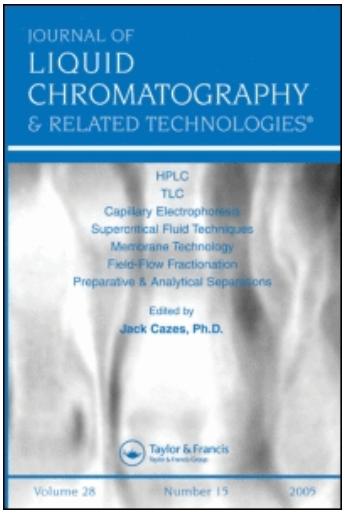

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Development and Validation of an HPLC Method for the Determination of 2-(4-Isobutylphenyl) Propionic Acid and 4-Isobutylacetophenone in a Gel Formulation

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Development and Validation of an HPLC Method for the Determination of 2-(4-Isobutylphenyl) Propionic Acid and 4-Isobutylacetophenone in a Gel Formulation

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Abstract: A simple isocratic reversed-phase high performance liquid chromatographic (HPLC) method was developed for the determination of 2-(4-isobutylphenyl) propionic acid and its major impurity 4-isobutylacetophenone in a gel formulation. The chromatographic separation was achieved with sodium phosphate buffer (pH 3.0) and methanol (40:60, v/v) as mobile phase, a ChromSpher C_{18} column (100 mm × 3 mm and 5 μm), and UV detection at 220 nm. The analysis time was less than 10 min. The method was validated with respect to linearity, range, precision, accuracy, specificity, and robustness. The calibration curves showed good linearity over the concentration range of 0.10–80 μg/mL. The correlation coefficients were ≥0.9997 in each case. The RSD values for intra- and inter-day precision studies were less than 0.50%. The procedure described here is simple, selective, and is suitable for routine quality control analysis.

Keywords: 2-(4-Isobutylphenyl) propionic acid, 4-Isobutylacetophenone, Gel formulation, Reversed-phase HPLC, Method development, Method validation

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INTRODUCTION

2-(4-Isobutylphenyl) propionic acid (Ibuprofen, Figure 1b) is a non-steroidal anti-inflammatory drug that is used extensively in the treatment of many forms of arthritis, and it is one of the top ten drugs sold worldwide.[1] Ibuprofen works by reducing hormones that cause inflammation and pain in the body. It is used to reduce fever and treat pain or inflammation caused by many conditions, such as headache, toothache, back pain, arthritis, menstrual cramps, or minor injury. It is a white crystalline powder with a molecular weight of 206.27, pKa (COOH) = 4.41, and low solubility in water and high solubility in organic solvents and diluted solutions of hydroxides or alkaline carbonates.[2]

A literature survey[3–12] revealed that there are a number of various analytical methods available for individual determination of ibuprofen, or combination with other drugs.

The United States Pharmacopoeia (USP) describes two different reversed-phase HPLC methods, one for impurities in raw materials using UV detection at 214 nm, and another one for ibuprofen determination in pharmaceuticals and in raw materials using UV detection at 254 nm. With regard to the related substances test, the area of an individual secondary chromatographic peak, which appears in the test sample, should not exceed 0.3% of the ibuprofen peak area, and the total area of all secondary peaks, 1% ibuprofen peak area (the compounds are not specified).[13] A similar HPLC method has been described for ibuprofen determination in the British Pharmacopoeia (BP) in pharmaceuticals and raw materials.[14] BP specifies five substances as possible impurities [one of them 4-Isobutylacetophenone (IBAP)].

