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Short communication

A simple and rapid high-performance liquid chromatographic method for the determination of bisoprolol fumarate and hydrochlorothiazide in a tablet dosage form

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ABSTRACT

A simple and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of bisoprolol fumarate (BF), and hydrochlorothiazide (HCTZ) in a tablet formulation. Chromatography was carried out at 25 °C on a 4.6 mm × 250 mm, 5 μm cyano column with the isocratic mobile phase of 0.1 M aqueous phosphate buffer, acetonitrile and tetrahydrofuran (85:10:5, v/v/v) at a flow rate of 1.0 ml/min. The UV detection was carried out at 225 nm. HCTZ and BF were separated in less than 10 min with good resolution and minimal tailing, without interference of excipients. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The method was linear in the range of 50–150 μg/ml for BF and 125–375 μg/ml for HCTZ.

1. Introduction

Tablet formulations containing the beta-blocker bisoprolol fumarate (BF) and the diuretic hydrochlorothiazide (HCTZ) are used in the therapy to treat high blood pressure. Several methods like HPLC with fluorescence detection, capillary liquid chromatography, liquid chromatography–tandem mass spectrometry (LC–MS/MS) have been reported for the determination of BF in plasma [1–4]. Several methods have also been reported for the determination of HCTZ alone or in combination with other drugs by HPLC and LC–MS/MS [5–9]. A HPLC method has also been reported for the simultaneous determination of BF and HCTZ in a tablet dosage form [10]. However, this method has a serious drawback of short retention time of BF (RT of BF and HCTZ 1.48 and 4.72 min, respectively). The United States Pharmacopoeia 2008 prescribes a HPLC method for the assay of BF and HCTZ in tablets using L11 packing and aqueous dibutyl ammonium phosphate and acetonitrile as the eluent in the gradient mode [11]. To avoid the obvious disadvantages of short retention time [10] and gradient elution [11] the aim of the present study was to develop a simple, specific, accurate and precise isocratic HPLC method with reasonable retention times for the simultaneous determination of BF and HCTZ in tablets.

2. Experimental

2.1. Instrumentation and chromatographic conditions

Integrated HPLC system LC-2010AHT from Shimadzu Corporation (Chromatographic and Spectrophotometric Division, Kyoto, Japan) consisted of a ternary gradient system, high speed autosampler, column oven and UV detector. Isocratic mobile phase consisted of 0.1 M aqueous potassium dihydrogen phosphate buffer, acetonitrile and tetrahydrofuran in the ratio 85:10:5 (v/v/v) filtered and degassed through membrane filter of 0.45 μm porosity. Spherisorb, 4.6 mm × 250 mm, 5 μm, cyano analytical column from Waters, USA, was used as stationary phase. The flow rate was l.0 ml/min and the detector was set at 225 nm. All analyses were made at 25 °C and the volume of solution injected was 10 μl. Chromatograms were recorded and integrated on PC installed with LC solution chromatographic software, Version 1.22 SP1 (Shimadzu, Kyoto, Japan).

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2.2. Reference substances, reagents and chemicals

Bisoprolol fumarate was obtained from Unichem Laboratories Ltd., India. Hydrochlorothiazide was obtained from Ipca Laboratories Ltd., India. Potassium dihydrogen orthophosphate (KH_2PO_4) was purchased from Panreac (Barcelona) Espana. Methanol and acetonitrile (HPLC grade) were obtained from J.T Bakers, Holland. Distilled water was prepared using a Milli-Q system, Millipore, Milford, MA, USA. All the chemicals and reagents were of analytical or reagent grade. Reference standards of bisoprolol fumarate and hydrochlorothiazide were obtained from United States Pharmacopoeia Convention, Rockville, MD, USA. The excipients, maize starch, crosspovidone, MCC pH 102, dicalcium phosphate, aerosil, magnesium stearate, were purchased from various suppliers.

2.3. Samples

Test samples were tablets prepared in-house and reference sample (Concor plus, Merck, Germany) purchased from the local market with following composition: BF 5 mg and HCTZ 12.5 mg/tablet.

2.4. Solution preparation

2.4.1. BF and HCTZ standard stock solution

BF and HCTZ standard stock solution were prepared by transferring accurately about 50 mg of BF and 125 mg of HCTZ reference standards to a 50-ml volumetric flask. Ten milliliters of methanol was added initially and sonicated for a few minutes to solubilize HCTZ. Then 30 ml of mobile phase was added, sonicated to dissolve BF. The solution was diluted to volume with the mobile phase and mixed.

2.4.2. Standard solution

A 5.0-ml portion of BF and HCTZ standard stock solution was transferred to a 50-ml volumetric flask and made up to volume with the mobile phase to obtain a solution containing 0.1 mg of BF and 0.25 mg of HCTZ/ml. The solution was mixed, filtered through 0.45 μm membrane filter and 10 μl was injected.

2.5. Determination from formulations

Five tablets, containing 5 mg BF and 12.5 mg HCTZ were transferred to a 50-ml volumetric flask. 10 ml of methanol is added and sonicated for a few minutes. A 30-ml portion of mobile phase was then added and sonicated for 15 min or until the tablets disintegrated completely followed by shaking on a shaker for 10 min to ensure complete extraction. The volume was made up to the mark with mobile phase and mixed. The solution was centrifuged at 4000 rpm for 8 min. A 10-ml portion of the supernatant solution was transferred to a 50-ml volumetric flask and diluted to volume with the mobile phase. The solution was filtered through 0.45 μm membrane filter and 10 μl was injected directly onto the column.

