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## Application Note SI-01023

# Productivity Enhancement of USP Assay for Ibuprofen Oral Suspension with the Varian 920-LC and Pursuit™ XRs C8 Column

Phuong Truong  
Varian, Inc.

### Introduction

Ibuprofen (iso-butyl-propanoic-phenolic acid) is a non-steroidal anti-inflammatory drug (NSAID) that is widely used around the world under a variety of trade names. It is used for the treatment of mild to moderate arthritis, pain, inflammation and fever. The release of prostaglandins in the body, in response to viral attack, promotes pain, fever and inflammation. Ibuprofen acts by blocking the cyclooxygenase enzyme that produces prostaglandins, as a consequence of the lower levels of prostaglandins, inflammation, pain and fever are reduced<sup>1</sup>.

There are several standard HPLC methods for the quantitative analysis of ibuprofen drugs. Among them, the United States Pharmacopoeia<sup>2</sup> (USP) monographs for Ibuprofen Oral Suspension were chosen to demonstrate a fast HPLC method using Varian Pursuit XRs columns and the Varian 920-LC liquid chromatograph. The results obtained from the experiment show that all performance requirements of the USP methods are satisfied and demonstrate a significant enhancement in laboratory productivity when using this system.

### Instrumentation

The experiment was carried out using a Varian 920-LC liquid chromatograph equipped with a low pressure quaternary gradient pump with built in 4 channel Degasser™, a diode array detector and an autosampler with 100 µL injection syringe.

The Varian 920-LC is fully controlled by Galaxie™ Chromatography Software.

### Materials and Reagents

Phosphoric acid 85% (AnalaR grade from BDH), water (18 MΩ, Milli-Q) and acetonitrile (HPLC grade) were used for the mobile phase, and diluent preparations.

A USP compliant ibuprofen standard and benzophenone (Reagent Plus 99%) were purchased from Sigma-Aldrich.

Ibuprofen oral suspension was purchased over-the-counter from a local pharmacy.

### Standard and Sample Preparation

Ibuprofen standard solutions and benzophenone internal standard solutions were prepared as in the USP method for the assay of ibuprofen oral suspension.

Ibuprofen oral suspension was also prepared as in the USP method for the assay of this product.

### Conditions

The chromatographic conditions for the analysis are summarized in Table 1.

Table 1. Chromatographic conditions

Column	Pursuit XRs C8, 5 µm, 150x4.6 mm Pursuit XRs C8, 3 µm, 50x4.6 mm Ambient temperature
Mobile phase	0.01 M Phosphoric Acid: Acetonitrile (55:45 % v/v)
Flow rate	2 mL/min
Wavelength (UV)	220 nm

### Results and Discussion

The USP method for assay of ibuprofen oral suspension used a column of 150 mm length and 4.6 mm ID with 5 µm packing L7. For a direct comparison we applied the USP method using a Varian Pursuit XRs C8 5 µm, 150x4.6 mm column. Figure 1 shows the chromatogram obtained from an ibuprofen oral suspension sample with the original USP method.

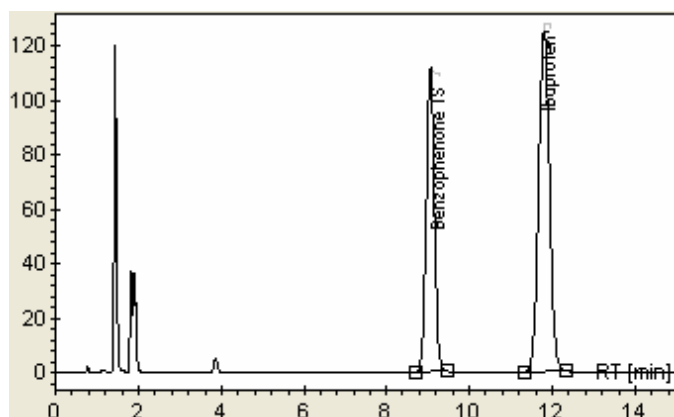


Figure 1. Chromatogram of ibuprofen from ibuprofen oral suspension

- Sample concentration: about 0.48 mg/mL
- Column: Pursuit™ XR8 C8, 5 µm, 150x4.6 mm
- Mobile phase and flow rate as in Table 1
- Injection volume: 5 µL

The USP method requires the resolution between benzophenone internal standard (IS) and ibuprofen to be greater than 1.5 and the tailing factor, a measure of peak symmetry, to be less than 2.0 for these peaks.

