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Development and validation of a stability-indicating high performance liquid chromatographic (HPLC) assay for biperiden in bulk form and pharmaceutical dosage forms

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Abstract

Current compendial (USP) methods of assay for the analysis of biperiden in bulk form and pharmaceutical dosage forms involve the use of titrimetric and spectrophotometric procedures, respectively. These are non-selective and non-stability-indicating techniques. In this work, a stability-indicating high performance liquid chromatographic assay procedure has been developed and validated for biperiden. The liquid chromatographic separation was achieved isocratically on a symmetry C8 column (150 mm × 3.9 mm i.d., 5 μm particle size) using a mobile phase containing methanol-buffer (50:50, v/v, pH 2.50) at a flow rate of 1 ml/min and UV detection at 205 nm. The buffer was composed of sodium dihydrogen phosphate (50 mM) and 1-heptanesulfonic acid sodium salt (5 mM). The method was linear over the concentration range of 0.5–25 μg/ml ($r = 0.9998$) with a limit of detection and quantitation 0.03 and 0.1 μg/ml, respectively. The method has the requisite accuracy, selectivity, sensitivity and precision to assay biperiden in bulk form and pharmaceutical dosage forms. Degradation products resulting from the stress studies did not interfere with the detection of biperiden and the assay is thus stability-indicating.

Keywords: Biperiden; Stability-indicating; HPLC-UV

1. Introduction

Biperiden, α-bicyclo[2.2.1]hepte-5-en-2-yl-α-phenyl-1-piperidinepropanol (Fig. 1) [1] is a synthetic, tertiary amine antimuscarinic antiparkinsonian agent [2]. Biperiden is used in the symptomatic treatment of parkinsonism including the alleviation of the extrapyramidal syndrome induced by drugs, such as phenothiazines, but, like other antimuscarinics, is of no value against tardive dyskinesias [3]. This agent is administered by mouth as the hydrochloride and by injection as the lactate; doses are expressed in terms of the relevant salt [3,4]. There is a dearth of analytical methods reported in the literature for the routine quantitative assay of biperiden in bulk form and pharmaceutical dosage forms. HPLC [5–7], gas chromatography [8–14], capillary electrophoresis [15] and radioreceptor assay [16] methods for the quantitative determination of biperiden in pharmaceutical dosage forms [14,15] or in biological samples have been reported. These methods [7–14] are complicated, costly and time consuming rather than a simple HPLC-UV method. The European [17] and British [18] Pharmacopeias determine the active pharmaceutical ingredient (API), in bulk form only, by gas chromatography-flame ionization detection. Also, current compendial (USP) methods [4] of assay for the analysis of biperiden in bulk form and pharmaceutical dosage forms involve the use of titrimetric and spectrophotometric procedures, respectively. These are non-selective and non-stability-indicating techniques. In an effort to improve drug product, drug substance, and excipient monographs to current scientific/regulatory standards, USP is seeking submission of proposals for improved methods for inclusion in some of USP-NF monographs to replace the current procedures that may be deficient, flawed, or unsafe. Currently, most of the separations

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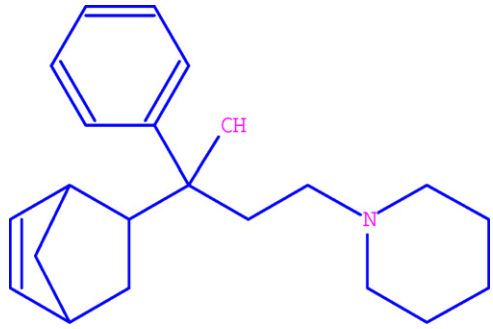

Fig. 1. Structural formulae for biperiden (MW = 311.46).

are performed by HPLC for reasons of robustness and familiarity of analysts with this technique. To our knowledge, no stability-indicating analytical method for the determination of biperiden in dosage forms has been published. The previous published methods are not directly applicable for this issue and need more investigation for method development and validation. Consequently, a simple, precise, accurate, specific stability-indicating HPLC-UV method for the quantitative determination of biperiden in pharmaceutical dosage forms was developed and applied to the assay of biperiden in tablets, injections and bulk form.

