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Full Length Research Paper

Simultaneous determination of ibuprofen and diphenhydramine HCl in orally disintegrating tablets and its dissolution by reversed-phase high performance liquid chromatography (RP-HPLC)

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Reversed-phase high performance liquid chromatography (RP-HPLC) methods were established for the simultaneous determination of ibuprofen (IBU) and diphenhydramine HCl (DPH) in orally disintegrating tablets (ODTs) and its dissolution in this work. The separation was performed on a Shim-pack VP-ODS C_{18} (150 × 4.6 mm, 5 µm) column. The mobile phases of determination and dissolution were a mixture of 0.05 mol/L potassium dihydrogen phosphate buffer (containing 0.2% triethylamine and 0.2% glacial acetic acid)-acetonitrile (54:46, v/v) and a mixture of 0.05 mol/L potassium dihydrogen phosphate buffer-acetonitrile-triethylamine (60:40:0.2), respectively. The mobile phase was delivered at a flow rate of 1.0 ml/min, and the detection was carried out at 263 nm under the column temperature of 30°C and the injection volume of 20 µl. The linear ranges of determination were 100 to 1000 µg/ml with the correlation coefficient of 0.9996 for IBU and 7.5 to 120 µg/ml with the correlation coefficient of 0.9998 for DPH. The average recoveries (n = 9) were 98.52% (relative standard deviation (RSD) = 0.22%) for IBU and 99.07% (RSD = 0.87%) for DPH, respectively. The linear ranges of dissolution were 50 to 800 µg/ml with the correlation coefficient of 0.9999 for IBU and 5 to 80 µg/ml with the correlation coefficient of 0.9999 for DPH; the dissolution both exceeded 80% of the labeled at 10 min. The results showed that the proposed methods were simple, sensitive, accurate and specific. To evaluate its potential application value, IBU and DPH in compound ODTs were simultaneously detected using this approach, and satisfied results were obtained.

Key words: Ibuprofen, diphenhydramine hydrochloride (HCl), Reversed-phase high performance liquid chromatography (RP-HPLC), determination, dissolution.

INTRODUCTION

Ibuprofen (IBU) and diphenhydramine HCl (DPH) orally disintegrating tablets (ODTs) is a pharmaceutical compound consisting of IBU and DPH. IBU, 2-(4-isobutylphenyl)-propionic acid, an important non-steroidal anti-inflammatory drug, possesses good analgetic, anti-inflammatory and antipyretic effects (Hersh et al., 2000; Gł´owka and Kara´zniewicz, 2005; Sujith et al., 2009; Issa et al., 2011; Mehlisch et al., 2003). DPH, 2-(diphenylmethoxy)-*N,N*-dimethylethylamine hydrochloride an antihistamine, also has central nervous system (CNS);depression and sedative properties (Pragst et al., 2006; Malathi et al., 2009).

Some studies have reported that the compound can be used for the relief of occasional sleeplessness associated with minor aches and pains. It is also helpful to fall asleep and stay asleep (Graham et al., 2002). Currently, there are two compound preparations consisting of IBU and DPH in USA. One is the Advil® PM Caplets containing 200 mg IBU and 25 mg DPH, and the other one is Advil® PM Liqui-Gels® containing 200 mg IBU and 38 mg diphenhydramine citrate. However, the compound preparation is currently under study in China.

The compound that composed of IBU and DPH, has

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not been recorded in British Pharmacopoeia (BP2009), United States Pharmacopoeia National Formulary (USP32) (2007), European Pharmacopoeia and the latest Chinese Pharmacopoeia (Chinese Pharmacopoeia, 2010). The reported literature about the quality of the compound was few, and it has not seen the report for the determination of content and dissolution of IBU and DPH ODTs. Many methods have been reported for the determination of either IBU or DPH in pharmaceutical samples and biological fluids (Ulu and Elmali, 2010), including capillary electrophoresis (Gomez et al., 2005; Hamoudová and Pospíšilová, 2006; Zhang et al., 2007), spectrophotometry (Issa et al., 2011; Yuan et al., 2009; Ebeshi et al., 2009), near infrared and Raman spectroscopy (Haag et al., 2009), spectrofluorimetry (Damiani et al., 2001), FT-Raman spectroscopy (Orkoula et al., 2006), high performance liquid chromatography (HPLC) (Wang et al., 2005; Whelan et al., 2002; Dönmez et al., 2011) and gas chromatography-mass spectrometry (GC-MS) and liquid chromatography- mass spectrometry (LC-MS) (Kintz et al., 2007). Whereas the methods described for their simultaneous estimation from formu-lation are very limited. Hence, a Shim-pack VP-ODS C_{18} column was used, and the mobile phase was screened and optimized on the basis of abundant references (Wang et al., 2005; Whelan et al., 2002; Dönmez et al., 2011; Gomez et al., 2005; Chinese Pharmacopoeia, 2010; Saville, 2001). Here, a rapid, accurate and validated method of RP-HPLC was deve-loped for simultaneous determination of IBU and DPH in combined dosage.

