Validation of Changes to the USP Assay Method for Ibuprofen Tablets

Extraction and Filtration Techniques

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This article discusses changes made to the *USP* assay preparation of ibuprofen (IBP) tablets, including the direct extraction of tablets and the filtration of extracts. In four formulations tested on the basis of recoveries exceeding 97% of nominal concentrations, the authors show that there are no differences in outcome between centrifugation used in the *USP* method and a filtration method using a 0.45-μm PTFE filter for separating solids from the extraction solution. No extractable filter-derived components were found in chromatograms. The results demonstrate the validation of recoveries of IBP after direct extraction of tablets without glass beads and filtration of extracts without centrifugation, which are the two significant changes made to the *USP* method.

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Changes were made to the *USP* method for the preparation of assay samples of ibuprofen (IBP) tablets (1). These changes included extraction and filtration techniques during sample preparation and required validation for the quantitation of IBP in assay samples. In this article, which is the first in a series about the validation of changes to the *USP* IBP assay method, we describe the validation of sample extraction and filtration techniques. Specifically, the extraction and filtration validation addresses the following:

- the effect of direct extraction (versus the powdering of tablets and extraction or the shaking of coated tablets with glass beads as per *USP* method) and shaking time on the disintegration of tablets in extraction solvent and the solubilization of the active ingredient as determined by the recovery of IBP from tablets
- the effect of filtration (versus a centrifugation technique as per the *USP* method) of extracts on the recovery of IBP from tablet assay preparations.

Materials and methods

Properties of IBP. IBP [α-methyl-4-(2-methylpropyl)benzeneacetic acid; CAS# 1567-27-1; molecular formula $C_{13}H_{18}O_2$; and molecular weight 206.28] is a colorless, crystalline, stable solid with a melting point of 75–77 °C. It is relatively insoluble in water (<1 mg/mL) but readily soluble in most organic solvents.

Reference standards. We obtained IBP reference standard–bulk drug substance from BASF Corporation (Bishop, TX). The equivalency of this standard to the *USP* IBP primary standard was demonstrated. 4-Isobutylacetophenone (4-IBAP) reference standard was manufactured by TCI America (Portland, OR). Valerophenone (internal standard [ISTD]) also was manufactured by TCI America.

IBP reference standard solution. We prepared a concentrated stock solution of IBP reference standard and added it to the placebo to achieve the desired concentration. The reference standard was prepared and used the same day.

4-IBAP standard stock solution. We prepared the 4-IBAP standard

Table I: Data from a representative system suitability run.*

	IBP				ISTD					4-IBAP				
Standard Injection #	RT (min)	TF	RRT (min)	Peak Area	RT (min)	TF	Res	RRT (min)	Peak Area	RT (min)	TF	Res	RRT (min)	Peak Area
1	4.087	1.8	0.76	2042.9	5.410	1.2	6.8	1.0	2641.3	6.365	1.2	4.9	1.18	128.7
2	4.085	1.8	0.76	2041.5	5.407	1.2	6.8	1.0	2643.8	6.363	1.1	4.9	1.18	126.0
3	4.085	1.8	0.76	2043.6	5.408	1.2	6.7	1.0	2641.9	6.366	1.1	4.9	1.18	124.2
4	4.088	1.8	0.76	2043.3	5.411	1.2	6.7	1.0	2643.8	6.368	1.1	4.9	1.18	124.3
5	4.088	1.8	0.76	2043.5	5.411	1.2	6.7	1.0	2645.1	6.368	1.1	4.9	1.18	124.2
	System precision (%RSD): 0.04				System precision (%RSD): 0.06					System precision (%RSD): 1.56				

*RT = retention time; TF = tailing factor; RRT = relative retention time = average RT of ISTD ÷ average RT of IBP or 4-IBAP; Res = resolution to preceding peak. Because IBP is the first peak in the chromatogram, no resolution is reported.

Table II: Validation of direct extraction method. IBP recovered as percentage of normal concentration, tablet replicate average (%), and RSD (%).