It is well known that IBAP (MW: 176.25844) causes adverse effects in the central nervous system and presents high dermal absorption. For these reasons, its control is necessary, especially for gels (a recent pharmaceutical preparation). However, it is worth noting the lack of official methods for assays of active ingredients in pharmaceutical preparations in reputed pharmacopoeias, especially when it is currently possible to find a plethora of methods based on reversed-phase HPLC for the determination of these compounds. As an example, Ghosh[15] has described HPLC methods for hundreds of active ingredients. However, only a few of the reported methods have been

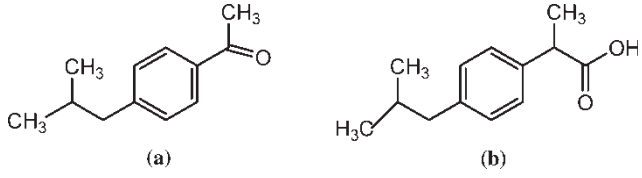

Figure 1. Chemical structure of the analytes in order of elution: (a) 4-isobutylacetophenone, (b) ibuprofen.

adequately validated.[16–20] The aim of this study was to develop and validate a simple isocratic reversed-phase HPLC method, which would be able to determine an ibuprofen drug substance and its major impurity IBAP in a gel formulation. It is shown that the developed method is applicable for the determination of these compounds in a gel formulation.

EXPERIMENTAL

Materials

Methanol (HPLC-grade) was obtained from Merck (Darmstadt, Germany). 2-(4-Isobutylphenyl) propionic acid, 4-isobutylacetophenone, sodium dihydrogen orthophosphate (NaH_2PO_4), and orthophosphoric acid (H_3PO_4) were purchased from Sigma chemicals (St. Louis, MO, USA). Deionised distilled water was used throughout the experiment.

Instruments

A PerkinElmer HPLC system equipped with a module LC 235C diode array detector (DAD), series 200 LC pump, series 200 autosampler, and series 200 peltier LC column oven (Norwalk, CT, USA) were used in this work. The data were acquired via PE TurboChrom Workstation data acquisition software using PE Nelson series 600 LINK interfaces.

Chromatographic Conditions

The chromatographic column used was ChromSpher polymeric octadecylsilane (ODS)-encapsulated spherical silica with dimensions of 100 mm × 3.0 mm with 5 μm particle size, obtained from Varian (Palo Alto, CA, USA). The isocratic method was employed, with the mobile phase consisting of 25 mM sodium dihydrogen orthophosphate (adjusted to pH 3.0 using orthophosphoric acid) and methanol (40:60, v/v). The column temperature was maintained at 25°C and the detection was monitored at a wavelength of 220 nm. Injection volume was 10 μL and the mobile phase flow was set at 1.0 mL/min.

Standard Preparation

An accurately weighed amount (50 mg) of ibuprofen, standard was placed in a 100 mL volumetric flask and dissolved in methanol (solution A). An accurately weighed amount (10 mg) of IBAP, standard was placed in a 100 mL

volumetric flask and dissolved in methanol (solution B). A 10 mL aliquot of stock solution A and 5 mL of stock solution B were added to a 100 mL volumetric flask and diluted in 45 mL methanol, and the volume made up with a phosphate buffer (working standard solution).

Sample Preparation

An accurately weighed amount (1.0 g) of gel sample was placed in a 100 mL volumetric flask and dissolved in methanol. A 10 mL aliquot solution was added to a 100 mL volumetric flask and diluted in 50 mL methanol, and the volume made up with a phosphate buffer (working sample solution).

RESULTS AND DISCUSSION

Method Development and Optimization

Impurity 4-isobutylacetophenone (IBAP) is a major impurity of ibuprofen drug substance. The main target of the chromatographic assay method development was to separate the impurity coeluted with ibuprofen. This impurity was coeluted by using different stationary phases such as C_{18}, C_8, and different mobile phases containing buffer like phosphate with different pH and temperatures, using organic modifiers like acetonitrile, methanol, and tetrahydrofuran in the mobile phase. The analytical column temperature has played a critical role in achieving the separation of this impurity IBAP and ibuprofen drug substance.

