Vol. 8, No. 3, 2017
ISSN 2233-4203/ e-ISSN 2093-8950
INVITED ARTICLE www.msletters.org | Mass Spectrometry Letters

Development of an Isotope Dilution Mass Spectrometry (IDMS)-Based Method for the Analysis of Ibuprofen

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Received July 25, 2017; Revised September 11, 2017; Accepted September 11, 2017
First published on the web September 30, 2017; DOI: 10.5478/MSL.2017.8.3.49

Abstract : Ibuprofen is one of the most popular analgesic and antipyretic drugs. An isotope dilution mass spectrometry method based on LC/MS was developed as a candidate reference method for the accurate determination of ibuprofen in pharmaceutical tablets. Isotope labelled ibuprofen, ibuprofen-d_3, was added as an internal standard into sample extracts. Ibuprofen and ibuprofen-d_3, was analysed by LC/MS in a selected ion monitoring (SIM) mode to detect ions at m/z 205 and 208, respectively. The repeatability and reproducibility of the developed ID-LC/MS method were tested for the validation and assessment of metrological quality of the method.

Keywords : Ibuprofen, ID-LC/MS, Method Validation, Pharmaceuticals

Introduction

Ibuprofen is a non-steroidal anti-inflammatory drugs (NSAID) that has anti-pyretic and non narcotic analgesic properties.[1,2]

The determination of ibuprofen has been performed using various analytical techniques including HPLC,[3,4] GC,[5] electrophoretic[6,7] or spectrophotometric methods, such as UV or IR.[8]

These analytical techniques have been advanced to analyze trace amounts of components in drugs[2,7] as well as biological[5,9] and environmental samples.[10,11]

Proper internal standards should be used to correct the biases resulting from the sample preparation process or instrumental analysis in order to assure the accuracy of quantification. Isotope dilution mass spectrometry (IDMS), which uses isotope labelled materials as an internal standard, is a superior analytical technique that accurately quantifies the target analyte after correcting these biases. Therefore, IDMS has been proposed to be one of the potential primary analytical methods in chemistry.[12]

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KRISS has developed IDMS methods to analyze various analytes in samples and applied them to certify target anlytes in matrix certified referenc materiasls (CRMs).[13-16]

This study aims to develop and validate an ID-LC/MS based method to accurately determine ibuprofen in pharmaceutical tablets, and which can be used for CRM certification.

Experimental

Chemicals and reagents

Ibuprofen (99.0%) was purchased from Dr. Ehrenstorfer (Augsburg, Germanty) as a primary reference material. Ibuprofen-d_3 was purchased from CDN Isotope (Pointe-Claire, Quebec, Canada). HPLC grade acetonitrile was purchased from Burdick and Jackson (Muskegon, MI, USA). Filter cartridges (PURDISC NYL 25 FILTER 25 mm 0.4 μm) were obtained from Whatman (Clifton, NJ, USA). Analgesic products (tablets) containing ibuprofen were purchased from a local pharmacy.

Calibration standard solutions

Multiple ibuprofen standard solutions and an isotope labelled ibuprofen, ibuprofen-d_3, standard solution (100 mg/kg) were gravimetrically prepared in acetonitrile. For each of the ibuprofen standard solutions, two isotope ratio standard solutions were prepared by mixing the ibuprofen standard solution and the ibuprofen-d_3 standard solution (1:1 isotope ratio). The isotope ratio standard solutions were diluted to 1 mg/kg, which was convenient for use in the LC/MS analysis. A total of eight isotope ratio standard solutions were cross-checked using the ID-LC/MS method, and one was selected for calibration of sample analysis.

Details of the process of calibration standard solution preparation was described in our previous studies.[13-16]

Sample preparation

To validate and evaluate the metrological quality of the method we developed, homogenized samples were required to minimize the ambiguity caused by sample inhomogeneity. Homogenized samples were prepared by in-house procedures as follow. Commercial ibuprofen tablets of a single brand were ground with a laboratory mill (FRITSCH, Model No. pulverisette 14, Germany). The materials were passed through 100-μm nylon sieve cloth and mixed in a V-mixer for more than 10 h. Next, 3 g of the homogenized samples were adeed to a 6-mL amber bottle for further study.