200 mg of Formula #1, Color #1

	Tablets					Placebo Spiked with IBP				
	Replicate #					Replicate #				
Time (h)	1	2	3	Average	RSD	1	2	3	Average	RSD
0.5	98.9	99.8	100.4	99.7	0.4	101.0	101.3	100.6	101.0	0.4
1	99.8	99.8	98.3	99.3	0.9	100.9	101.3	100.3	100.9	0.5
2	99.5	98.7	100.3	99.5	0.5	101.2	100.9	101.0	101.0	0.1
3	99.5	99.2	100.2	99.6	0.5	102.0	101.6	100.8	101.5	0.6
5	99.8	98.4	100.8	99.7	1.2	101.0	101.4	101.5	101.3	0.2

200 mg of Formula #1, Color #2

	Tablets					Placebo Spiked with IBP				
	Replicate #					Replicate #				
Time (h)	1	2	3	Average	RSD	1	2	3	Average	RSD
0.5	88.1	87.5	82.0	85.9	3.9	99.9	99.4	98.6	99.3	0.7
1	98.2	97.5	97.7	97.8	0.3	99.5	99.0	100.0	99.5	0.5
2	98.3	98.2	99.3	98.6	0.6	99.4	99.2	100.1	99.6	0.5
3	99.2	99.3	98.2	98.9	0.6	99.2	99.8	99.8	99.6	0.3
5	99.6	97.6	100.2	99.1	1.3	99.9	99.4	100.0	99.6	0.3

400 mg of Formula #2

	Tablets					Placebo Spiked with IBP				
	Replicate #					Replicate #				
Time (h)	1	2	3	Average	RSD	1	2	3	Average	RSD
0.5	98.1	95.2	95.1	96.1	1.8	99.2	99.9	99.6	99.6	0.4
1	100.1	99.8	100.0	100.0	0.1	98.8	99.2	99.8	99.2	0.5
2	99.9	100.7	100.1	100.2	0.4	99.9	99.4	99.8	99.2	0.5
3	99.7	100.2	100.2	100.0	0.3	99.1	99.5	98.6	99.0	0.4
5	100.6	98.8	99.9	99.8	0.9	99.9	99.4	99.7	99.7	0.3

600 mg of Formula #2

	Tablets					Placebo Spiked with IBP				
	Replicate #					Replicate #				
Time (h)	1	2	3	Average	RSD	1	2	3	Average	RSD
0.5	96.7	97.1	98.5	97.4	1.0	99.2	99.9	99.6	99.6	0.4
1	98.5	99.3	99.4	99.1	0.5	98.8	99.2	99.8	99.2	0.5
2	99.5	98.6	99.3	99.1	0.5	99.9	99.4	99.8	99.2	0.5
3	99.3	98.8	99.6	99.2	0.4	99.1	99.5	98.6	99.0	0.4
5	100.7	98.9	98.7	99.4	1.1	99.9	99.4	99.7	99.7	0.3

stock solution in acetonitrile (ACN) at a 0.6 mg/mL concentration. The 4-IBAP stock solution was kept refrigerated.

Extraction solution. We prepared ISTD in ACN and chloroacetic acid, the two components of the mobile phase. ISTD (3.5 mL) was added to 4000 mL of ACN and mixed well. We then added 2000 mL of ACN and 4000 mL of 1% chloroacetic acid and mixed the solution well again (60% of ACN and 40% of 1% chloroacetic acid). This extraction solution was stored at room temperature in a light-resistant container and was used to extract tablets (tablet assay preparation).

Standard mix solution. We added 2 mL of 4-IBAP standard stock solution to a 100-mL volumetric flask containing 1200 mg of IBP. The contents were diluted to volume with extraction solvent, which contained ISTD. System suitability and calibration (quantitation of IBP and 4-IBAP) determinations were made using this standard mix (designated as "standard" [STD]), which was prepared fresh daily and in duplicate. The STD solution was prepared and used the same day.

