Efficient HPLC Analysis in the USP Assay of Ibuprofen and in the Limit Test of Ibuprofen-related Compound C in Ibuprofen Tablets with the Varian 920-LC and Pursuit™ C18 Column

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Introduction

Identification and quantification of the Active Pharmaceutical Ingredient (API) and related impurities in pharmaceutical products are the main focus of many pharmaceutical applications of HPLC.

The concentrations of impurities in the products are normally much lower than that of the API. Consequently, it is necessary to use highly sensitive and selective instruments to comply with the modern regulatory requirements.

The ibuprofen-related compound C (4-isobutylacetophenone) causes adverse effects in the central nervous system[1]. It presents in ibuprofen products as a degradation of the API[2] and needs to be monitored and controlled over the shelf life of the product. In the United States Pharmacopoeia (USP) monographs for ibuprofen tablets[3], the Limit Test requires the amount of ibuprofen-related compound C in ibuprofen tablets to be not more than 0.1% per tablet.

In this experiment, the USP monographs' Limit Test for ibuprofen-related compound C was carried out with Varian 920-LC liquid chromatograph and a Varian Pursuit C18 column. The result shows a fast HPLC analysis achieved by using Varian Pursuit XRs columns and the Varian 920-LC system.

Furthermore, the level at which ibuprofen-related compound C can be quantified is at least ten times lower than the level required by the USP monographs. The analysis exceeded the performance requirements stated in the USP method for Ibuprofen Assay and Limit Test of ibuprofen-related compound C and permits a significant improvement in laboratory productivity without compromising analytical quality.

Instrumentation

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

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

Materials and Reagents

Chloroacetic Acid (A.C.S. Reagent from Sigma-Aldrich), ammonium hydroxide (Analytical reagent from Univar), water (18 MΩ, Milli-Q) and acetonitrile (HPLC grade) were used for the mobile phase and as the diluent for internal standard solution preparations.

Ibuprofen (meets USP testing specifications) and valerophenone (99%) were purchased from Sigma-Aldrich. Ibuprofen-related Compound C was supplied from U.S. Pharmacopoeia.

Ibuprofen tablets were purchased over-the-counter from a local pharmacy.

Standard and Sample Preparation

Ibuprofen standard solutions, valerophenone internal standard solutions, ibuprofen-related compound C were prepared as in the USP monographs for Ibuprofen Tablet Assay and Limit of Related Compound C.

Ibuprofen samples were also prepared as in the USP monographs for ibuprofen tablets.

Conditions

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

Table 1. Chromatographic conditions

Column	Pursuit XRs C18, 5 µm, 250x4.6 mm Pursuit XRs C18, 3 µm, 100x4.6 mm Both at ambient temperature
Mobile phase	Chloroacetic acid in water (1% w/v) pH=3 : acetonitrile (40:60 % v/v)
Flow rate	2 mL/min
Wavelength (UV)	254 nm

Results and Discussion

The USP method for Assay of Ibuprofen Tablets specifies a column of 250 mm length and 4.6 mm ID with 5 µm packing. For a direct comparison we applied the USP method using a Varian Pursuit XRs C18 5 µm, 250x4.6 mm column. Figure 1 shows the chromatogram of an ibuprofen tablet sample obtained using the above chromatographic conditions.

We also used a Varian Pursuit™ C18, 3 µm, 100x4.6 mm column to achieve a faster separation for ibuprofen in the tablets using the same conditions as in the USP method. The injection volume was scaled down to keep the same peak height as in the original method. Figure 2 shows the chromatogram of the ibuprofen tablet sample with the shorter column and smaller particle size.

