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Research Article

DEVELOPMENT AND VALIDATION OF A SIMPLE HPLC METHOD FOR THE DETERMINATION OF IBUPROFEN STICKING ONTO PUNCH FACES

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ABSTRACT

Objective: Ibuprofen (IPB) tends to stick to punch surfaces during tablet compression, a problem that leads to the production of low quality tablets and can cause damage to compression tooling. The purpose of this study was to develop and validate a simple method for the detection and quantitation of IPB adhesion to punch faces and to study the relationship between IPB sticking tendency and compression time.

Methods: IBP determination was carried out using an HPLC instrument supplied with a C18 column (250 mm × 4 mm; 4.6 µm particle size) and a UV detector at 214 nm. The mobile phase was composed of a mixture of water adjusted to pH 2.5 with phosphoric acid and acetonitrile (40/60, v/v). To examine the method, a formulation containing IBP was prepared and compressed, and the amount of IBP adhered to punch faces was determined.

Results: The retention time of IBP was 5.9 ± 0.3 min. The method was linear over the range 6.1–200 µg/mL with a correlation coefficient (r^2) of 0.9998. The method was demonstrated to be highly reproducible, specific, precise, and accurate. LOD and LOQ were found to be 1.70 and 6.05 µg/ml, respectively. A significant relationship was established between the amount of adhering ibuprofen and the compression run time.

Conclusion: The developed method is suitable for the routine control of IPB punch adhesion, and because of the simplicity of the material and instruments used it can be applied in every laboratory equipped with a basic HPLC system.

Keywords: HPLC; Ibuprofen; Method validation; Punch adhesion; Sticking.

INTRODUCTION

Ibuprofen (IPB), α-methyl-4-(2-methylpropyl)-benzene acetic acid, is one of the most common non-steroidal anti-inflammatory drugs (NSAIDs). It is widely used as an analgesic in mild to moderate pain, and in the treatment of rheumatoid arthritis and osteoarthritis [1]. The conventional daily dose of this NSAID is 600–1200 mg per day [2].

IPB is a crystal powdered drug and the common needle-shaped crystal form shows disadvantages regarding compressibility and sticking on press punches [3]. Its sticking tendency has been found to stem from low melting point (75°C), and sublimation characteristics [4-5]. As a consequence, tiny proportions of IBP powder adhere (stick) onto the surface of compression punches [6]. Sticking is a serious problem in the compression process because it leads to the production of low quality tablets with weight variation and appearance irregularities [7]. It also contributes to loss in materials and time and can cause damage to compression tooling [7]. Therefore, it is important to quantify the amount of adhered IBPon punch faces using an appropriate analytical method [8]. Such a method will help to determine sticking tendency, which can be used in formulation development aiming at reducing the sticking effect. Sticking tendency of IBP was previously investigated using a specrtophotometric method after the extraction from punches with ethanol 95% [9]. The extract was analyzed spectrophotometrically at 264 nm, and sticking was reported as the amount of IBP adhered in micrograms. This method was not validated in terms of accuracy, precision, and linearity. Furthermore, this method may lack selectivity as the extract may retain other materials, from the formulation components, that could interfere with the analysis.

Several analytical methods are available for the determination of IBP in pharmaceutical samples including electrophoretic [10], titrimetric [11], and chromatographic [12-16] methods. In general, these methods are designed to measure high IBP content which is usually found in IBP dosage forms and, therefore, they may lack sensitivity making them unsuitable to detect the sticking initiation. In addition, a number of analytical methods combined with extraction methods to determine IBP concentration in biological samples (serum, plasma, and urine) have been also describe [17-20]. However, these methods require complex and expensive methods or instrumentation such as HPLC-MS and UPLC-MS [21].

All previous methods may not be adaptable for appropriate determination of IPB sticking in a quality assurance laboratory equipped with a basic HPLC system. Thus, the aim of this study was to develop and validate a sensitive, simple, and cost-effective method that is suitable for in-process control and can be utilized to determine small changes in the amount of IBP adhered to punch surfaces during compression process. Another objective was to study the relationship between IPB sticking tendency and tablet compression time.