Preparation of the mobile phase. The mobile phase was similar to the extraction solvent. We prepared chloroacetic acid in water (40 g in 4000 mL of water [1%]), adjusted the solution with ammonium hydroxide to a pH of 3.0, and filtered the solution. Chloroacetic acid and ACN were degassed in a high-performance liquid chromatography (HPLC) system using a vacuum degasser (Agilent 1100, Agilent Technologies, Wilmington, DE) and were mixed on-line during HPLC analysis in a ratio of 40:60%, respectively.

Preparation of IBP tablets for assay. Tablets from four formulations were used. The following drug product tablet sam-

(Table II Continued)

800 mg of Formula #3

	Tablets					Placebo Spiked with IBP				
	Replicate #					Replicate #				
Time (h)	1	2	3	Average	RSD	1	2	3	Average	RSD
0.5	98.1	98.8	99.1	98.6	0.5	100.9	100.8	100.7	100.8	0.2
1	98.5	99.1	98.7	98.8	0.3	100.5	100.8	100.5	100.6	0.2
2	99.7	98.4	98.5	98.8	0.7	100.8	100.8	101.5	101.0	0.4
3	99.2	98.7	98.9	98.9	0.3	100.6	101.0	101.2	100.9	0.3
5	99.2	98.5	98.3	98.7	0.5	100.6	101.2	100.9	100.9	0.3

200 mg of Formula #4

	Tablets					Placebo Spiked with IBP				
	Replicate #					Replicate #				
Time (h)	1	2	3	Average	RSD	1	2	3	Average	RSD
0.5	98.7	98.3	98.5	98.5	0.2	100.7	100.6	101.1	100.8	0.2
1	100.2	98.7	97.6	98.8	1.3	100.9	100.4	100.8	100.7	0.3
2	98.5	100.3	98.6	99.1	1.0	99.9	99.9	100.0	99.9	0.1
3	99.5	97.5	99.4	98.8	1.2	99.3	99.6	99.3	99.4	0.2
5	98.2	99.7	98.9	98.9	0.8	102.1	101.8	101.1	101.7	0.5

400 mg of Formula #4

	Tablets					Placebo Spiked with IBP				
	Replicate #					Replicate #				
Time (h)	1	2	3	Average	RSD	1	2	3	Average	RSD
0.5	99.0	99.4	97.3	98.6	1.2	100.7	100.6	101.1	100.8	0.2
1	101.6	98.3	99.2	99.7	1.7	100.9	100.4	100.8	100.7	0.3
2	98.7	98.5	98.4	98.5	0.1	99.9	99.9	100.0	99.9	0.1
3	99.4	99.9	99.1	99.5	0.4	99.3	99.6	99.3	99.4	0.2
5	100.1	99.4	97.5	99.0	1.4	102.1	101.8	101.1	101.7	0.5

600 mg of Formula #4

	Tablets					Placebo Spiked with IBP				
	Replicate #					Replicate #				
Time (h)	1	2	3	Average	RSD	1	2	3	Average	RSD
0.5	100.5	98.9	101.2	99.9	0.9	100.7	100.6	101.1	100.8	0.2
1	99.3	99.3	98.9	99.2	0.3	100.9	100.4	100.8	100.7	0.3
2	99.5	99.2	100.4	99.7	0.6	99.9	99.9	100.0	99.9	0.1
3	98.6	98.2	98.7	98.5	0.3	99.3	99.6	99.3	99.4	0.2
5	99.5	99.6	99.6	99.6	0.1	102.1	101.8	101.1	101.7	0.5