MATERIALS AND METHODS

Apparatus

The following apparatus were used in the sample preparation: Sartorius Basic PH Meter PB-10 (Sartorius A G, Germany); Mettler Toledo Classic Balance Line AB-S (Mettler Toledo instruments Co. LTD, Switzerland); RCZ-6B2 Drug dissolution instrument (Huanghai Medicine Checking Instrument Co. Ltd, Shanghai, China).

Reagents

IBU reference standard (99.5% purity, Lot 100179-200804) and DPH reference standard (99.9% purity, Lot 100066-200807) were obtained from National Institute for Food and Drug Control (China). The IBU and DPH ODTs used contain 200 mg IBU and 25 mg DPH per tablet and were manufactured by our laboratory (Lot 20110416, 20110417, 20110418). Acetonitrile of HPLC grade (Tianjin Shield Fine Chemicals Company, Tianjin, China). Potassium dihydrogen phosphate, analytical-grade glacial acetic acid and triethylamine (Kelong Chemical Reagent Company, Chengdu, China). Ultra-pure water (18.2 MΩ) was purified by ELGA PURELAB classic system (Veolia Water Solutions and Technologies Co. Ltd.), and used throughout this investigation.

Chromatography conditions

Chromatographic analysis was performed using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of LC-20AD pump, an autosampler (Model SIL-20A) and photodiode array UV-Vis detector (Model SPD-M20A). A Shim-pack VP-ODS C_{18} (150 × 4.6 mm, 5 µm) column was used. Mobile phases of determination consist of 0.05 mol/L potassium dihydrogen phosphate buffer (containing 0.2% triethylamine and 0.2% glacial acetic acid) and acetonitrile (54:46, v/v); while the mobile phase of dissolution was a mixture solution of 0.05 mol/L potassium dihydrogen phosphate buffer (containing 0.2% triethylamine) and acetonitrile (60:40, v/v). The flow rate was 1.0 ml/min and the injection volume was 20 µl. The column temperature was maintained at 30°C. The detection wavelength was fixed at 263 nm. The mobile phase was degassed and filtered through 0.22 µm membrane filter before used.

Dissolution

Dissolution was conducted on a Chinese Pharmacopoeia (2010) (Vol II, Method 1, rotating basket) apparatus with a speed of 100 rpm. D2 was used as the dissolution media at a volume of 500 ml with the temperature maintained at 37 ± 0.5°C. Five milliliter aliquots of the dissolution media were collected and quickly filtered through 0.45 µm membrane after 10 min. Then 20 µl of filtrate was injected into the column.

Preparation of solutions

Standard solutions

Stock solutions of 1 mg/ml of IBU and 0.75 mg/ml DPH were prepared by dissolving 100 mg of IBU and 75 mg of DPH in 100 ml diluents, respectively. The standard solutions were prepared by diluting the stock solutions with the same solvent to reach concentration of 500 µg/ml (IBU) and 62.5 µg/ml (DPH). The diluent of determination was the mixture of pH 6.8, 0.05 mol/L potassium dihydrogen phosphate buffer and acetonitrile (40:60, v/v) (D1), while the diluent of dissolution was pH 7.2, 0.2 mol/L potassium dihydrogen phosphate buffer (D2). All of the standard solutions were filtered through 0.22 µm membrane filter before flowing into the chromatographic system.

Sample preparation

Ten tablets were weighed and finely powdered in a mortar, an amount of the tablets powder equivalent to 50 mg IBU and 6.25 mg DPH was dissolved in a 100 ml standard volumetric flask with D1. The mixture was homogenized ultrasonically for 30 min and filtered through 0.45 µm membrane.

Validation

In order to demonstrate the suitability of the developed methods, validation was carried out following State Food and Drug Administration (SFDA) recommendations.