A portion of the homogenized sample (0.2 g) was weighed and added to a 120-mL glass bottle, and 100 mL of methanol was added. The exact amounts of sample and extraction solvent were determined by weighing the bottle before and after the addition of each of them. After sonication for 2 h in a water bath (35°C), 0.5 mL of the sample extract was pipetted into a vial. A portion of the ibuprofen-d_3 solution was spiked into this vial so that isotope ratio was close to 1.0. The isotope spiked sample was passed through a filter cartridge, and then diluted with acetonitrile for analysis.

Instrumentation and MS analysis

A ThermoElectron TSQ Quantum mass spectrometer (San Jose, CA, USA) connected with an Agilent 1100 Series (Waldbronn, Germany) was used for analysis. The chromatographic separation was carried out on a Waters XBridge C18 (4.6 mm × 150 mm, 3.5 μm) connected to a C18 guard column. The mobile phase was run isocratic at 0.3 mL/min in a solution of 10% of 10 mmol/L ammonium acetate in H_2O and 90% acetonitrile. The injection volume was 10 μL. MS analysis was conducted in the negative mode of electrospray ionization (ESI). In order to obtain the highest precursor ion abundance in the selected ion monitoring (SIM) mode, the following optimized conditions were used: ionspray voltage, 3500 V; capillary temperature, 350°C; sheath gas pressure, 20 μL/min; auxiliary gas pressure, 10 μL/min; skimmer offset, 6.

For the LC/MS analysis, the MS was operated on SIM mode to monitor the $[M-H]^-$ ions of ibuprofen and ibuprofen-d_3 at *m/z* 205 and *m/z* 208, respectively. For the LC/MS/MS analysis, selected reaction monitoring (SRM) mode was run to monitor the collisionally induced dissociation (CID) channels of *m/z* 205 → *m/z* 161 and *m/z* 208 → *m/z* 164, which resulted from the CO_2 loss from the $[M-H]^-$ ions of ibuprofen and ibuprofen-d_3, respectively. For SRM mode, the collision energy was 10 eV in the collision cell.

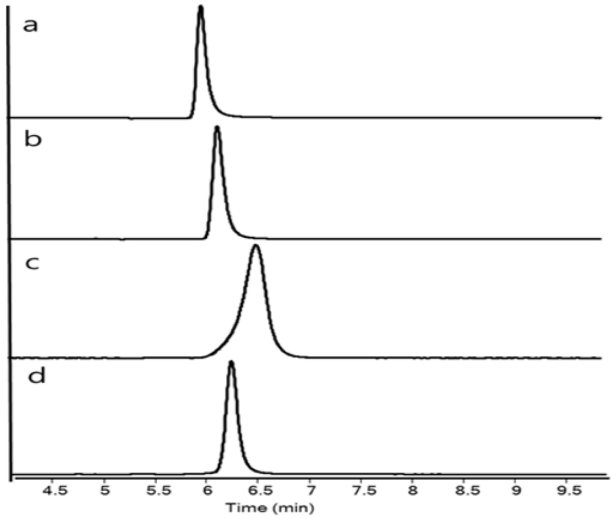

Figure 1. SIM chromatograms of ibuprofen observed from different compositions of 10 mmol/L ammonium acetate and organic solvent. a: Acetonitrile (90%, v/v), b: Acetonitrile (80%, v/v), c: Methanol (90%, v/v), d: Acetonitrile:Methanol (1:1, 90%, v/v).