MATERIAL AND METHODS

Materials

IBP was purchased from Abbott GmbH & Co.KG BASF (Germany). Lactose monohydrate (Pharmatose 200M), Aerosil 200, and magnesium stearate were supplied by Fonterra limited (New Zealand), Degussa (UK), and BDH (UK), respectively. HPLC grade acetonitrile and phosphoric acid were purchased from Merck KGaA (Germany). HPLC grade water was obtained from chem.-lab (Belgium).

Apparatus and chromatographic conditions

Analyses were performed using a Shimadzu prominence UV/Vis HPLC connected to a C18 column (Nucleodur C18; 250 mm length × 4 mm width; 4.6 µm particle size) supplied with an isocratic pump (Shimadzu-LC-20AD prominence) and an auto-sampling device (Shimadzu-SIL-20A prominence). The experiments were operated at ambient temperature. The mobile phase was composed of a mixture of water adjusted to pH 2.5 with phosphoric acid and acetonitrile (40/60, v/v). The mobile phase flow-rate was 1.7 mL/min. The detection was performed using a UV detector (Shimadzu UV-Vis Abs) at wavelength 214 nm.

Standard solution preparation

IBP solution was prepared by transferring an accurately weighed 5 mg of IBP reference material into a 25-mL volumetric flask. 15 mL of acetonitrile was added followed by sonication for 1 min using an

ultrasonic source. The solution was brought up to final volume with the aqueous component of the mobile phase (water adjusted to pH 2.5 with phosphoric acid) in order to reach an IBP concentration of 0.2 mg/mL. Aliquots of the IBP stock solutions were transferred into separate volumetric flasks in order to obtain dilutions as appropriate. Injection volume was 40 µL.

Method validation

System suitability

IBP solution with a concentration of 0.2 mg/mL was injected five times to evaluate the analytical response. The mean and relative standard deviation (RSD%) were calculated. RSD% of no more than 2% was recommended. Theoretical plates number and tailing factor were also observed.

Linearity and range

The linearity of the method was checked using a series of freshly prepared calibration standard samples. Five concentrations of Ibuprofen were prepared in triplicate (6.1, 12, 48, 100, 200 µg/mL), as described in **Standard solution preparation** section, and three linearity curves were plotted; each one consisted of five concentration points. Thus, three linear equations were extrapolated and correlation coefficient, slope, intercept, and RSD% of the slopes and the intercepts were determined for each curve. A correlation of more than 0.999 was sought for all of the three calibration plots.

Specificity

The specificity of the method was tested according to the ICH guidelines [22]. A sample of standard IBP, within the linearity range described in *Linearity and range* section, was first injected into the apparatus and analyzed. Using the same concentration of IBP, a sample of a given formula containing the drug and a number of excipients as illustrated in Table 1 was prepared, with appropriate amounts, and examined by the same method. Finally, a blank sample containing the matrix [all the formula ingredients (Table 1) excluding IBP] was also analyzed to assess any interference by other formulative ingredients at the retention time of ibuprofen. All measurements were performed in triplicate.

Table 1: Formulative ingredients and their corresponding proportions/amounts used in specificity, accuracy, and precision measurements and in the evaluation of ibuprofen sticking to press punches

Material	Proportion/amount used	
	Specificity, accuracy, precision determinations (%)	Compression experiment (mg per tablet)
Ibuprofen	66.66	600
Lactose monohydrate	32.33	291
Magnesium stearate	0.5	4.5
Aerosil 200	0.5	4.5
Total	100	900

Accuracy and precision

The accuracy and precision of the method were evaluated by analyzing the intra- and inter-day variability of three sets of freshly prepared samples on the same day and on three different days using five different concentrations of IBP (6.1, 12, 48, 100, 200 µg/mL). A matrix consisting of several ingredients, as used in the specificity determinations in *Specificity* section, with appropriate amounts, as demonstrated in Table 1, was added to each sample. Inter- and intra-day accuracy was determined by comparing the measured concentration and the actually used concentration of each sample. Precision was determined by computing RSD% for each sample.

Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were determined by comparing acquired signals from samples with known low concentrations of the analyte with those of blank samples. These measurements establish the minimum concentrations at which the analyte can be reliably identified and quantified. A typical signal-to-noise ratio is 10:1 and 3:1 for LOQ and LOD, respectively. Replicate analysis (n = 6) was performed on spiked samples with LOD and LOQ concentrations. The mean coefficient of variation and recovery were also determined in order to check precision and accuracy, respectively.

Application of analysis

This analytical method was primarily developed to detect tiny amounts of IBP adhered onto punch faces of tablet press machines. To examine the method, a formulation containing IBP was prepared and compressed and the amount of adhered IBP was determined. IBP was mixed with lactose monohydrate, magnesium stearate, and Aerosil 200 using the amounts specified in Table 1 (amounts used in compression experiment). The powder admixture was compressed using a pre-cleaned single punch machine (ERWEKA GmbH, EK0, Germany). Compression was performed for five different running times (1, 2, 4, 8, and 16 minutes). The test was repeated three times.

Sample preparation

Following each compression run, both upper and lower punches were immediately detached and the loose powder on the body of the punches was carefully removed with a vacuum cleaner and a damp cloth without disturbing the punch faces. Punch tips were immersed in a 50-mL beaker containing 15 mL of acetonitrile in order to dissolve the residual layer adhered to the tip surfaces. To ensure complete removal, a small spatula was used to scrap the residual layer and, then, also soaked in the same beaker. After complete dissolution, the punches and the spatula were removed and the mixture was stirred for 1 minute. Consequently, the mixture was transferred into a 25-mL volumetric flask and the volume brought up to the mark with the aqueous component of the mobile phase (water adjusted to pH 2.5 with phosphoric acid). Finally, the mixture was filtered using 0.22 µm Millipore® filter and 40 µL of the filtrate was injected into the HPLC system and analyzed. Samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The method was developed based on a United States pharmacopeia (USP) assay procedure [15] with the objective to enhance the method sensitivity to allow measurement of small changes in the amounts of ibuprofen adhered to punch faces during compression. It was sought to develop a simple and easy-to-apply method that can be used for routine analysis in pharmaceutical industry laboratories. Initially, the mobile phase composition of the USP assay method (400:600; chloroacetic acid solution in water 0.01 g/mL: acetonitrile) was modified by replacing chloroacetic acid solution with water adjusted to pH 2.5 using diluted phosphoric acid. While chloroacetic acid comes as a solid material that must be dissolved prior to use, phosphoric acid is available as liquid making it easier to use. The pH value (pH 2.5) was selected in order to maintain IBP carboxylic group in its unionized form; ibuprofen has a Pka value of 4.5. To select the optimal mobile phase ratio, several water (adjusted to pH 2.5 using phosphoric acid) to acetonitrile volumetric ratios (80:20, 60:40, 50:50, 40:60, 20:80) were

evaluated by checking IBP peak as well as its sharpness and retention time. Optimum conditions were achieved with water:acetonitrile 40:60 (v/v).

Method validation

System suitability

As demonstrated in Fig. 1 and Table 2, the retention time of IBP standard was 5.9 ± 0.3 min, the tailing factor 1.15 ± 0.04, the theoretical plates number 2471 ± 171, and the relative standard deviation of peak area (RSD%) 0.23% (n = 5).

Linearity and range

Quantitative parameters of the analysis are shown in Table3. A linear relationship was found between the peak area and the concentration of IBP over the studied range between 6.1 – 200 µg/mL (n = 3). The mean linear regression equation was y = 56,504.5 (slope) x + 27,731.9 (intercept) with a correlation coefficient (r^2) of 0.9998. RSD values of the slope and the intercept were 0.43% and 22.82%, respectively.