Chromatographic separation was achieved on C_{18} stationary phase, ChromSpher ODS, 100 mm × 3.0 mm, 5 μm column by using a mobile phase consisting of the mixture of 25 mM sodium dihydrogen orthophosphate (adjusted to pH 3.0 using orthophosphoric acid) and methanol (40:60, v/v), with column temperature set at 25°C. In the optimized conditions, the impurity namely IBAP and the drug substance ibuprofen were well separated with a resolution of more than 4.25. The optimal wavelength for IBAP and ibuprofen detection was established using UV absorbance scans over the range of 190 to 360 nm. It was shown that 220 nm is the optimal wavelength to maximize the signal. Figure 2 shows the UV absorption spectrum of IBAP and ibuprofen drug substance.

To assess the quantitative nature of the method, a series of samples with different amounts of IBAP and ibuprofen were run to investigate the best assay concentration. Six standard solutions of impurity IBAP and ibuprofen were prepared in the range of 0.02–200 μg/mL and a 10 μL injection was drawn from each solution. The integrated peak areas were plotted versus amount injected. The calibration curve was found to be linear from the concentration range 20–80 μg/mL with a correlation coefficient of 0.9999 for ibuprofen and

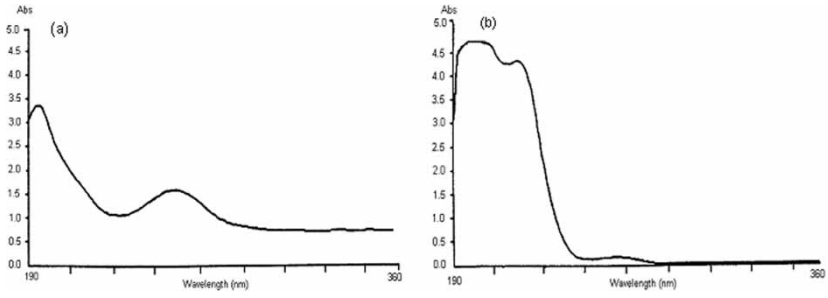

Figure 2. UV spectra: (a) 4-isobutylacetophenone; (b) ibuprofen.

0.10–50 μg/mL for IBAP ($r^2 = 0.9997$). On the bases of this data, the best concentrations, 5 μg/mL for impurity IBAP and 50 μg/mL for ibuprofen drug substance, were chosen as a working concentration for the assay. The chromatograms obtained for standard and gel sample are shown in Figures 3 and 4, respectively. The impurity peak IBAP was eluted at 6.0 min and ibuprofen (principal peak) was eluted at 7.72 min.

The system suitability testing was performed to determine the accuracy and precision of the system from six replicate injections of a solution containing 5 μg IBAP/mL and 50 μg ibuprofen/mL. The percent relative standard deviation (%RSD) of the peak areas was found to be less than 0.16% in each case. The separation factor (α) was calculated using the equation, $\alpha = k_2/k_1$ where k_1 and k_2 are the retention factors for the first and last eluted peaks, respectively. The separation factor for ibuprofen peak obtained was 2.16. The plate number (also known as column efficiency, N) was calculated as $N = 5.54\ (t_r/w_{0.5})^2$, where $w_{0.5}$ is the peak width at half

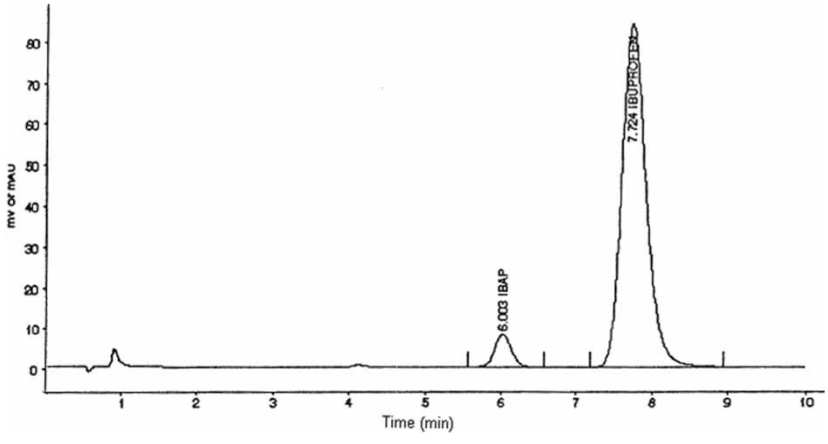

Figure 3. HPLC chromatogram of standard 4-isobutylacetophenone and ibuprofen.