800 mg of Formula #4

	Tablets					Placebo Spiked with IBP				
	Replicate #					Replicate #				
Time (h)	1	2	3	Average	RSD	1	2	3	Average	RSD
0.5	99.3	98.3	100.7	99.4	1.2	100.7	100.6	101.1	100.8	0.2
1	100.4	99.8	99.5	99.9	0.5	100.9	100.4	100.8	100.7	0.3
2	102.1	99.5	99.2	100.2	2.0	99.9	99.9	100.0	99.9	0.1
3	99.5	99.6	98.2	99.1	0.8	99.3	99.6	99.3	99.4	0.2
5	99.6	99.1	99.2	99.3	0.2	102.1	101.8	101.1	101.7	0.5

ples were used in the methods validation study:

- 200 mg (formulation 1, two colors)
- 400 mg and 600 mg (formulation 2)
- 800 mg (formulation 3)
- 200, 400, 600, and 800 mg (formulation 4).

We varied the number of tablets used and the amount of extraction solution added for each tablet label claim (200, 400, 600, and 800 mg) to obtain a final concentration of 12 mg of IBP/mL of ISTD for the assay. The tablets and the ISTD solution were shaken for the appropriate time defined during the methods validation and were filtered. Samples were prepared in triplicate with one HPLC injection per replicate.

Preparation of placebos. We prepared placebos for all formulations listed in the previous section. Each placebo was prepared in the extraction solution by weighing the excipients and mixing in proportion to the tablet assay preparation. To calculate the amount of excipient required per sample solution equivalent to the tablet assay preparation, the amount of excipient (mg/tablet) was multiplied by the number of tablets used and divided by the volume (mL) of extraction solution as follows:

Excipient (mg/tablet) ×
Number of tablets ÷
Volume of extraction solution (mL)
= Concentration of excipient (mg/mL)
as in the sample matrix.

The following equation was used to calculate the amount of excipient used for various volumes of solutions prepared:

Concentration of excipient in
the sample matrix (mg/mL) ×
Volume of solution (mL)
= Weight of excipient (mg)

If the weight of any excipient was <1 mg, then 1 mg was weighed.

HPLC equipment and conditions. We performed an HPLC analysis of the samples using a UV detector set at a wavelength of 254 nm. The column was 4.6 mm × 25 cm and contained packing material L1 with a column temperature of 40 °C. The flow rate was 2 mL/min. Agilent Chemstation software was used to analyze HPLC peak responses for the quantitation of the peaks of interest in standards and samples. We used

Table III: Validation of filtration technique during IBP tablet assay sample preparation for determination of IBP content. IBP recovered as percentage of nominal concentration, tablet replicate average (%), and RSD (%).

	Unfiltered and Centrifuged Samples				Filtered Samples				% Difference
Formulation	Rep #1	Rep #2	Rep #3	AVG (RSD)*	Rep #1	Rep #2	Rep #3	AVG (RSD)*	in Recovery
200 mg, formula #1 color #1 tablets	99.9	98.3	101.0	99.7 (1.4)	99.8	98.4	100.8	99.7 (1.2)	0.0
200 mg, formula #1 color #1 placebo	101.0	101.2	101.5	101.2 (0.2)	101.0	101.4	101.5	101.3 (0.3)	0.1
200 mg, formula #1 color #2 tablets	99.9	97.9	100.6	99.5 (1.4)	99.6	97.6	100.2	99.1 (1.4)	0.4
200 mg, formula #1 color #2 placebo	99.4	99.8	99.6	99.6 (0.2)	99.9	99.4	100.0	99.8 (0.3)	0.2
600 mg, formula #2 tablets	100.5	98.8	98.7	99.3 (1.0)	100.7	98.9	98.7	99.4 (1.1)	0.1
600 mg, formula #2 placebo	99.7	99.0	99.7	99.5 (0.4)	99.9	99.4	99.7	99.7 (0.3)	0.2
800 mg, formula #3 tablets	98.9	98.1	98.4	98.5 (0.4)	99.2	98.5	98.3	98.7 (0.5)	0.2
800 mg, formula #3 placebo	100.6	100.6	100.9	100.7 (0.2)	100.6	101.2	100.9	100.9 (0.3)	0.2
800 mg, formula #4 tablets	99.1	99.5	99.0	99.2 (0.3)	99.6	99.1	99.2	99.3 (0.3)	0.1
800 mg, formula #4 placebo	101.6	101.1	100.7	101.2 (0.0)	102.1	101.8	101.1	101.7 (0.3)	0.5

*AVG = average; RSD = relative standard deviation (%)

an Agilent photodiode array detector to determine the purity of the peaks of interest.