Linearity

The calibration curves were prepared on the basis of the peak areas and the working solution concentrations. A series of working standard solutions of content (dissolution) at concentrations of 100, 200, 400, 800 and 1000 µg/ml (50, 100, 200, 400 and 800 µg/ml) for IBU and 7.5, 15, 30, 60 and 120 µg/ml (5, 10, 20, 40 and 80 µg/ml) for DPH, were prepared by diluting the stock solutions with D1(D2), then injected into HPLC and calculating the slope, Y-

intercept and correlation coefficient. Triplicate injections were applied.

Selectivity and sensitivity

The selectivity of the method was estimated by preparation and analysis of the blank sample (short of the two active ingredients) and the samples. The sensitivity of the method was assessed by limits of detection (LODs) and limits of quantitation (LOQs).

Accuracy

The accuracy of the method was evaluated by determining recoveries of IBU and DPH at three concentration levels, 120, 100 and 80% of target test concentration (500 µg/ml of IBU and 62.5 µg/ml of DPH) in simples by triplicate analysis. The recovery of the method was calculated by comparing the determined concentration of spiked sample to the theoretical concentration.

Precision and stability

Repeatability (intra-day)

The precision of the assay method was evaluated by carrying out six independent assays of IBU and DPH standard solution (500 µg/ml of IBU and 62.5 µg/ml of DPH) by one person at the same condition.

Intermediate precision (inter-day)

Different analysts from the same laboratory evaluated the intermediate precision of the method. This was performed by analyzing six samples of IBU and DPH tablets.

Sample stability

The solution of IBU and DPH were stored at room temperature and light proof place for 24 h. The peak areas of IBU and DPH were determined at 0, 4, 8, 12, 18 and 24 h by injecting 20 µl of each sample solution.

RESULTS

Linearity

Content

The linear calibration curves were obtained over the concentration ranges of 100 to 1000 µg/ml for IBU and 7.5 to 120 µg/ml for DPH. The correlation coefficient (R^2) obtained was greater than 0.999 for both drugs, which showed that an excellent correlation existed between the peak area and concentration of the analyte over the calibration ranges. The linear regression equations was A = 1505.1C + 18067 (R^2 = 0.9996) for IBU and A = 1297.8C + 860.96 (R^2 = 0.9998) for DPH.

Dissolution

The concentration ranges of IBU was 50 to 800 µg/ml, the linear regression equations was A = 1753C + 244.82 (R^2 = 0.9998); while the calibration ranges of DPH was 5 to 80 µg/ml and the linear regression equations was A = 1206.4C - 1104.4 (R^2 = 0.9998).

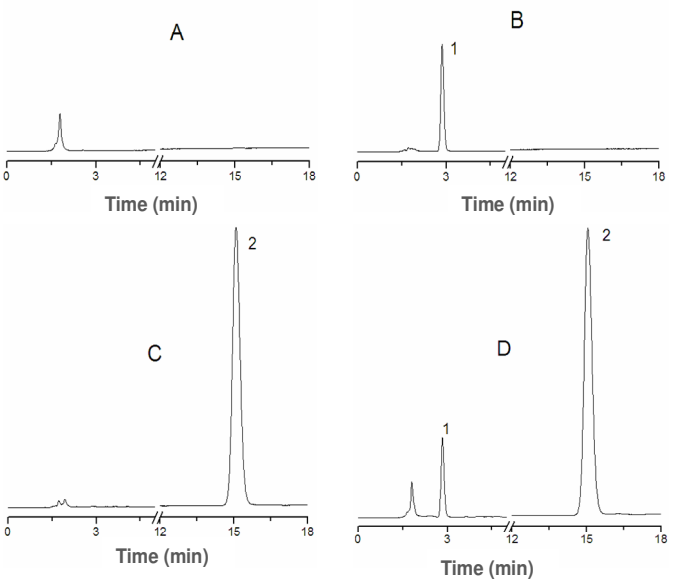

Figure 1. Chromatograms of samples (content). A-Additives; B-Diphenhydramine HCL reference substance; C-Ibuprofenreference substance; D-Sample of content; 1- Diphenhydramine HCL; 2-Ibuprofen.

$A = aC + b$

Where A is the peak area of the analytes, C is the concentration of the analytes (µg/ml), a is the slope and b is the Y-intercept.

Selectivity and sensitivity

From Figures 1 and 2, it is known that the excipients used in the tablets did not interfere with the retention times of the two active ingredients at the wavelength of 263 nm. The peaks of the two drugs were separated well without any overlap.