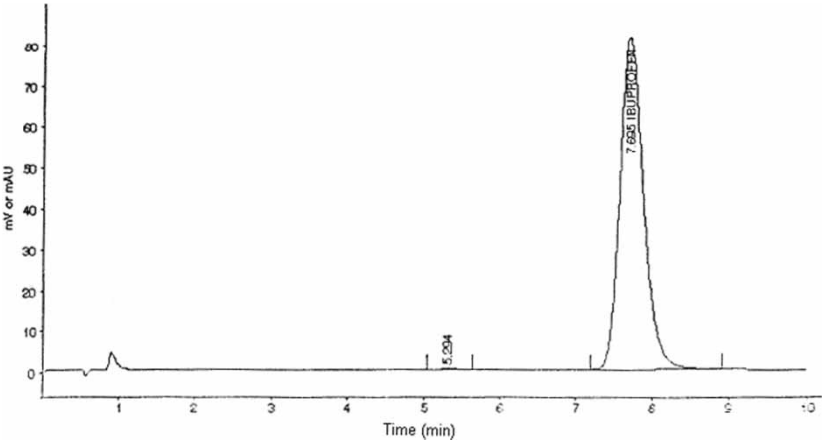

Figure 4. HPLC chromatogram of gel sample.

peak height. In this study, the theoretical plate number was 3456. Resolution is calculated from the equation $R_s = 2(t_2 - t_1)/(t_{w1} + t_{w2})$, where t_1 and t_2 are retention times of the first and second eluted peaks, respectively, and t_{w1} and t_{w2} are the peak widths. The resolution between the closely eluted impurity IBAP peak to ibuprofen peak was 4.25. The asymmetry factor (A_s) was calculated using the USP method. The peak asymmetry value for each ibuprofen peak was 1.08.

For the determination of method robustness within a laboratory during the method development phase, a number of chromatographic parameters were evaluated, such as flow rate, column temperature, mobile phase composition, columns from different batches, and the quantitative influence of the variables were determined. For each parameter studied, two injections of both standard and sample solutions were chromatographed. In all cases, the influence of the parameters were found within a previously specified tolerance range. This shows that the method for determination of ibuprofen and its impurity IBAP in gel formulation was reproducible and robust.

Method Validation

Linear Concentration Range

A linearity test was performed using six different amounts of ibuprofen in the range of 80–120% around the theoretical values (50 μg/mL) and in the range 0.05–0.50% of ibuprofen content for IBAP. Solutions corresponding to each concentration level were injected in triplicate and the following equations were found by plotting peak areas (y) versus concentration (x)

Table 1. Linearity details for ibuprofen and IBAP

Compound name	Concentration range (μg/mL)	Slope	*y*-Intercept	Correlation coefficient
Ibuprofen	20–80	10732	330697	0.9999
IBAP	0.10–50	5585.3	14500	0.9997

expressed in μg/mL: $y = 10732x + 330697$ ($r^2 = 0.9999$) for ibuprofen and $y = 5585.3x + 14500$ ($r^2 = 0.9997$) for IBAP. The determination coefficient (r^2) obtained (Table 1) for the regression line demonstrates the excellent relationship between peak area and the concentration of ibuprofen and IBAP.

Precision (Repeatability and Intermediate Precision)

The precision of the analytical method was evaluated in terms of repeatability and intermediate precision. Repeatability (intra-day precision) was assessed injecting six replicate injections at 100% test concentration (50 μg/mL ibuprofen and 5 μg/mL IBAP). The %RSD values of the results corresponding to the peak areas and retention times were found in the range of 0.13–0.15 and 0.16–0.35% (Table 2).