2. Experimental

2.1. Chemicals and reagents

Biperiden working standard powder was purchased from Abbot Laboratories, UK and was used without further purification. Akineton® tablets containing 2 mg biperiden hydrochloride and Akineton® injections containing 5 mg/ml biperiden lactate as per labels claim were obtained from Tehran Chemie pharmaceutical company, Tehran, Iran. Methanol, phosphoric acid, sodium dihydrogen phosphate, 1-heptanesulfonic acid sodium salt, sodium hydroxide, hydrochloric acid and hydrogen peroxide were obtained from Merck (Darmstadt, Germany). All chemicals were at least of analytical grade and used as received.

Purified HPLC grade water was obtained by reverse osmosis and filtration through a Milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all solutions.

2.2. HPLC instrumentation and conditions

The HPLC system consisted of a Younglin 930D controller solvent delivery module (Younglin instrument, Korea), a manual rheodyne injection system, a Groton 20/20 PDA detector (Groton technology Inc., MA, USA). The software was an Autochro 2000 and Groton Winsolo acquisition version 2.0.1 (Groton technology Inc., MA, USA). Chromatographic Data System was coupled to the detector via an ADM Module (Younglin Chromatography Division) and used to record and evaluate the data collected during chromatographic analysis. The chromatographic separation was performed using a Waters®symmetry C8 column (150 mm × 3.9 mm i.d., 5 μm particle size). Separation was achieved using a mobile phase consisting of methanol-buffer (50:50, v/v, pH 2.50) at a flow rate of 1 ml min^{-1} and UV detection at 205 nm. The buffer was composed of sodium dihydrogen phosphate (50 mM) and 1-heptanesulfonic acid sodium salt (5 mM). The column was maintained at ambient temperature and an injection volume of 20 μl was used. The mobile phase was filtered through 0.45 μm Chrom Tech Nylon-66 filter and degassed in ultrasonic bath prior to use. For analysis of forced degradation samples, the photodiode array detector was used in scan mode with a scan range of 200–500 nm. Peak homogeneity was expressed in terms of peak purity values, and was obtained directly from spectral analysis report obtained using the instrument software.

2.3. Preparation of stock and standard solutions

A stock solution of biperiden (1 mg/ml) was prepared in HPLC grade methanol. The stock solution was protected from light using aluminium foil and stored for 1 week at 4 °C and was found to be stable during this period. Aliquots of the standard stock solution of biperiden were transferred using A-grade bulb pipettes into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 0.5, 1, 5, 10, 15, 20 and 25 μg/ml.

2.4. Preparation of tablets and injections for assay

Twenty tablets were weighed, crushed and mixed in a mortar and pestle for 20 min. A portion of powder equivalent to the weight of one tablet was accurately weighed into each of six 20 ml A-grade volumetric flasks and 8 ml of mobile phase was added to each flask. The volumetric flasks were sonicated for 20 min to effect complete dissolution of the biperiden and the solutions were then made up to the volume with mobile phase. Suitable aliquots of solution were filtered through a 0.45 μm nylon filter. One microlitre of the filtered solution was transferred to a volumetric flask and made up to the volume with mobile phase to yield concentration of biperiden in the range of linearity previously described.

For injections assay, the content of 10 ampoules were poured into a volumetric flask and mixed well. An aliquot of the resultant solution equivalent to the volume of one injection was added into each of six 100 ml volumetric flask, made up to the volume with mobile phase and mixed well. One microlitre of the diluted solution was transferred to a 10 ml volumetric flask and made up to the volume with mobile phase to yield concentration of biperiden in the range of linearity previously described.

2.5. Forced degradation studies of API and tablet contents

In order to determine whether the analytical method and assay were stability-indicating, biperiden tablets and biperiden API powder were stressed under various conditions to conduct forced degradation studies [19]. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21 CFR section 211 all require the devel-

opment and validation of stability-indicating potency assays. Unfortunately, the current guidance documents do not indicate detailed degradation conditions in stress testing. However, the used forced degradation conditions, stress agent concentration and times of stress, were found to effect a degradation, preferably not less than 10% and not complete degradation of active materials. The discovery of such conditions was based on trial and error. As biperiden is practically insoluble in water and is readily soluble in methanol, methanol was used as co-solvent in all studies [20]. All solutions prepared for use in forced degradation studies were prepared to yield starting concentrations of biperiden of 10 μg/ml.