The amount of BF and HCTZ per tablet was calculated from the peak areas of BF and HCTZ in the chromatograms of the test solution and standard solution, respectively.

3. Results and discussion

3.1. Chromatography

Initially, a Lichrospher 100 C-18, 5 μm column 20 cm × 4.6 mm in isocratic mode, with mobile phase containing water, acetonitrile and tetrahydrofuran in proportion of 80:20:5 (v/v/v) at a flow rate of 1.0 ml/min at a detection wavelength of 225 nm was used. This resulted in too early elution of BF. Changing the chromatographic system to Spherisorb, 4.6 mm × 250 mm, 5 μm, cyano column and 0.1 M aqueous potassium dihydrogen phosphate, acetonitrile and tetrahydrofuran 85:10:5 (v/v/v) as the mobile phase, at a flow rate of 1.0 ml/min resulted in the increase of the retention of polar compounds, symmetrical peak shapes, good separation of the peaks of BF and HCTZ from each other, the solvent front peak and the peak of chlorothiazide, an impurity of HCTZ. The resolution between BF and HCTZ and between chlorothiazide and HCTZ were about 7.8 and 2.0, respectively, meeting the resolution criteria specified in USP 2008. Decreasing the concentration of potassium dihydrogen phosphate in the buffer from 0.1 to 0.05 M resulted in poor resolution between BF, HCTZ and impurity peak. The cyano column showed excellent resistance to hydrolysis under the given experimental conditions: no retention loss was observed after 3 months usage. A typical chromatogram of test solution is shown in Fig. 1.

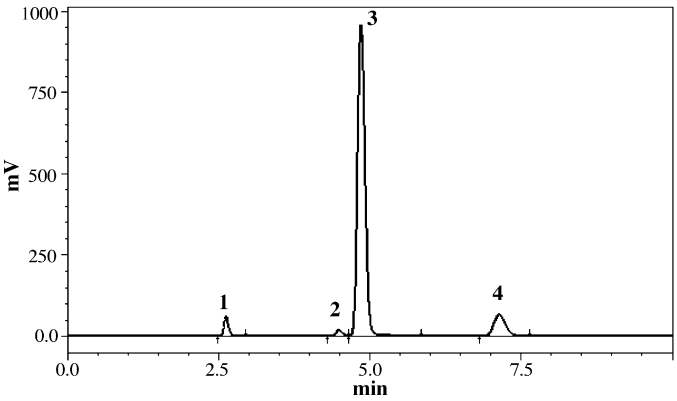

Fig. 1. HPLC chromatogram showing separation of fumarate, chlorothiazide impurity from HCTZ and BF by the proposed method.

3.2. Method validation

The method was validated according to the ICH guidelines [12].

3.2.1. Specificity

Excipients (maize starch, crosspovidone, MCC pH 102, dicalcium phosphate, aerosil and magnesium stearate) did not interfere with the assay. As seen in Fig. 1, an impurity of HCTZ (chlorothiazide) was well separated from the peak of HCTZ.

3.2.2. Linearity

BF and HCTZ showed linear calibration curves in the range of 50–150 μg/ml and 125–375 μg/ml, respectively ($r^2 > 0.9999$).

3.2.3. Accuracy

The accuracy of the method was evaluated from the recovery results of spiked placebo samples.

Appropriate portions of stock solution of BF and HCTZ were spiked into blank placebo matrix to produce concentrations of 50, 75, 100, 125 and 150% of the theoretical concentration. Mean recovery of spiked samples was 99.96% for BF and 100.40% for HCTZ.

3.2.4. Precision

Instrumental precision was determined by six replicate determinations of standard solution and the relative standard deviations were 0.21% for BF and 0.24% for HCTZ.

Method precision or intra-assay precision was performed by preparing six different samples involving different weightings. Each solution was injected in triplicate under the same conditions and the mean values of peak area responses for each solution were taken. The R.S.D. values were 0.23% for BF and 0.62% for HCTZ.

Intermediate precision was performed by analyzing the samples by two different analysts employing different instruments. R.S.D.

Table 1
System suitability and validation parameters

Parameters	HCTZ	BF
Retention time (min)	4.86 ± 0.04	7.14 ± 0.03
Linearity range (μg/ml)	125–375	50–150
Correlation coefficient (r^2)	0.9999	0.9999
Regression equation ($Y = mx + c$)		
Slope (m)	124,900	27,420
Intercept (c)	−101,700	−10,510
Tailing factor	1.16	1.15
Theoretical plates	7240	6311
% R.S.D. ($n = 6$)	0.24	0.21
Mean recovery (%)	100.40 ± 0.68	99.96 ± 0.42

HCTZ = hydrochlorothiazide; BF = bisoprolol fumarate; Y = peak area; x = concentration in μg/ml; R.S.D. = relative standard deviation.

values obtained from 12 assay results were 0.52% for BF and 0.50% for HCTZ.

3.2.5. Robustness

Robustness of the proposed method was estimated by changing: (i) mobile phase composition from buffer:acetonitrile:tetrahydrofuran (85:10:5, v/v/v) to buffer:acetonitrile:tetrahydrofuran (80:15:5, v/v/v); (ii) changing the column brand; (iii) changing the flow rate from 1.0 ml to 1.2 ml/min. System suitability parameters in Table 1 were found to be within acceptable limits.

System suitability and validation parameters are summarized in Table 1.

3.3. Application of the proposed method and conclusions

The proposed HPLC method is simple, rapid, specific, accurate and precise for simultaneous determination of BF and HCTZ in tablets in the presence of chlorothiazide impurity. The proposed method is isocratic unlike the USP method which involves gradient elution.

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