Calculation of IBP content in IBP bulk drug substance and in tablets. We determined IBP content in IBP bulk drug substance and in tablets by using the chromatograms of bulk drug and tablet assay preparations and chromatograms of the STD solution. The quantity as percentage IBP is calculated using the following equation:

$$\text{Injection result} = (R_u \div R_s) \times \%\text{IBP}$$

in which R_u is the area response of IBP in the sample injection divided by the area response of ISTD in the sample injection; R_s is the area response of IBP in the standard injection divided by the area response of ISTD in the sample injection; and

$$\%\text{IBP} = C_{\text{IBP}} \div 12 \times 100 \times K\epsilon$$

in which

$$C_{\text{IBP}} = W_{\text{IBP-STD}} \times 1000 \div 100$$

$W_{\text{IBP-STD}}$ is the amount of IBP in grams transferred into IBP standard, Kϵ is the equivalency factor of IBP standard material (0.996), and 12 is the established theoretical amount in milligrams per milliliter of IBP.

System suitability specifications. System suitability results were taken from the "Statistics Report" of the Agilent Chemstation, which was used to integrate and analyze the HPLC peak responses for quantitation. We performed system suitability with each run sequence of samples (daily) using five injections of each of the two standard mix (STD) preparations. System suitability results were evaluated for the following specifications:

- relative retention times of ~0.75 for IBP, 1.0 for ISTD, and 1.2 for 4-IBAP
- tailing factors for individual peaks of not more than 2.5
- resolution *R* between the IBP, ISTD, and 4-IBAP peaks not less than 2.5
- relative standard deviation (RSD) for replicate injections of not more than 2.0%.

Validation of direct extraction procedure and shaking time. We studied the effectiveness of direct extraction, without glass beads, of tablets and the shaking time (shaking for 0.5, 1, 2, 3, and 5 h using wrist-action shakers [model 75, Burrell, Pittsburgh, PA]) on the disintegration of tablets, solubilization, and recovery of IBP from tablets of various strengths and spiked placebo. Tablets containing IBP at strengths of 200 (two colors), 400, 600, and 800 mg representing four formulations (see section "Preparation of IBP tablets for assay") were placed in extraction solution for a final IBP concentration of 12 mg/mL. Tablets from the fourth formulation (200, 400, 600, and 800 mg) were prepared in a similar manner. Respective placebo matrix formulations were prepared in extraction solution and were spiked with IBP reference standard stock solution at a final concentration similar to that targeted for tablet preparations (~12 mg of IBP/mL of extraction solution). Spiked placebo matrixes were shaken

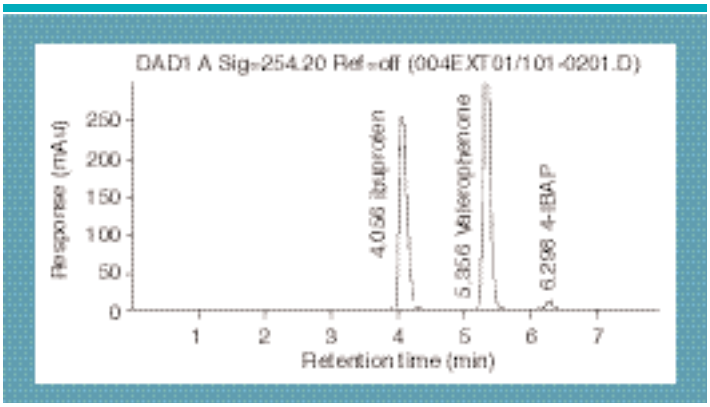

Figure 1: Representative chromatogram of typical system suitability runs.