The chromatographic performance for the above ibuprofen sample is summarized in Table 2.

Table 2. Chromatographic performance from ibuprofen oral suspension sample using USP method and Pursuit XR8 5 µm 150x4.6 mm

	Resolution	Asymmetry	Selectivity
Benzophenone IS		1.00	
Ibuprofen	6.5	1.01	1.33

As can be seen from Table 2, the performance of the Pursuit XR8 5 µm 150x4.6 mm column went well beyond the requirements specified in the USP method. However, the retention times are such that 15 minute run times are required and this can limit productivity in terms of sample throughput.

A shorter column with the same bonded phase but with smaller particle size, the Varian Pursuit XR8 C8, 3 µm 50x4.6 mm, was used to improve productivity. The same chromatographic conditions were used except the injection volume was scaled down to 3 µL to account for the change in lower overall column volume. The results obtained (Table 3) also clearly surpass the USP method requirements but the run times can be reduced to below 5 minutes. Figure 2 shows the chromatogram obtained for a sample of ibuprofen from the ibuprofen oral suspension using this column.

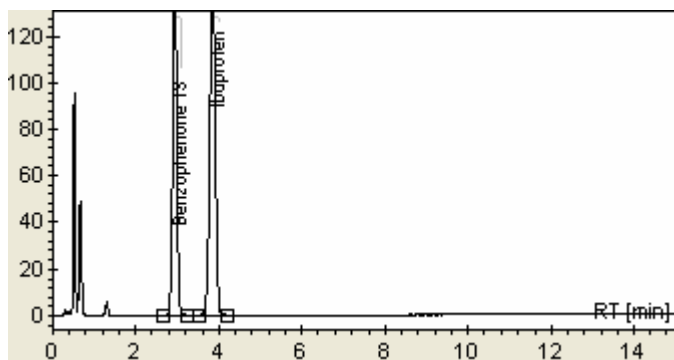


Figure 2. Chromatogram of ibuprofen from ibuprofen oral suspension

- Sample concentration: about 0.48 mg/mL
- Column: Pursuit XR8 C8, 3 µm 50x4.6 mm
- Mobile phase and flow rate as in Table 1
- Injection volume: 3 µL

Table 3. Chromatographic Performance from ibuprofen oral suspension sample applying USP method and Pursuit XR8 3 µm 50x4.6 mm

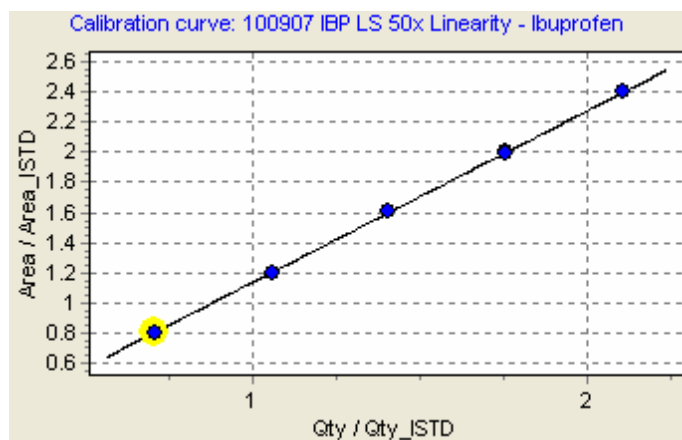
	Resolution	Asymmetry	Selectivity
Benzophenone IS		0.99	
Ibuprofen	4.3	0.99	1.34

The USP method for this assay specifies a required level of precision for both retention time and area. For an acceptable analysis, the relative standard deviation for replicate injections should be less than 2.0%. Table 4 shows the results obtained from six replicate injections of standard solutions. Excellent precision in both retention time and peak area was obtained.