2.5.1. Oxidation

Solutions for use in oxidation studies were prepared in methanol and 3% H_2O_2 (20:80, v/v), protected from light and stored at room temperature for 3 days.

2.5.2. Acid degradation studies

Solutions for acid degradation studies were prepared in methanol and 1 M hydrochloric acid (20:80, v/v), protected from light and stored at room temperature for 3 days.

2.5.3. Alkali degradation studies

Solutions for alkali degradation studies were prepared in methanol and 1 M sodium hydroxide (20:80, v/v), protected from light and stored at room temperature for 3 days.

2.5.4. Neutral degradation studies

Solutions for neutral degradation studies were prepared in methanol and water (20:80, v/v), protected from light and stored at room temperature for 3 days.

2.5.5. Temperature stress studies

Tablets and API powder were exposed to dry heat (90 °C) in an oven for 3 days. The tablets and API powders were removed from the oven and 20 tablets were crushed and mixed and an aliquot of powder equivalent to the weight of one tablet and API powder were then prepared for analysis as previously described.

2.5.6. Photostability

Tablets, API powder and solutions of biperiden were prepared and exposed to light to determine the effects of irradiation on the stability of biperiden in solution and in the solid state. Approximately, 50 mg of API was spread on a glass dish in a layer that was less than 2 mm in thickness. A solution of API (1 mg/ml) was prepared in methanol and HPLC grade water (20:80, v/v). Tablets were prepared in the same way. All samples for photostability testing were placed in a light cabinet (Thermolab, India) and exposed to light for 40 h resulting in an overall illumination of ≥200 w h/m^2 at 25 °C with UV radiation at 320–400 nm. Control samples, which were protected from light with aluminium foil, were also placed in the light cabinet and exposed concurrently. Following removal from the light cabinet, all samples were prepared for analysis as previously described.

3. Results and discussion

3.1. HPLC method development and optimization

A Waters® symmetry C8 column (150 mm × 3.9 mm i.d., 5 μm particle size), maintained at ambient temperature (25 °C) was used for the separation and the method validated for the determination of biperiden in pharmaceutical dosage forms. The stressed samples were initially analyzed using a mobile phase consisting of methanol–sodium dihydrogen phosphate (50 mM) buffer (50:50, v/v, pH 6) at a flow rate of 1 ml min^{-1} and UV detection at 205 nm. Under these conditions, as the peak shape was not optimal, the pH was changed from 6 to 2.5. An improvement was observed in the peak shape, but column efficiency was diminished. An attempt to improve peak shape while retaining column efficiency was made by adding a negatively charged paired ion reagent, 1-heptanesulfonic acid sodium salt, to the mobile phase. The presence of the ion-pair reagent in the mobile phase resulted in excellent overall chromatography with appropriate peak symmetry and complete baseline resolution. Eventually, a mobile phase consisting of methanol-buffer (50:50, v/v, pH 2.50) provided the best chromatographic response and was used for further studies. The buffer was composed of sodium dihydrogen phosphate (50 mM) and 1-heptanesulfonic acid sodium salt (5 mM).

3.2. Validation

The method was validated with respect to parameters including linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and recovery.

3.2.1. Linearity

The calibration curves constructed for biperiden were linear over the concentration range of 0.5–25 μg/ml. Peak areas of biperiden were plotted versus biperiden concentration and linear regression analysis performed on the resultant curve. Three correlation coefficients of $R1 = 0.9997$, $R2 = 0.9997$ and $R3 = 0.9998$ with %R.S.D. values ranging from 0.1 to 2.75% were obtained following linear regression analysis. Typically, the regression equation for the calibration curve was found to be $y = 34197x + 2159$.

3.2.2. LOQ and LOD

The LOQ and LOD were determined based on signal-to-noise ratios and were determine using an analytical responses of 10 and three times the background noise, respectively [21]. The LOQ was found to be 0.1 μg/ml with a resultant %R.S.D. of 0.4% ($n = 5$). The LOD was found to be 0.03 μg/ml.

