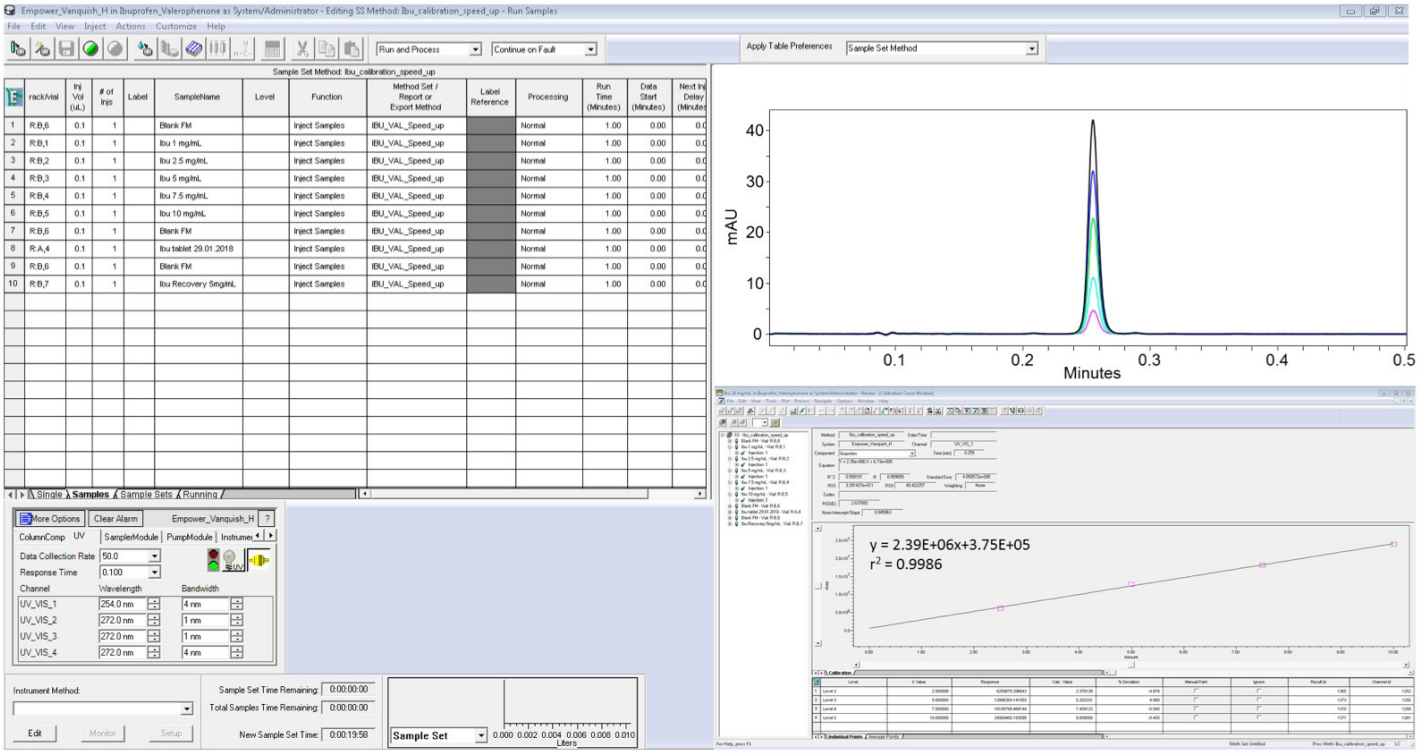

Figure 4. Insight to the Empower 3 software with an example sample set and the instrument controlling status on the left (section run samples) and an example of overlaid chromatograms and a calibration curve on the right (section data review)

The quantitative results are summarized in Table 5. Good recovery could be achieved with 103% for ibuprofen with the USP method and 108% for the fast method, respectively. The content of ibuprofen in the tablet was calculated to be 397 mg/tablet in the USP method and 391 mg/mL with method 2, already corrected by recovery rate. This corresponds to an ibuprofen content of 99% and 98%, respectively. Both methods therefore meet easily the USP requirements of 90–110% labeled amount of ibuprofen in the tablet.

Table 5. Quantitative results of ibuprofen in tablet with each method. The measured amount was corrected by the recovery rate.

	Stated amount on label [mg/tablet]	Measured amount [mg/tablet]	Recovery [%]	% Amount ibuprofen in tablet
USP method	400	397	103	99
Fast method	400	391	108	98

The fast method shows comparable results with the USP method, demonstrating clear advantages in analysis throughput and solvent consumption. Instrumentation and software from multiple vendors are commonly employed within pharmaceutical laboratories, and this workflow improvement enables greater flexibility.

Conclusion

- The analysis of ibuprofen in a tablet with the fast method and the USP method provides comparable results in terms of RRT, resolution, and tailing factors.
- The measured amount of ibuprofen in the tablet with the fast method was 98%, which is in line with the USP requirements of 90–110%.
- Analysis time could be reduced by 94%, along with solvent reduction of 97% and a 69% cost per sample savings.
- The Vanquish UHPLC systems can be controlled by the combination of DII for Empower software and Empower 3 software.

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