Intermediate precision (interday precision) was demonstrated by two analysts using two HPLC systems on different days and evaluating the peak area data across the HPLC systems at three concentration levels (75%, 100%, and 150%) that cover the assay method range 20–80 (μg/mL) for ibuprofen and 0.10–50 (μg/mL) for IBAP. The mean and %RSD across the systems and analysts were calculated from the individual peak area mean values at the 75%, 100%, and 150% of the test concentration. The %RSD values for both instruments and analysts were $<0.50\%$ (Table 3) for each drug, and illustrated the good precision of the analytical method.

Table 2. Repeatability studies data for ibuprofen and IBAP

Injection no.	Ibuprofen		IBAP	
	Peak area (μV s)	RT (min)	Peak area (μV s)	RT (min)
1	870137	7.724	45587	6.003
2	873247	7.715	45622	6.002
3	871234	7.731	45696	6.008
4	872276	7.724	45598	6.041
5	871003	7.752	45625	6.011
6	870836	7.727	45766	6.050
Mean	871455	7.729	45649	6.019
RSD (%)	0.13	0.16	0.15	0.35

Table 3. Intermediate precision studies data for ibuprofen and IBAP

Injection no.	Analyst 1, day 1, HPLC system 1			Analyst 2, day 2, HPLC system 2		
	75[a]	100	150	75	100	150
Ibuprofen						
1	761539[b]	870167	973376	762154	870023	974024
2	761532	870261	973598	762004	870238	973653
3	763671	872276	977232	764662	872852	977302
Mean	762247	870901	974735	762940	871038	974993
RSD (%)	0.16	0.14	0.22	0.20	0.18	0.20
IBAP						
	75	100	150	75	100	150
1	37598	42174	48432	37243	42353	48387
2	37587	42322	48471	37438	42423	48422
3	37742	42536	48668	37278	42746	48727
Mean	37642	42344	48524	37320	42507	48512
RSD (%)	0.23	0.43	0.26	0.28	0.49	0.39

[a]Percent of nominal.
[b]Peak area (μV s).

Accuracy

To study the reliability and suitability of the developed method, recovery experiments were carried out. Placebo samples were spiked with different amounts of ibuprofen at 75, 100, and 150% in triplicate for each one ($n = 9$) over the theoretical values and IBAP at 0.1% over the ibuprofen content. The contents of ibuprofen and IBAP were once again determined by the proposed method by recording the chromatogram. From the amount of the drug present, percent recoveries were calculated using the Eq. (1.) The mean values of the percent recoveries obtained are shown in Table 4.

$$\%\text{Recovery} = \left(\frac{\text{b} - \text{a}}{\text{c}}\right) \times 100 \tag{1}$$

where, a = amount found before addition, b = amount found after addition, and c = amount of standard drug added.

Specificity

Injections of the extracted placebo were performed to demonstrate the absence of interference with the elution of the ibuprofen and IBAP. These results demonstrate (Figure 5) that there was no interference from the other

Table 4. Recovery studies of ibuprofen and IBAP from samples with known concentration

	Ibuprofen			IBAP		
Sample	75%	100%	150%	75%	100%	150%
1	100.2	99.8	100.3	98.4	99.7	100.1
2	100.4	100.3	100.6	97.8	99.6	99.8
3	100.6	100.2	99.7	99.8	100.2	99.6
Mean recovery (%)	100.4	100.1	100.2	98.6	99.8	99.8
RSD (%)	0.20	0.26	0.46	1.06	0.32	0.25

materials in the gel formulation and, therefore, confirm the specificity of the method.

Limits of Detection and Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) tests for the procedure were performed on samples containing very low concentrations of analyte. LOD is defined as the lowest concentration of analyte in a sample that can be detected above baseline noise. It is expressed as a concentration at a specified signal-to-noise (s/n) ratio, typically, three times the noise level. LOQ is defined as the lowest concentration of analyte in a sample that can be reproducibly quantitated above the baseline noise that gives $s/n > 10$. The LOD and LOQ values for ibuprofen and IBAP were found to be $\mu g/mL$ ($s/n = 3.2$; $s/n = 10.35$) and ($s/n = 3.06$; $s/n = 10.2$), respectively.