Results and Discussion

LC/MS Optimization

LC/MS conditions were optimized to produce reliable quantification results for the analysis of ibuprofen in pharmaceutical tablets. A Waters XBridge C18 column (4.6 mm i.d., 150 mm length, 3.5-μm particle size) was chosen as an analytical column. The chromatogram of ibuprofen on the selected column is illustrated in Figure 1 showing the effect of organic phase composition which consisted of acetonitrile (a,b) or methanol (c,d). Aectonitrile was more acceptable compared to methanol based upon the peak width of ibuprofen. Furthermore, the ibuprofen peak sharpened with 90% of acetonitrile was used compred to 80% of acetonitrile. Consequently, ibuprofen was analyzed by isocratic running with 90% acetonitrile.

In ESI/MS analysis, ibuprofen was analyzed in negative mode. $[M-H]^-$ ions at *m/z* 205 and *m/z* 208 was dominant ion in MS spectrum of ibuprofen and ibuprofen-d_3, respectively. Collisionally induced dissociation (CID) of $[M-H]^-$ ions produced $[M-H-CO_2]^-$ as a dominant product ion and its intensity was various depend on the collison energy (Figure 2). For the proper SRM analysis, 10 eV was set for the collision cell and CID channels of *m/z* 205 → *m/z* 161 and *m/z* 208 → *m/z* 164 were monitored to detect ibuprofen and ibuprofen-d_3, respectivley.

Prior to selecting between SIM and SRM modes for the quantification method, the prepared samples (n=10) were analyzed by both method. As shown in Figure 3, both of ibuprofen and ibuprofen-d_3 were clearly anlayzed in both SIM and SRM modes without noticeable interference in

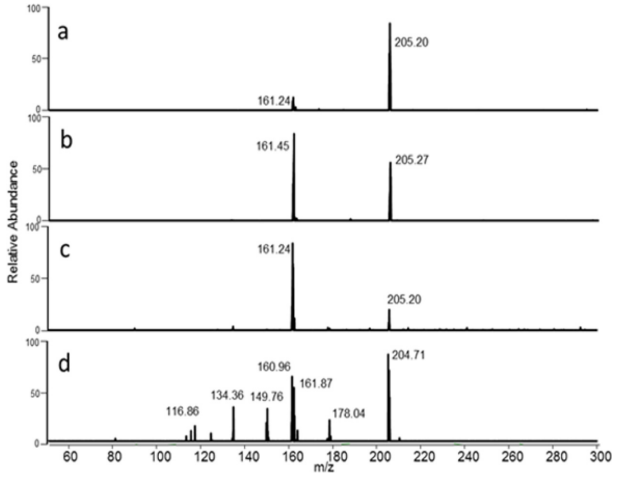

Figure 2. Collisionally induced dissociation spectra of $[M-H]^-$ ions of ibuprofen depended on various collision energy. a: 5 eV, b: 8 eV, c: 10 eV, d: 15 eV.

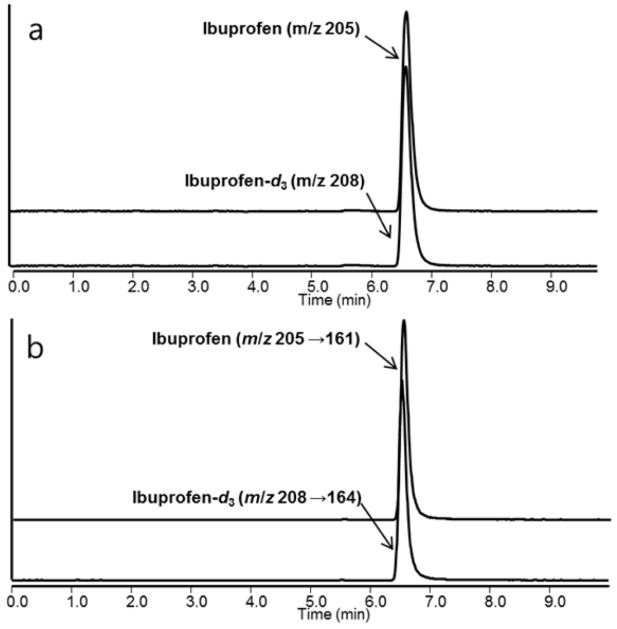

Figure 3. SIM (a) and SRM (b) chromatograms of ibuprofen and ibuprofen-d_3 in homogenized analgesic tablets.

the chromatograms. As shown in Table 1, the measurement results obtained by using two different modes agreed wihtin their uncertainties. Based on the chromatorgraphic quality and quantification results, both the SIM and SRM mode can be applied to quantify ibuprofen in pharmaceutical tablets. SIM mode was selected for the main approach for the analytical method, and it validated the measurement quality. SRM mode was also monitored for entire study to confirm the data.