Table 4. Precision in retention time and Peak Area of standard solution of 0.24 mg/L (50% level), 0.48 mg/mL (100% level) and 0.72 mg/mL (150% level)

	%RSD	
	Retention time Ratio between Ibuprofen and IS	Peak Area Ratio between Ibuprofen and IS
Standard solution at 50% level (n=6)	0.09	0.98
Standard solution at 100% level (n=6)	0.07	0.28
Standard solution at 150% level (n=6)	0.14	0.25

The linearity test for standard solutions was also conducted with 5 standard solutions: at 50%, 75 %, 100%, 125% and 150% level. Figure 3 shows the regression coefficient for the curve is 1.0000 and Table 5 shows the calibration points. Therefore, the fast LC method can be used to quantify ibuprofen in the linear range from 50 to 150 % standard level.



X: Qty/Qty<sub>ISTD</sub>, Y: Area/Area<sub>ISTD</sub>, Polynom: y = bx+a  
Regression coefficient = 1.000, a = 0.01165, b = 1.12899

Figure 3. Regression curve for ibuprofen standard solutions with Varian Pursuit XR8, 3 µm, 50x4.6 mm

Table 5. Standards 1-5 calibration points

	Qty/QTY_ISTD	Area/Area_ISTD
Standard 1	0.70	0.81
Standard 2	1.06	1.20
Standard 3	1.41	1.61
Standard 4	1.76	2.00
Standard 5	2.11	2.40

To measure the reproducibility of sample analysis with Pursuit™ XRs C8, 3 µm, 50x4.6 mm, six ibuprofen oral suspension samples were assayed as USP method and duplicate injections were made for each sample. Table 5 summarizes the results obtained and Figure 4 shows the results went beyond USP acceptance criteria and very good reproducibility achieved for 6 sample replicates or 12 sample injections.

Table 6. Results from the assay of ibuprofen oral suspension using USP method with Pursuit XRs C8, 3 µm, 50x4.6 mm

	Average Assay % Labeled Amount*	%RSD (n =12)	
		Quantity of Ibuprofen in each mL	Selectivity
Ibuprofen	96.0	0.72	0.14

\*USP method stated ibuprofen oral suspension contains not less than 90.0% and not more than 110.0% of the labelled amount of ibuprofen.

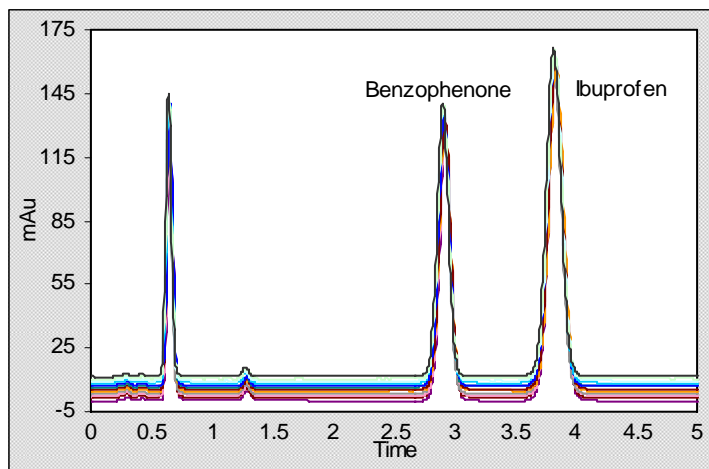


Figure 4. Reproducibility of Sample Assay for ibuprofen oral suspension using USP method and Pursuit XRs C8, 3 µm, 50x4.6 mm. The assays were performed with 6 sample replicates and duplicate injections for each sample.

Benzophenone	%RSD (Rt) = 0.28 %RSD (Area) = 0.83
Ibuprofen	%RSD (Rt) = 0.19 %RSD (Area) = 1.33
RS = 4.4	

## Conclusion

The standard USP method for the assay of ibuprofen in an oral suspension can be applied and transferred to a faster HPLC method using the Pursuit XRs C8 3 µm 50x4.6 mm column with excellent results and superior sample throughput. The significant reduction in run time, without compromising analytical quality, can lead to major savings in operator time and solvent usage and therefore represents a major advance.

## References

<sup>1</sup> Wikimedia Foundation, Inc. Last modified 12:52, 24 September 2007. Wikipedia. Last viewed September 20, 2007. <http://en.wikipedia.org/wiki/Ibuprofen>

<sup>2</sup> United States Pharmacopeia. Published November 2006, Official May 1, 2007. USP30-NF25, USP Monographs: Ibuprofen Oral Suspension.

These data represent typical results.

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