The LODs of the two analytes were 0.4 and 0.03 µg/ml for IBU and DPH, respectively. The LOQs of the two analytes were 1.5 and 0.1 µg/ml for IBU and for DPH, individually.

Accuracy

The mean recoveries were 98.52% (relative standard deviation (RSD) = 0.22%, n = 9) for IBU and 99.07% (RSD = 0.87%, n = 9) for DPH which were all in the range of 98 to 102%. Satisfactory recoveries with small RSD were obtained, which indicated the high accuracy of the

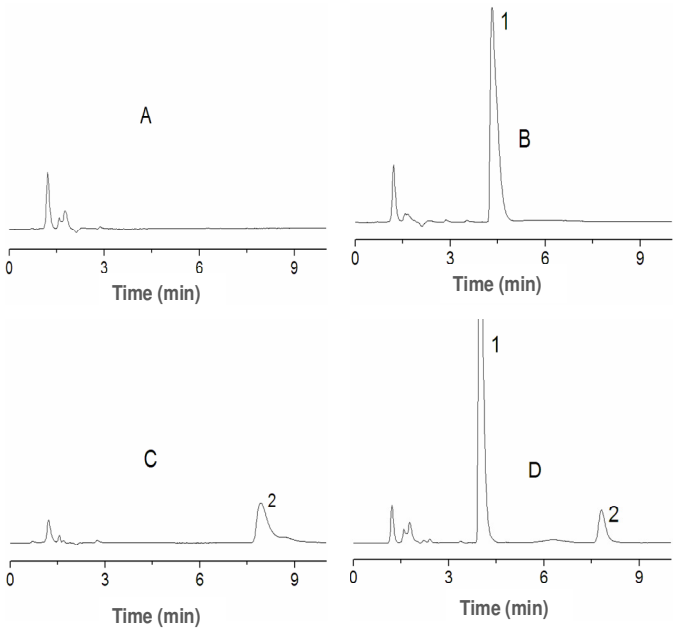

Figure 2. Chromatograms of samples (dissolution). A, Additives; B, ibuprofen reference substance; C, diphenhydramine HCL reference substance; D, sample of dissolution; 1, ibuprofen; 2, diphenhydramine HCL.

two proposed methods.

Precision and stability

Content

The corresponding intra-day and inter-day (n = 6) variations (RSD) were found to be 0.98 and 0.07 for IBU and 0.97 and 0.12% for DPH.

Dissolution

The RSD of precision (n = 6) was 0.08 and 0.84% for IBU and DPH, respectively. The result showed good precision of the two methods.

The percentage RSD values for the stability study of content were 0.63 and 0.58% for IBU and DPH, correspondingly, and the values of dissolution were 0.07 and 1.30% for IBU and DPH, respectively. The results showed that the solutions of IBU and DPH were stable for at least 24 h with keeping at room temperature and away from highlight.

Analysis of ODTs

In order to evaluate the applicability and reliability of the proposed methods, a total of three batch samples, manufactured by us, were analyzed by the described method. The results of content and dissolution are as shown in Table 1, and satisfactory results were obtained for both drugs and were in a good agreement with the labeled amount (200 mg IBU and 25 mg DPH).

DISCUSSION

The chromatographic separation was optimized by testing different mobile phase compositions. Using the potassium dihydrogen phosphate buffer and methanol as a mobile phase, IBU and DPH were well separated. However, the peak of DPH was so early that it was not separated from additives. Hence, the mobile phase of content was changed into 0.05 mol/L potassium dihydrogen phosphate buffer (containing 0.2% triethylamine and 0.2% glacial acetic acid) and acetonitrile (54:46, v/v). Under this condition, IBU and DPH were well separated from additives (as shown in Figure 1). However, IBU was not well separated from additives when the same mobile phase was used for dissolution. So, the ratio of potassium dihydrogen phosphate buffer (containing 0.2% triethylamine) and acetonitrile was adjusted (40:60, v/v), the well separated IBU and DPH were attained (as shown in Figure 2).

IBU is an acidic drug (pKa 4.4) and practically insoluble in water (Costa et al., 2004; Yong et al., 2004; Eichie et al., 2009; Plakkot et al., 2011). DPH, containing basic group, is very soluble in water (Chinese Pharmacopoeia, 2010). Thus, all kinds of solvents were used to optimize the efficiency of solubility. A mixture of potassium dihydrogen phosphate buffer (0.05 mol/L) and acetonitrile (40:60, v/v) was adopted as the solvent of content, and the best peak shapes and resolution of the two drugs were obtained at pH 6.8. According to reports (Liu and Guo, 2005; Gryczke et al., 2011; Alvarez et al., 2011), phosphate buffer was the excellent solvent for the poorly water-soluble IBU. The concentration and pH of phosphate buffer were screened and optimized. 0.2 mol/L potassium dihydrogen phosphate buffer with pH 7.2 was the ultimate solvent of dissolution.