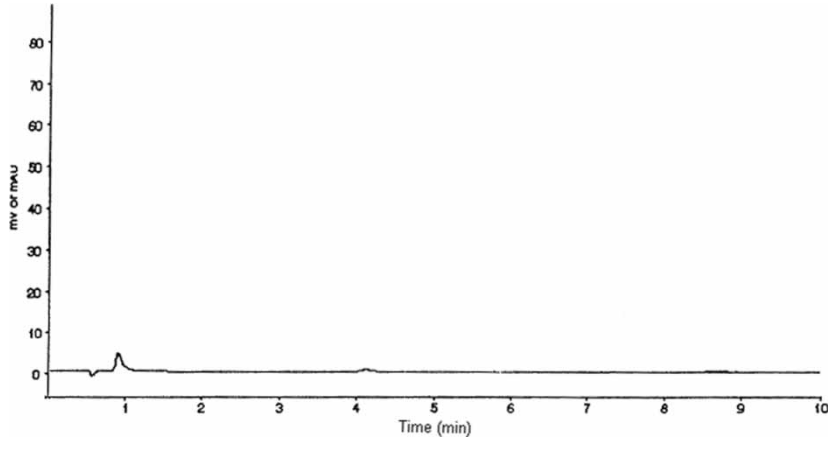

Figure 5. HPLC chromatogram of placebo.

Table 5. Method validation data for robustness study

Parameter altered	RT of principal peak (min)	Resolution between impurity and principal peak
Standard chromatogram obtained with method optimized conditions	7.72	4.25
Increased organic modifier (38:62)	5.46	4.35
Decreased organic modifier (42:58)	9.10	4.41
Flow at 0.8 mL/min	11.34	4.16
Flow rate at 1.2 mL/min	5.56	4.11
Column temperature at 20°C	9.43	4.62
Column temperature at 30°C	6.08	4.05

Method Robustness

The method robustness was studied by deliberately changing the experimental conditions such as percentage of organic modifier, flow rate, and column temperature.

Change in the organic modifier, methanol content was studied by varying its content to a ratio of buffer/methanol (42:58, v/v) and (38:62, v/v) from the original optimized ratio of (40:60, v/v). It was observed that by increasing the organic modifier, the principal peak retention time is decreased to 5.46 min and by decreasing the content the retention time is increased to 9.10 min against 7.72 min retention time obtained with original optimized conditions. The resolution between the closely eluted IBAP impurity peaks was observed as 4.35 and 4.41, respectively, against the standard resolution of 4.25. The data are presented in Table 5.

The change in the flow rate to 0.8 and 1.2 from 1.0 mL/min does not affect the chromatography/resolution between the closely eluted impurity peaks. The resolutions were observed as 4.16 and 4.11, respectively. However, the retention times were shifted to 11.34 and 5.56 min, respectively. The data are presented in Table 5.

The change in column temperature to 20 and 30 from 25°C does not affect the resolution between the closely eluted peaks corresponding to the IBAP and ibuprofen. The resolutions were observed as 4.62 and 4.05, respectively. However, the retention times of the principal peaks were shifted to 9.43 and 6.08 min, respectively. The data corresponding to the variations in temperature are presented in Table 5.

CONCLUSIONS

A simple isocratic reversed-phase HPLC method has been developed and validated for the determination of ibuprofen drug substance and its major

impurity IBAP in a gel formulation. The developed method has been found to be selective, sensitive, accurate, precise, and robust. The method was used reliably in quality control laboratories for routine analysis of ibuprofen drug substance and its major impurity IBAP in bulk, raw materials, and final gel products formulations.