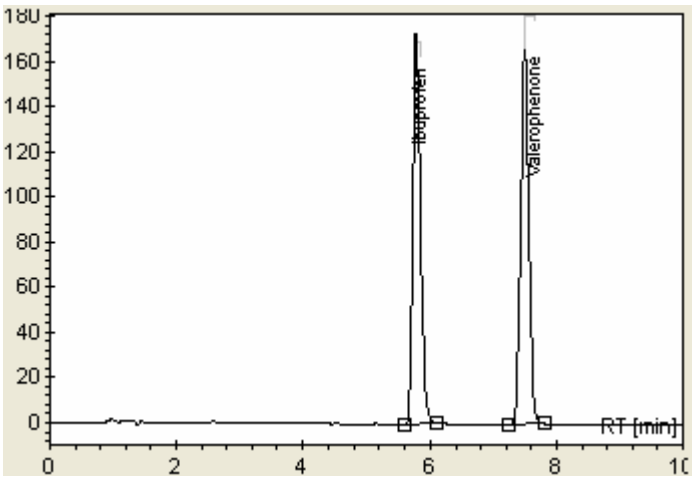

Figure 1. Chromatogram of ibuprofen from ibuprofen tablets using the Pursuit XRs C18, 5µm, 250x4.6 mm column, ~12 mg/mL ibuprofen in tablet sample solution with an injection volume of 5 µL, mobile phase and flow rate as in Table 1.

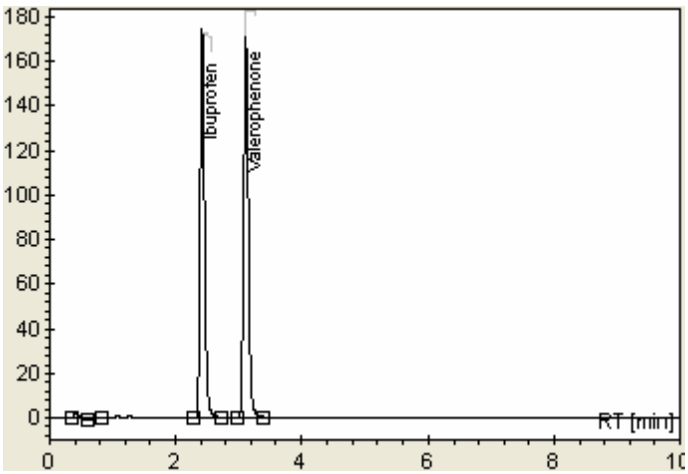

Figure 2. Chromatogram of ibuprofen from ibuprofen tablets using the Pursuit XRs C18, 3 µm, 100x4.6 mm column, ~12 mg/mL ibuprofen in tablet sample solution with an injection volume of 3 µL, mobile phase and flow rate as in Table 1.

Figures 3 and 4 show the chromatograms of ibuprofen-related compound C in standard solution using the USP method with a Pursuit XRs C18 5 µm 250x4.6 mm column and a Pursuit XRs C18 3 µm 100x4.6 mm column respectively.

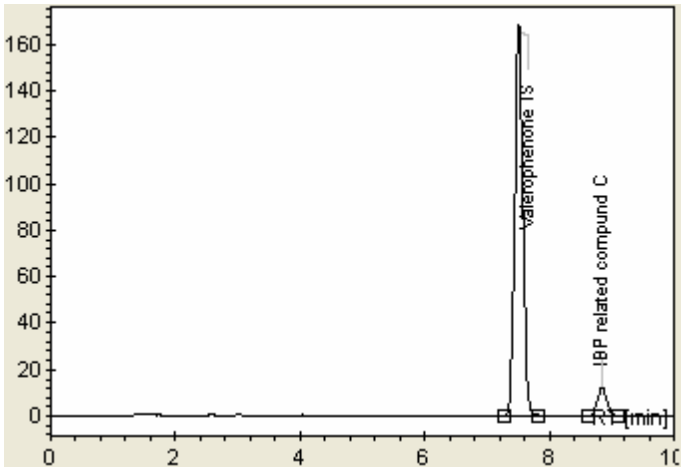

Figure 3. Chromatogram of ibuprofen-related compound C using the Pursuit XRs C18, 5 µm, 250x4.6 mm column, ~12 mg/mL ibuprofen-related compound C with an injection volume of 5 µL, mobile phase and flow rate as in Table 1.