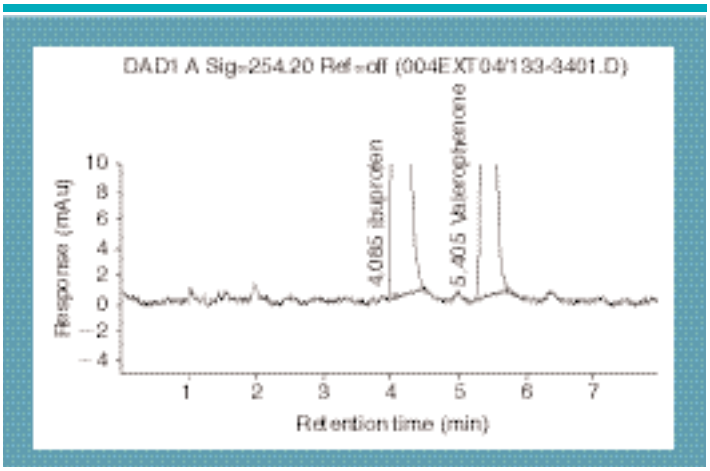

Figure 2: Representative chromatogram of centrifuged assay preparations.

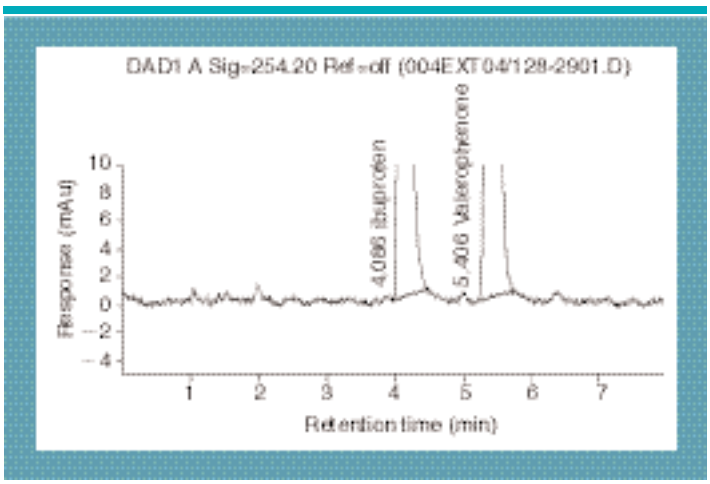

Figure 3: Representative chromatogram of assay preparation (filtered) showing the absence of any filter-related components in assay preparation.

and processed in a manner similar to that for tablets. Aliquots of all samples were filtered using a 0.45-μm PTFE membrane filter (Whatman, Inc., Clifton, NJ). The filtered samples were assayed by HPLC. IBP in samples was expressed as the percentage of nominal concentration recovered.

Validation of filtration technique during sample preparation for the determination of IBP content. To facilitate proper HPLC injection and analysis, we filtered samples using the 0.45-μm PTFE filter to remove undissolved and particulate excipient material. The objective of this process was to show that there was no loss of IBP as a result of filtration and that there were no differences between centrifugation (used in the *USP* method) and filtration. The filtered samples for the IBP tablets and the placebo were derived from the direct extraction validation experiments described previously. Three aliquots of the unfiltered solutions were centrifuged for comparison with these filtered samples. The filtrate and the supernatant of each sample were subjected to HPLC analysis, and the differences in percentage of IBP recovered from these two matrixes were estimated. The HPLC-UV chromatograms were examined for any components extracted from filter materials and their potential interference with the IBP, ISTD, and 4-IBAP peaks.