3.2.3. Precision

Precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability was investigated by injecting nine replicate samples of each of the 0.5, 5 and 25 μg/ml standards where the mean concentrations were found to be 0.51, 5.09 and 25.15 with associated %R.S.D. values of 2.1, 1.3 and 0.22, respectively. Inter-day precision was assessed by

injecting the same three concentrations over 3 consecutive days, resulting in mean concentrations of biperiden of 0.512, 5.06 and 25.15 μg/ml and associated %R.S.D. of 2.35, 2.45 and 0.34%, respectively. The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results for biperiden that has been performed by two analysts. The %R.S.D. values for intra- and inter-day assays of biperiden in the cited formulations performed in the same laboratory by the two analysts did not exceed 3%, thus indicating the ruggedness of the method. The mean retention time of biperiden was 14.9 min with %R.S.D. of 0.1%.

3.2.4. Accuracy

Accuracy of the assay was determined by interpolation of replicate ($n = 6$) peak areas of three accuracy standards (0.5, 5 and 25 μg/ml) from a calibration curve prepared as previously described. In each case, the percent relevant error was calculated. The resultant concentrations were 0.513 ± 0.010 μg/ml (mean ± S.D.), 5.12 ± 0.064 μg/ml and 25.07 ± 0.05 μg/ml with percent relevant errors of 2.58, 2.47, and 0.26%, respectively.

3.2.5. Selectivity

The results of stress testing studies indicated a high degree of selectivity of this method for biperiden. The degradation of biperiden was found to be similar for both the tablets and API powder. Typical chromatograms obtained following the assay of pure bulk sample and stressed samples are shown in Fig. 2. Photodiode array detection was also used as an evidence of the selectivity of the method, and to evaluate the homogeneity of the drug peak. Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 0.999 indicates a homogenous peak. The peak purity values for biperiden in chromatograms of stressed samples were in the range of 0.999–1 for both the tablets and API powder, indicating homogenous peaks and thus establishing the selectivity of assay method. Selectivity was also checked by monitoring co-injection of a similarly structured compound, trihexyphenidyl, into analysis system. The mean retention time of trihexyphenidyl was 17.85 min with %R.S.D. of 0.12%.

3.2.6. Recovery

A known amount of biperiden standard powder was added to aliquots ($n = 20$) of tablet contents, mixed and the powder was extracted and diluted to yield a starting concentration of 20 μg/ml as previously described in the Section 2.4. This solution was analyzed as previously described. The assay was repeated ($n = 9$) over 3 consecutive days to obtain intermediate precision data. The observed concentration of biperiden was found to be 19.99 ± 0.23 μg/ml (mean ± S.D.). The resultant %R.S.D. for this study was found to be 1.15% with a corresponding percentage recovery value of 99.95%.

3.2.7. Stability studies

All stressed samples in both solid and solution state remained colorless. Biperiden API was found to be more stable rather than biperiden tablets under dry heat condition, resulted in 13.2 and 53.6% decomposition, respectively. No decomposition was seen on exposure of solid drug powder biperiden and also API and tablets in methanol and water (20:80, v/v) to light in a photostability chamber. Under storage at room temperature and in methanol and 3% H_2O_2 (80:20, v/v) for 3 days, around 37% of