Specificity

Specificity of the method can be defined as the capability to accurately determine the response of the analyzed compound without interferences from sample matrix. The method's specificity was examined by comparing a standard IBP with a proposed formulation containing IBP. The recovery of IBP was 99.28% ± 0.05% and 99.93% ± 0.05% for standard and formulation samples, respectively. Furthermore, blank samples (IBP-free matrix) showed an insignificant response. These findings suggest that IBP measurement using this method was not interfered by the formulation ingredients.

Accuracy and precision

Intra- and inter-day variability of the method was assessed through analysis of IBP samples on the same day and on three different days. The accuracy and precision data are summarized in Table 4. For a concentration range between 6.1 – 200 µg/mL, intra- and inter-day accuracy was within 95 – 105 %. The precision of all concentrations was less than 3.5%. Based on these data, the method proved to be accurate, reproducible, and precise.

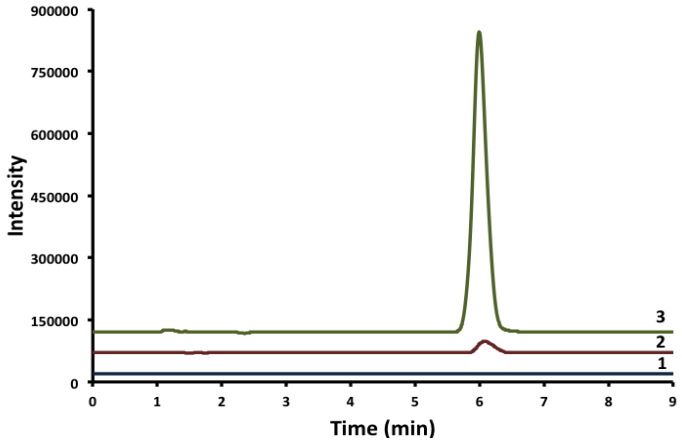

Fig. 1: Chromatograms of (1) blank (ibuprofen-free formulation containing lactose monohydrate, magnesium stearate, and Aerosil 200), (2) ibuprofen standard with concentration 6.1 µg/mL, and (3) ibuprofen standard with concentration 200 µg/mL.

Table 2: Constitutional standards in the chromatogram of 200 µg/mL ibuprofen standard solution (n = 5).

Area (Mean ± SD)	11,426,934 ± 25,962
RSD%	0.23
Theoretical plates	2,471 ± 171
Tailing factor	1.15 ± 0.04
Retention time ± SD	5.9 ± 0.3

Table 3: Quantitative parameters of ibuprofen calibration plot (n = 3).

Parameter	Value
Linearity range (µg/mL)	6.1 – 200
Slope ± SD	56,504.5 ± 241.2
Intercept ± SD	27,731.9 ± 6,329
Correlation coefficient (r^2)	0.9998
RSD% of slope	0.43
RSD% of intercept	22.82

Table 4: Precision and accuracy of ibuprofen HPLC assay.

Sample concentration. (µg/mL)	Concentration determined					
	Inter-day assays (n=3)			Intra-day assays (n=9)		
	Mean	Accuracy (D/A) × 100	Precision (RSD%)	Mean	Accuracy (D/A) × 100	Precision (RSD%)
6.1	6.1	101.05	1.53	6.2	102.05	1.37
12	12.6	104.80	1.03	12.4	103.29	2.24
48	49.2	102.47	0.67	48.8	101.58	3.27
140	139	99.57	1.69	138.6	98.97	0.81
200	200	100.22	0.77	199.3	100.68	1.42

LOD and LOQ

LOD and LOQ were determined experimentally (Table 5). LOD, defined as the concentration with a signal to noise ratio equal to 3, was found to be 1.70 ± 0.04 µg/ml. LOQ, which is the lowest concentration in the linearity range that have a signal to noise ratio superior to 10, was found to be 6.05 ± 0.06 µg/ml.

Table 5: Limit of detection (LOD) and limit of quantitation (LOQ) results (n=6).