Table 1. Ibuprofen contents in homogenized analgesic tablets determined by using the SIM or SRM modes.

Sample	Ibuprofen Contents (% in kg/kg)	
	SIM mode	SRM mode
1	29.65 ± 0.37[a]	29.53 ± 0.89
2	29.71 ± 0.36	29.59 ± 0.74
3	29.48 ± 0.36	29.16 ± 0.44
4	29.08 ± 0.38	29.36 ± 0.93
5	29.16 ± 0.36	29.29 ± 0.55
6	29.37 ± 0.31	29.66 ± 0.74
7	29.51 ± 0.54	29.49 ± 0.45
8	29.63 ± 0.29	29.74 ± 0.42
9	29.57 ± 0.29	29.25 ± 0.48
10	29.43 ± 0.31	29.40 ± 0.54
Average	29.46 ± 0.51	29.45 ± 0.49

[a.] The values following "±" are the expanded uncertainties of the preceding values at the 95% level of confidence and coverage factor, *k*, was 2.1.

Table 2. Repeatability and reproducibility of the ID-LC/MS method for analysis of ibuprofen in homogenized samples.

Period	Sub-sample	Measurement Results (g/kg)
1	1	29.53 ± 0.88[a]
	2	29.59 ± 0.74
	3	29.16 ± 0.44
	Average	29.43
	Standard deviation	0.23
	Expanded Uncertainty	0.88
2	1	29.51 ± 0.26
	2	28.97 ± 0.26
	3	29.26 ± 0.25
	Average	29.25
	Standard deviation	0.27
	Expanded Uncertainty	1.28
3	1	30.04 ± 0.66
	2	29.50 ± 0.68
	3	29.74 ± 0.83
	Average	29.76
	Standard deviation	0.27
	Expanded Uncertainty	0.92
Average		29.48
Standard deviation among period		0.26

[a.] The values following "±" are the expanded uncertainties of the preceding values at the 95% level of confidence and coverage factor, *k*, was 2.1.

Repeatability and Reproducibility

To test the repeatability of the method, multiple well-homogenized samples were measured within 1 day. The reproducibility of the method was evaluated by conducting the same repeatability test on different days at 6-months interval.

For each timepoint of analysis, a new set of multiple standard solutions were prepared and verified using the consistency test as described in the Experimental section. As illustrated in Table 2, the averages for the measurement results in each time period were 29.43, 29.25, and 29.48 g/kg. The relative standard deviation within a period was 0.8, 0.9, and 0.3%, respectively, indicating that good repeatability was maintained over the long-term. Measurements from the three time points showed a relative standard deviation of 0.9%, and each measurement agreed with each other within their uncertainties, indicating that the method has a good reproducibility over the time period tested. Additionally, the results at the three different time points were in agreement within their overall uncertainties.

Conclusions

The currently developed ID-LC/MS-based method can be used to accurately detect ibuprofen in pharmaceutical tablets. After comparing both analytical datasets obtained from the SIM and SRM modes, the SIM mode was selected for use in the quantification method. Good results for repeatability and reproducibility show that the method we developed can produce reliable data over a long period of time to identify the presence ibuprofen in pharmaceutical tablets. Therefore, this analytical method has a higher-order metrological quality as a reference method and can be applied to certify the presence of ibuprofen in pharmaceutical tablet CRMs.

Acknowledgments

This work was supproted by Korea Research Institute of Standards and Science under the project ‘Establishing measurement standards for organic anlaysis’, grant 17011053.

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