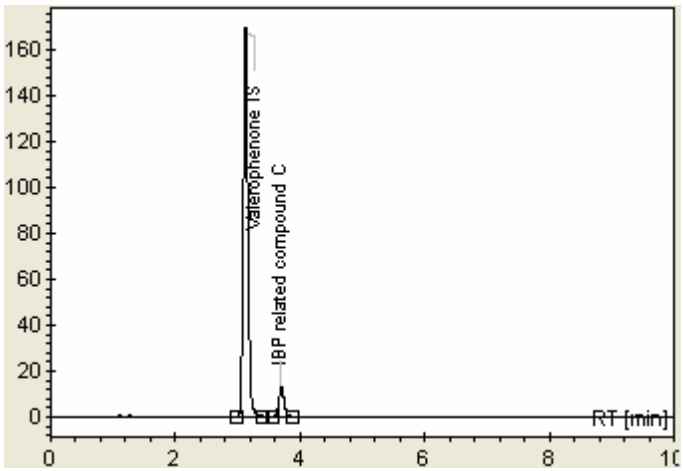

Figure 4. Chromatogram of ibuprofen-related compound C using the Pursuit XRs C18, 3 µm, 100x4.6 mm column, ~12 mg/mL ibuprofen-related compound C with an injection volume of 3 µL, mobile phase and flow rate as in Table 1.

Tables 2 and 3 summarize the chromatographic performance obtained from the ibuprofen (IBP) tablet sample solution and the ibuprofen-related compound C standard solution using the original USP method (a) and the faster HPLC analysis (b).

Table 2. Chromatographic performance from ibuprofen tablet applying USP method and Pursuit™ XRs C18 5 µm 250x4.6 mm column

(a)	Resolution	Asymmetry	Selectivity
Ibuprofen		1.29	
Valerophenone IS in IBP tablet sample solution	7.3.	1.00	1.38
Valerophenone IS in IBP related compound C standard solution		1.06	
Ibuprofen-related compound C	5.1	1.01	1.21

Table 3. Ibuprofen tablet applying USP method and Pursuit XRs C18 3 µm 100x4.6 mm column

(b)	Resolution	Asymmetry	Selectivity
Ibuprofen		1.27	
Valerophenone IS in IBP tablet sample solution	5.3.	1.01	1.29
Valerophenone IS in IBP related compound C standard solution		1.13	
Ibuprofen-related compound C	4.0	1.10	1.18

The USP method requires the resolution between ibuprofen and valerophenone internal standard (IS) and between valerophenone and ibuprofen-related compound C to be greater than 2.5; the tailing factors (a measure of peak asymmetry) for the individual peaks are less than 2.5.

Tables 2 and 3 show that the performance of the Pursuit XRs C18 5 µm 250x4.6 mm column went well beyond the acceptance criteria specified in the USP method. A shorter column with the same bonded phase and smaller particle size, the Varian Pursuit XRs C18 3 µm 100x4.6 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 clearly surpass the requirements of the USP method. Furthermore, the run times can be reduced to below 4 minutes, less than half the run times from the original method.

Table 4 shows the results obtained from 6 replicate injections of standard solutions and sample solutions. Excellent precision in both retention time and peak area was obtained.

Table 4. Precision in retention time and peak area of ibuprofen, ibuprofen-related compound C and valerophenone IS

	%RSD (Rt) (n=6)	%RSD (Area) (n=6)
	Ibuprofen standard solutions 12 mg/mL	
Ibuprofen	0.48	0.44
Valerophenone IS	0.37	0.16
	Ibuprofen-related compound C standard solutions of 0.012 mg/mL	
Valerophenone IS	0.27	0.22
Ibuprofen-related compound C	0.25	0.51
	Ibuprofen tablet sample solutions ~12mg/mL	
Ibuprofen	0.25	0.14
Valerophenone IS	0.17	0.65

Each analysis was performed using the same chromatographic conditions as in Table 1 with a Pursuit XRs C18 3 µm, 100x4.6 mm column.