Parameter	LOD	LOQ
Mean (µg/ml)	1.70	6.05
SD	0.04	0.06
CV% (precision)	2.52	0.99
Recovery% (Accuracy)	101.69	102.19

Application of analysis

The major objective of the work was to develop an analytical method suitable to study the sticking properties of IBP on punch faces. When compression commences, part of the formulation starts to adhere to the punch surfaces. As tabletting run continues, the adhered material builds up on punch face surfaces. It has been previously demonstrated that the amount of a drug material layered on the punch faces increases linearly with the number of tablets produced or compression time (cycles) [8]. Using the method developed in this study, a significant relationship was established between the amount of adhering ibuprofen and the compression run time (Fig. 2) with a line equation of y = 0.635x + 0.973 and a regression coefficient (r^2) of 0.9793. These findings were in agreement to those expected [8] and confirmed that the HPLC method is appropriate for the determination of IBP adherence to punch faces.

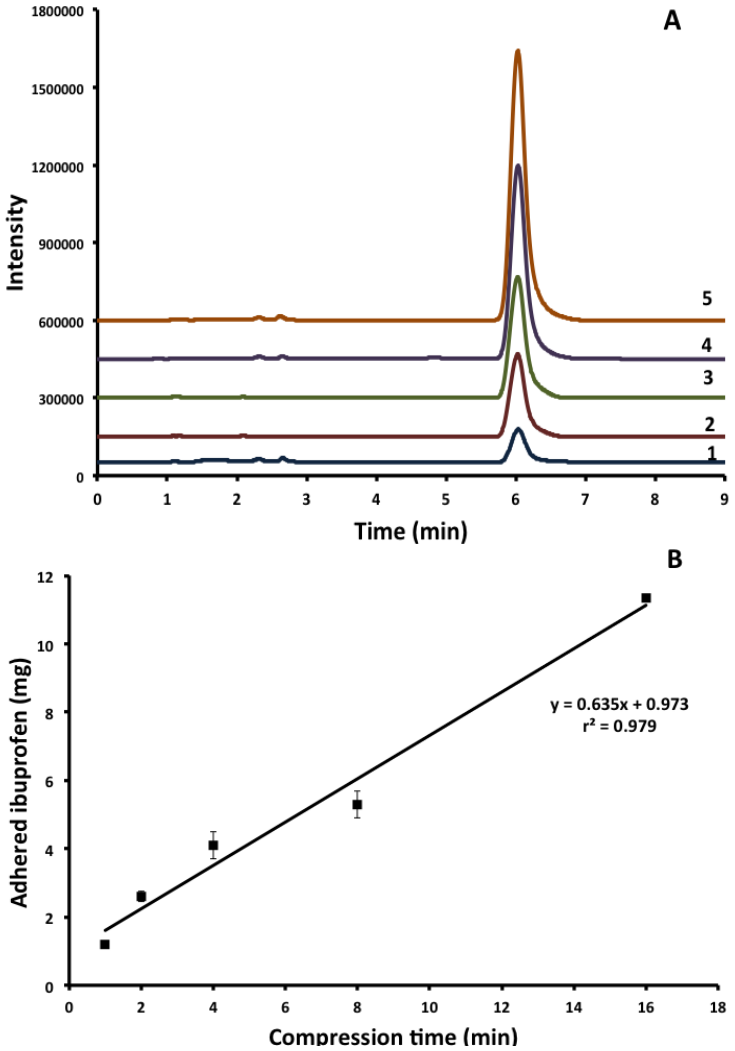

Fig. 2: The relationship between the amount of ibuprofen adhered to punch faces and compression run time determined using the developed analytical method. (A) Chromatograms of ibuprofen adhered to punch faces after five different compression run times: (1) 1, (2) 2, (3) 4, (4) 8, and (5) 16 minutes. (B) the amount of adhered ibuprofen vs. compression run time (n=3).

CONCLUSION

In this work, a simple analytical method was successfully developed and validated for the detection and quantitation of ibuprofen adherence to press punches. The method was demonstrated to be highly reproducible, specific, precise, and accurate. Because of the simplicity of the material and instruments used in this method, it can be applied in every laboratory equipped with a basic HPLC system. Furthermore, the HPLC method could also be suggested for the routine analysis of ibuprofen in bulk drug as well as in pharmaceutical dosage formulations.

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