At present, not a standard dissolution method was proposed for the determination of ODTs. The time of risperidone ODTs was 5 min (Shukla et al., 2009), ODTs of Olanzapine-2-Hydroxypropyl-beta-Cyclodextrin inclusion complex was 6 min (Shankarrao et al., 2010). In order to select the detection time of the dissolution, the process was carried out using the method of dissolution, however, the sampling times were reset. 5 ml aliquots of the dissolution media were collected at 0.5, 1, 5, 10, 20, 30 and 45 min, and were replaced with equivalent volumes of fresh media, and the amounts released at determined times were calculated. Figure 3 displayed the dissolution profile of IBU and DPH from ODTs. Within the first 10 min, accumulated dissolution percent of IBU and

Table 1. Results of the content and dissolution of IBU and DPH ODTs (n = 6, $\bar{x}$ ±s %).

Batch number	Content (%)		Dissolution (%)	
	IBU	DPH	IBU	DPH
20110416	97.73±0.11	98.83±0.26	99.23±2.32	88.63±3.26
20110417	97.78±0.17	93.71±0.21	98.86±2.30	91.13±5.27
20110418	98.68±0.40	93.76±0.06	99.36±2.55	80.27±4.63

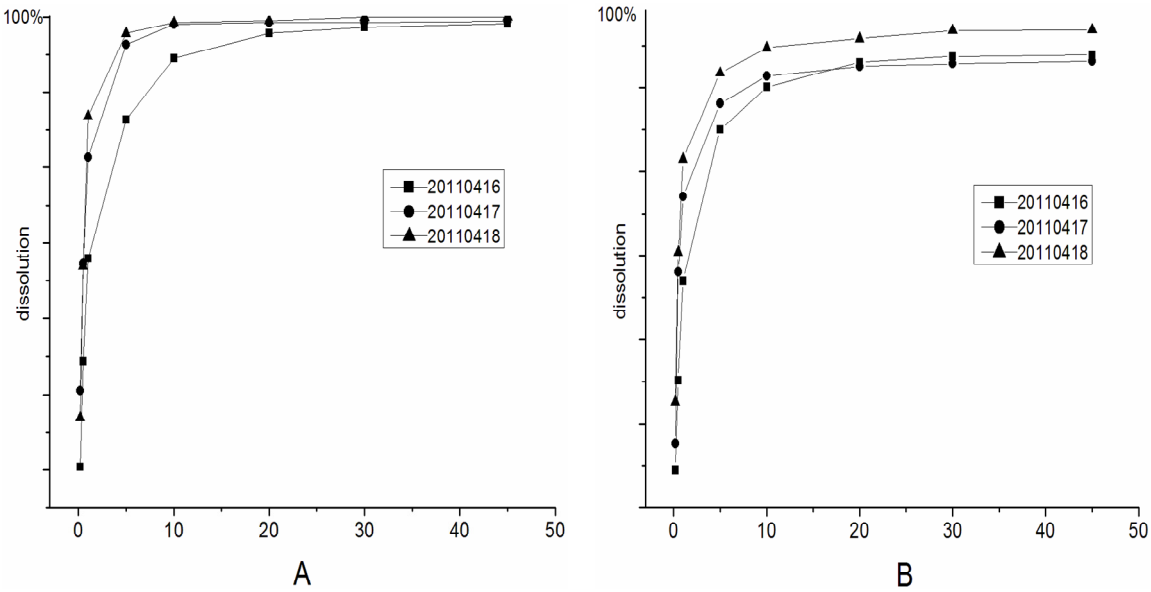

Figure 3. Dissolution curves of samples. A. ibuprofen; B. Diphenhydramine HCL.

DPH were both over 80%. So, 10 min was selected to determine the dissolution of this compound, and it was concordant with the detecting time of ondansetron ODTs recorded in USP (US.P, 2007).

The methods were validated and found to be specific, precise, accurate and linear for the detection and quantification of the two pharmaceutical compounds, IBU and DPH, in their pharmaceutical preparations where several excipients are present.

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