Results and discussion

System suitability specifications. System suitability samples were included for each analytical run during the methods validation study. We used five standard injections and presented the tailing factor, retention times, and resolution for each standard run. We calculated the relative retention times of IBP and 4-IBAP with respect to ISTD (1.0) using the retention-time values. The system suitability criteria were met for all runs. Table I shows the results from a representative system suitability run. The relative retention times for IBP, ISTD, and 4-IBAP were 0.76, 1.0, and 1.18, respectively. The tailing factors for IBP, ISTD, and 4-IBAP were 1.82, 1.19, and 1.13, respectively. The resolution *R* of the ISTD and 4-IBAP peaks with respect to the IBP and the ISTD peaks was 6.75 and 4.90, respectively. The system precision was demonstrated with %RSD of the peak areas of IBP, ISTD, and 4-IBAP for the five injections being 0.04, 0.06, and 1.56, respectively (see Table I, Figure 1).

Validation of direct-extraction procedure and shaking time on the recovery of IBP. The average direct-extraction recoveries of IBP from tablets from all four formulations ranged from 90 to 100%, with the exception of 200-mg color #2 tablets, in which the average extraction recovery was 85.9% (82.0–88.1% range across three replicate determinations) after 0.5 h of shaking. However, another 200-mg formulation at 0.5 h had 99.7% IBP, with a range of 98.9–100.4%. At a shaking time of 1 h, the mean recoveries across all tablet strengths from all formulations ranged from 97.8 to 100%, with the individual replicate values for IBP recovery across the spectrum ranging from 97.5 to 102%. No significant differences were evident between the recoveries observed after a 1-h shaking time and those after longer shaking times (e.g., at 2, 3, and 5 h). Recoveries of IBP from spiked placebo samples exceeded 98%, with a range of 98.4–102.1% across the spectrum, thereby further confirming the results obtained from the experiments with the tablets and supporting the use of a 1-h shaking time for extraction. On the basis of these results (97.8–100%), a shaking time of 1 h was selected for the remaining phases of the methods validation study (see Table II).

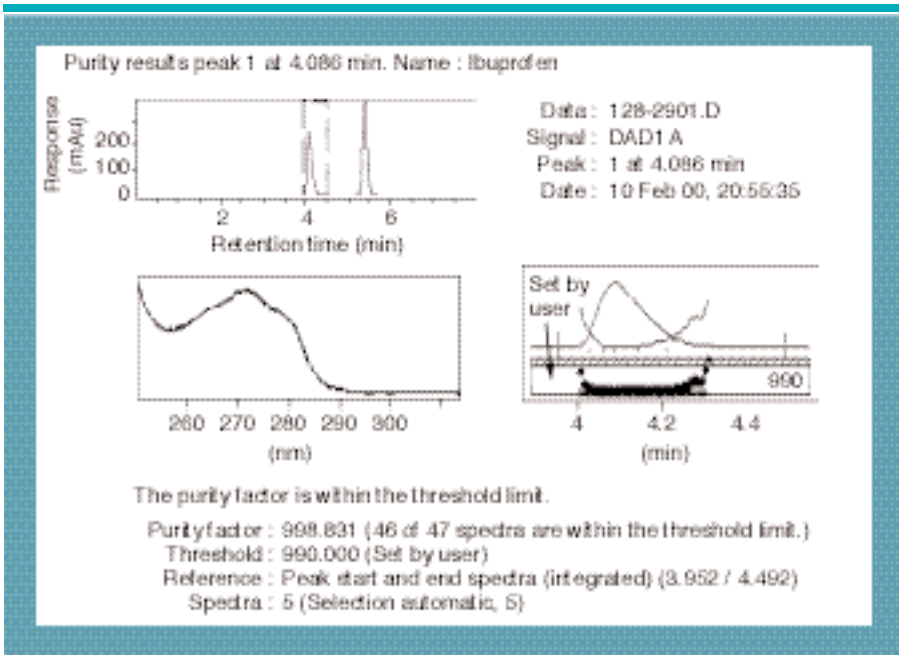

Figure 4: Photodiode array detector scans of ibuprofen peak in filtered samples showing purity of IBP peak.