REFERENCES

1. Cleij, M.; Archelas, A.; Furstoss, R. Microbiological transformations 43. Epoxide hydrolases as tools for the synthesis of enantiopure α-methylstyrene oxide: A new and efficient synthesis of (*S*)-Ibuprofen. J. Org. Chem. **1999**, *64*, 5029–5035.
2. *The Merck Index*, 13th ed.; Merck & Co., Inc., 2001.
3. Gasco-Lopez, A.I.; Izquierdo-Hornillos, R.; Jimenez, A. LC method development for ibuprofen and validation in different pharmaceuticals. J. Pharm. Biomed. Anal. **1999**, *21*, 143–149.
4. Farrar, H.; Letzig, L.; Gill, M. Validation of a liquid chromatographic method for the determination of ibuprofen in human plasma. J. Chromatogr. B **2002**, *780*, 341–348.
5. Huidobro, A.L.; Rupérez, F.J.; Barbas, C. Tandem column for the simultaneous determination of arginine, ibuprofen, and related impurities by liquid chromatography. J. Chromatogr. A **2006**, *1119*, 238–245.
6. Franciszek, K.G.; Karaźniewicz, M. High performance capillary electrophoresis method for determination of ibuprofen enantiomers in human serum and urine. Anal. Chim. Acta **2005**, *540*, 95–102.
7. Jonkman, J.H.G.; Schoenmaker, R.; Holtkamp, A.H.; Hempenius, J. Determination of ibuprofen in human plasma by solid phase extraction and reversed-phase HPLC. J. Pharm. Biomed. Anal. **1985**, *3*, 433–438.
8. Ginman, R.; Thomas, H.K.; Perrin, J. Simultaneous determination of codeine and ibuprofen in plasma by HPLC. J. Pharm. Biomed. Anal. **1985**, *3*, 439–445.
9. Dorte, R.K.; Ulla, G.S.; Tjornelund, J.; Steen, H.H. Simultaneous quantitative determination of the major phase I and II metabolites of ibuprofen in biological fluids by HPLC on dynamically modified silica. J. Chromatogr. B: Biomed. Sci. Appl. **1997**, *696*, 235–241.
10. Kang, S.H.; Chang, S.Y.; Ki-Chan, D.; Sang-Cheol, C.; Chung, D.S. HPLC with a column-switching system and capillary electrophoresis for the determination of ibuprofen in plasma. J. Chromatogr. B: Biomed. Sci. Appl. **1998**, *712*, 153–160.
11. Vermeulen, B.; Remon, J.P. Validation of a HPLC method for the determination of ibuprofen enantiomers in plasma of broiler chickens. J. Chromatogr. B: Biomed. Sci. Appl. **2000**, *749*, 243–251.
12. Madelaine, R.W.; Ford, J.L.; Powell, M.W. Simultaneous determination of ibuprofen and hydroxypropylmethylcellulose using HPLC and evaporative light scattering detection. J. Pharm. Biomed. Anal. **2002**, *30*, 1355–1359.
13. *The United States Pharmacopeia*, 29th ed.; The United States Pharmacopoeial Convenction: Rockville, MD, 2007, 1100.
14. *The British Pharmacopoeia*, 3rd ed.; Stationary Office, Health Ministry: London, 2000, 843 and 2023.
15. Ghosh, M.K. *HPLC Methods for Drug Analysis*; Springer-Verlag: Berlin, 1992.
16. Shabir, G.A. Validation of HPLC methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the

U.S. Food and Drug Administration, the U.S. Pharmacopoeia and the International Conference on Harmonization. J. Chromatogr. A **2003**, *987*, 57–66.
17. International Conference on Harmonization, Draft Guideline on validation of analytical procedures, Federal Register 60, 1995, 11260.
18. United States Pharmacopoeia 29-National Formulary 24, General chapter 1225, Validation of compendial procedures. United States Pharmacopeial Convention, Rockville, MD, 2007, p. 3050.
19. Shabir, G.A. Step-by-step analytical methods validation and protocol in the quality system compliance industry. J. Validation Technol. **2004**, *10*, 314–324.
20. Shabir, G.A.; Lough, J.W.; Shafique, A.A.; Bradshaw, T.K. Evaluation and application of best practice in analytical method validation. J. Liq. Chromatogr. & Rel. Technol. **2007**, *30*, 311–333.

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