The assay was carried out with duplicate samples of the powered tablets. Duplicate injections were made for each sample solution. Results of the assay analysis for ibuprofen tablets are shown in Table 5.

Table 5. Results from the assay of ibuprofen tablets applying USP method with Pursuit XRs C18, 3 µm, 100x4.6 mm column

% Labeled Amount of Ibuprofen*	Asymmetry of Ibuprofen	Asymmetry of IS	Resolution between Ibuprofen and IS
Mean= 101.9 %RSD = 0.68 (n=4)	1.27	1.08	5.4

*The USP method stated ibuprofen tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of ibuprofen.

The ibuprofen-related compound C standard solution in Figure 4 has a concentration of 0.012 mg/mL or 0.1% per tablet. Figure 5 shows the chromatogram and peak results obtained from the tablet sample solution in the Limit Test for ibuprofen-related compound C.

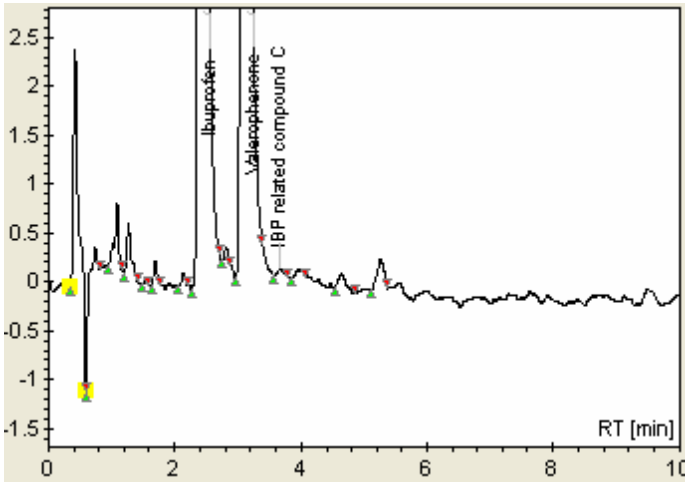

Figure 5. Chromatogram and peak results of ibuprofen tablet sample solution using Pursuit XRs C18, 3 µm, 100x4.6 mm column and USP method

Name	Time (min)	Area (mAu s)	Height (mAu)	As. USP	Res. USP	Selec-tivity	Width 50%	Area (%)
Unknown	0.42	18.304	2.774	1.32	0.00	0.00	0.07	1.041
Unknown	0.59	0.978	1.242	0.00	2.80	1.40	0.02	0.056
Unknown	1.09	2.954	0.664	0.66	11.05	1.84	0.05	0.168
Unknown	1.26	2.452	0.533	1.35	1.87	1.16	0.06	0.139
Unknown	1.54	0.209	0.069	0.81	3.05	1.22	0.05	0.012
Unknown	1.69	0.884	0.251	1.20	1.69	1.10	0.06	0.050
Unknown	2.14	0.432	0.126	0.85	4.84	1.27	0.05	0.025
Ibuprofen	2.42	849.701	174.753	1.26	2.55	1.13	0.07	48.316
Unknown	2.82	0.569	0.161	0.86	3.57	1.17	0.06	0.032
Valerophenone IS	3.11	877.297	168.767	1.11	2.54	1.11	0.08	49.885
IBP related compound C	3.67	0.550	0.081	1.05	3.54	1.18	0.10	0.031
Unknown	3.96	0.629	0.074	1.14	1.52	1.08	0.14	0.036
Unknown	4.65	1.391	0.167	1.21	3.44	1.17	0.13	0.079
Unknown	5.26	2.301	0.306	0.95	3.10	1.13	0.12	0.131

The UV spectra of ibuprofen-related compound C in the standard solution and in the tablet sample solution were obtained using a UV-Vis spectrophotometer with a diode array detector and the Galaxie™ Chromatography Software. Figure 6 shows the UV spectra of those chromatographic peaks. The spectra allowed the confirmation of known impurity ibuprofen-related compound C in the sample solution.