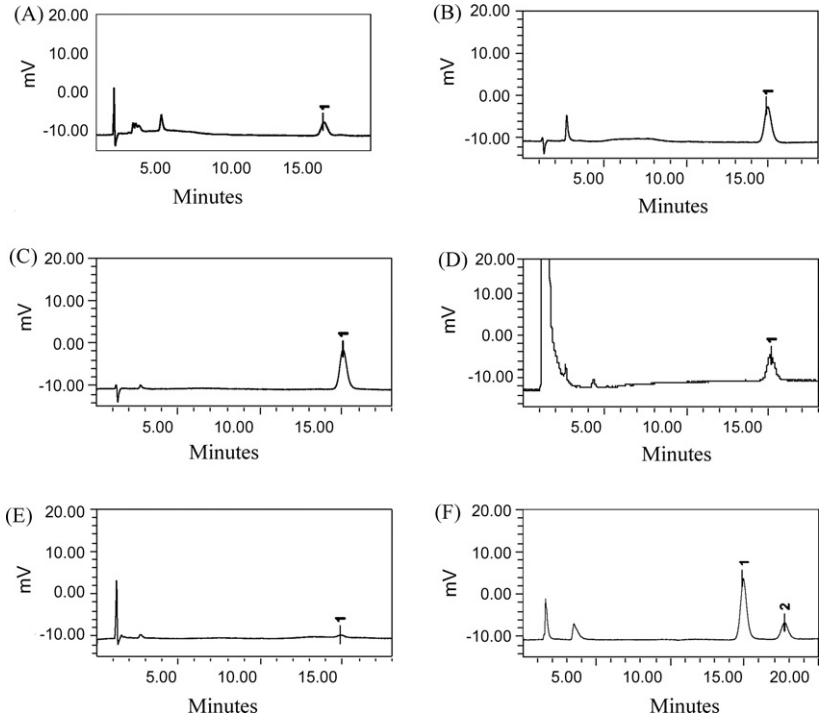

Fig. 2. Typical HPLC chromatograms of: (A) acid hydrolysis-degraded active pharmaceutical ingredient (API); (B) neutral-hydrolysis-degraded API; (C) photodegraded API; (D) oxidative degraded API; (E) base hydrolysis-degraded API and (F) untreated API showing biperiden (1); trihexyphenidyl (2).

drug was only degraded. In spite of acidic condition, the drug was found to be more unstable in alkaline condition falling by 92% upon storage at room temperature for 3 days. Three days after storage at room temperature and in methanol and water (80:20, v/v), 23.6% of drug was degraded. It was not the intention of the study to identify degradation products, but merely to show that they would not interfere if and when present. Degradation products did not appear on chromatograms and the peak purity values for biperiden in chromatograms of stressed samples were in the range of 0.999–1 for both the tablets and API powder, indicating homogenous peaks and thus establishing the selectivity of assay method. The indication is that the drug was degraded to nonchromophoric products. The stability of the stock solution was determined by quantitation of biperiden and comparison to freshly prepared standard. No significant change (<2%) was observed in stock solution response, relative to freshly prepared standard.

3.2.8. Assay

The proposed method was applied to the determination of biperiden in Akineton® tablets and injections. A typical chromatogram obtained following the assay of biperiden tablets and injections is depicted in Fig. 3.The result of these assays yielded 100.04% (%R.S.D. = 1.250%) and 99.96% (%R.S.D. = 1.4%) of label claim for the tablets and injections, respectively. The results of the assay indicate that the method is selective for the assay of biperiden without interference from the excipients used in these dosage forms.

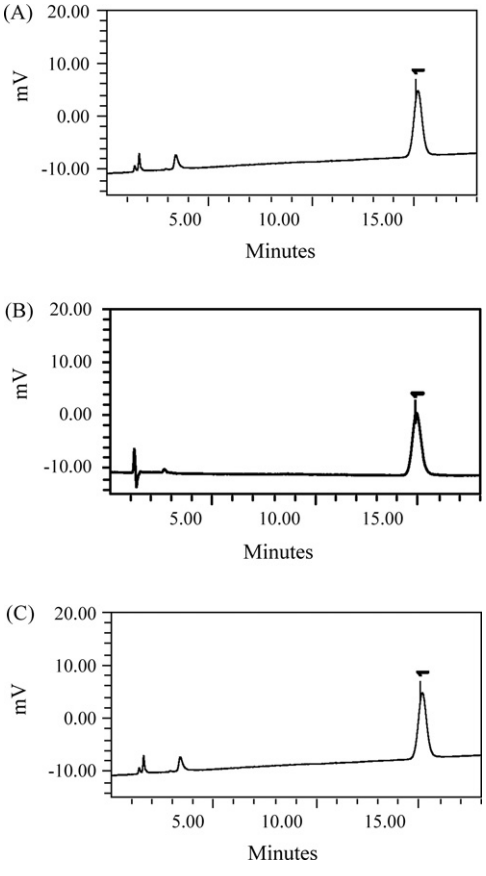

Fig. 3. Resultant HPLC chromatograms following the analysis of a standard solution of biperiden (10 μg/ml) (A), biperiden tablets (B) and biperiden injections (C) showing biperiden (1).

4. Conclusions

A validated stability-indicating HPLC analytical method has been developed for the determination of biperiden in API and dosage forms. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating. The proposed method is simple, accurate, precise, specific, and has the ability to separate the drug from degradation products and excipients found in the tablet and ampoule dosage forms. The method is suitable for the routine analysis of biperiden in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments, such as LC–MS or GC–MS. These methods are complicated and costly rather than a simple HPLC-UV method. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiry dates of pharmaceuticals.

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