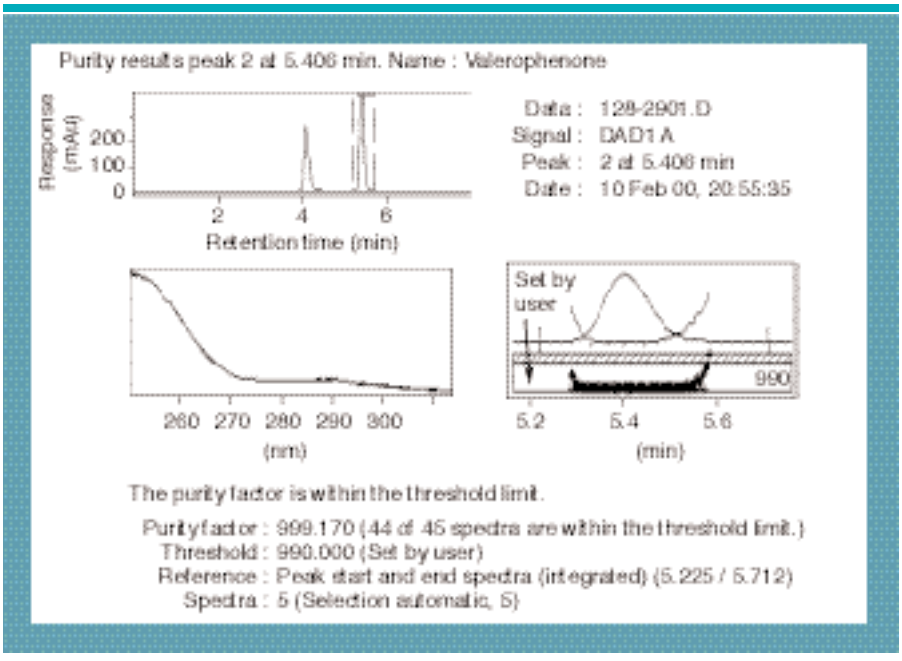

Figure 5: Photodiode array detector scans of ISTD (Valerophenone) peak in filtered samples showing purity of ISTD peak.

Validation of filtration technique for sample preparation for IBP content. To validate the filtration technique, we examined tablets from the four formulations and corresponding placebos spiked with IBP (see Table III). The largest difference between the amount of IBP in filtered and unfiltered centrifuged samples was 0.5%. No extractable filter-derived components were present or interfered with the analytes of interest (IBP, 4-IBAP, and ISTD) in the HPLC chromatograms of these preparations. In the expanded scale, base-line noise and IBP and ISTD peaks were seen. The chromatograms were similar to those of the centrifuged samples (see Figures 2 and 3). Photodiode array detector scans of IBP and ISTD from filtered samples showed no evidence of coeluting peaks and confirmed peak purity (see Figures 4 and 5) of the respective peaks. The observed peak resolution was >2.5.

Summary and conclusion

This study investigated the effectiveness of the direct extraction of tablets and the shaking time (0.5, 1, 2, 3, and 5 h) on the disintegration of tablets, solubilization, and recovery of IBP from tablets of various formulations, strengths, and spiked placebo. The direct extraction and shaking time of 1 h was selected for all tablet strengths of four formulations tested on the basis of recoveries exceeding 97% of nominal concentrations. Filtration of sample extracts (tablet and spiked placebo samples) through a 0.45-μm PTFE filter showed that no IBP was lost as a result of filtration and no differences exist between centrifugation (used in *USP* method) and filtration methods. The largest difference between the amount of IBP in filtered and unfiltered centrifuged samples was 0.5%, and no extractable filter-derived components were present in the chromatograms. These results demonstrate the validation of recoveries of IBP after the direct extraction of tablets (without glass beads) and filtration of extracts (without centrifugation), which are the two significant changes made to the *USP* method for IBP tablet assay sample preparation.

Reference

1. "Ibuprofen Tablets," in *USP 25–NF 20* (The United States Pharmacopeial Convention, Rockville, MD, 2002), pp. 886–887. **PT**