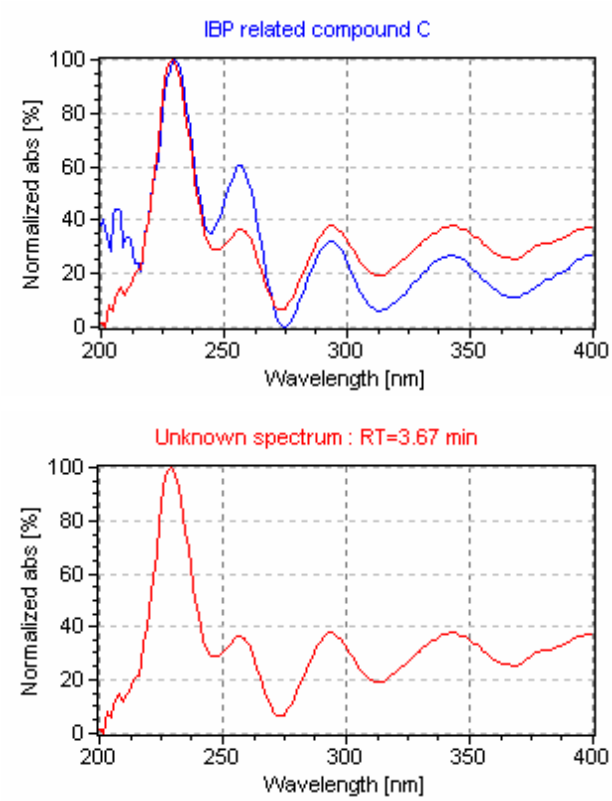

Figure 6. Normalized and lay out UV spectra of ibuprofen-related compound C (Rt = 3.67 min) in ibuprofen tablet sample solution and in standard solution

The peak height and peak area of the ibuprofen-related compound C in the ibuprofen tablet sample solution were both very small (Figure 5) compared to the peak height and peak area of the 0.012 mg/mL ibuprofen-related compound C in the standard solution. This indicates that the concentration of ibuprofen-related compound C in these tablets was well below the 0.1% limit.

A diluted ibuprofen-related compound C was prepared with a concentration of 0.0012 mg/L, 10 times less than the concentration limit specified in the USP method, to test the analytical performance at this level. Five replicate injections were made for this solution, which also included the internal standard. The precision in retention time and peak area are given in Table 6, which shows that excellent precision was obtained. This method is well able to quantify the ibuprofen-related compound C at levels well below the acceptance limit set in the USP method.

Table 6. Chromatographic performance for replicate injections of ibuprofen-related compound C standard solution (0.0012 mg/mL) using a Pursuit™ XRs C18 3 µm 100x4.6 mm column and USP method

	Valerophenone IS	Ibuprofen-related compound C
Peak symmetry	1.14	1.07
Resolution		4.04
Avarage Peak Area (millabsorbance seconds), n = 5	883.295	7.165
%RSD of Rt (n = 5)	0.25	0.23
%RSD of Area (n = 5)	0.29	1.51

Conclusion

The assay for ibuprofen tablets and the Limit Test for Ibuprofen-related compound C specified in the standard USP monographs can be applied using the Pursuit XRs C18 3 µm 100x4.6 mm column and Varian 920-LC liquid chromatograph. This provides a significant reduction in run time, along with excellent results for ibuprofen-related compound C at a concentration below the limit set by the USP method. Data are collected by a UV-Vis spectrophotometer with diode array detector and processed by the Galaxie Chromatography Software, for preliminary confirmation of identification of known impurities or even unknown impurities having a spectrum similar to that of the API.

Using the Varian 920-LC, Varian column and Galaxie Chromatography Software provided efficient analysis not only for identification, but also for quantitation of the impurities in pharmaceutical substances with improved laboratory productivity and quality results.

Acknowledgements

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References

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[3] United States Pharmacopeia. Published November 2006, Official May 1, 2007. USP30-NF25, USP Monographs: Ibuprofen Oral Suspension.

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