STABILITY-INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF METOPROLOL SUCCINATE AND HYDROCHLOROTHIAZIDE IN COMBINATION DRUG PRODUCTS

VIJAYA P. GODSE*, ASHOK V. BHOSALE, YOGESH S. BAFANA and DHANARAJ D. BORKAR

Pharmaceutical Analysis Department, Seth Govind Raghunath Sable College of Pharmacy, SASWAD - 412 301 (Pune), Tal- Purandar, Dist- Pune (M. S.) INDIA

ABSTRACT

A stability-indicating HPLC method has been developed and subsequently validated for the simultaneous determination of metoprolol succinate and hydrochlorothiazide in commercial tablets. The proposed HPLC method utilizes Kya Tech corporation® HiQ sil C18 W (250 mm × 4.6 mm i. d., 10 µm) and mobile phase consisting of acetonitrile-10 mM potassium dihydrogen phosphate pH 3.0 (17 : 83, v/v) for 3 min, then changed to 55 : 45 (v/v) for next 20 min and finally equilibrated back to 17 : 83 (v/v) from 20 to 25 min. at a flow rate of 1.0 mL min⁻¹. Quantitation was achieved with UV detection at 270 nm and 224 nm for metoprolol succinate and hydrochlorothiazide, respectively, based on peak area with linear calibration curves at concentration ranges 100-1000 µg mL⁻¹ for metoprolol succinate and 50-500 µg mL⁻¹ for hydrochlorothiazide (R² > 0.999 for both drugs). The method was validated in terms of accuracy, precision, linearity and robustness. This method has been successively applied to pharmaceutical formulation and no interference from the tablet excipients was found. Metoprolol succinate, hydrochlorothiazide and their combination drug product were exposed to acid, base, neutral hydrolysis; oxidation, dry heat, photolytic stress conditions and the stressed samples were analyzed by the proposed method. As the proposed method could effectively separate the drug from its degradation products, it can be employed as stability-indicating method for the determination of instability of these drugs in bulk and commercial products.

Key words: Column liquid chromatography, Stability-indicating method, Degradation products, Metoprolol succinate, Hydrochlorothiazide

INTRODUCTION

Safety and efficacy of pharmaceuticals are two fundamental issues of importance

* Author for correspondence
in drug therapy. Instability of pharmaceuticals can cause a change in physical, chemical, pharmacological and toxicological properties of the active pharmaceutical ingredients (API); thereby affecting its safety and efficacy. Hence, the pharmacists should take cognizance of various factors such as drug stability, possible degradation products, mechanisms and routes of degradation and potential interactions with excipients utilized in the formulation to ensure the delivery of their therapeutic values to patients. In order to assess the stability of a drug product, one needs an appropriate analytical methodology, so called the stability-indicating methods, which allow accurate and precise quantitation of the drug, its degradation products and interaction products, if any. In recent times, the development of stability-indicating assays has increased enormously\textsuperscript{1–3}, using the approach of stress testing as outlined in the International Conference on Harmonization (ICH) guideline Q1AR2\textsuperscript{4} and even this approach is being extended to drug combinations\textsuperscript{5–7}. This ICH guideline requires that stress testing on API and drug products should be carried out to establish their inherent stability characteristics, which should include the effect of temperature, humidity, light, oxidizing agents as well as susceptibility across a wide range of pH. However, there are no detailed regulatory guidelines that direct how stress testing is to be done and hence, stress testing has evolved into an “artful science” that is highly dependent on the experience of the pharmaceutical industries or the individuals directing the studies\textsuperscript{8}. The knowledge gained from stress testing can be useful for (i) The development of stable formulation and appropriate packaging design, (ii) Controlling of manufacturing and processing parameters, (iii) Identification and isolation of toxic degradants during API synthesis, (iv) Recommendation of appropriate storage conditions and shelf-life determination and (v) Designing and interpreting environmental studies, as the degradation of the drug in the environment will often be similar to degradation observed during stress-testing studies. It is also recommended that analysis of stability samples should be done through the use of a validated stability-indicating testing method.

Metoprolol (MET; Fig. 1a), 1-[4-(2- methoxyethyl) - 1- phenoxy] -3- [(1- methyl ethyl) amino] -2- propanol (CAS, 37350–58-6; MW, 267.36), a cardioselective adrenergic beta-1-blocking agent. It is used in the management of angina pectoris, hypertension, cardiac arrhythmias and myocardial infarction. Hydrochlorothiazide (HCTZ; Fig. 1b), 6 – Chloro – 3, 4 – dihydro – 2H – 1, 2, 4, - benzothiadiazine – 7 – sulphonamide 1, 1 – dioxide (CAS, 58–93-5; MW, 297.74), a thiazide diuretic often considered the prototypical member of this class. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride and magnesium. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus and hypoparathyroidism\textsuperscript{9}. Combination drug products of metoprolol succinate and hydrochlorothiazide are hence widely marketed and successfully
used in the treatment of hypertension. Several HPLC methods have been cited in the literature for the estimation of MET\textsuperscript{10–13}, spectrophotometry\textsuperscript{14} and ion-pair extraction\textsuperscript{15}, are also reported. HCTZ is estimated by several HPLC methods\textsuperscript{16–21} individually and to our knowledge, no analytical method for the simultaneous determination of these two drugs in dosage forms has been published. Further, no stability – indicating method has been reported in literature for simultaneous determination of MET and HCTZ in presence of their degradants. Therefore, the present study targets the development and subsequent validation of a stability – indicating HPLC method for the simultaneous determination of MET and HCTZ in presence of their degradants. To establish the stability indicating nature of the method, forced degradation of each API and drug product was performed under stress conditions and stressed samples were analyzed by the proposed method. The proposed LC method was able to separate both drugs from degradants generated during forced degradation studies.

![Chemical structures]

**Fig. 1(a): Metoprolol**  
**Fig. 1(b): Hydrochlorothiazide**

**EXPERIMENTAL**

**Chemicals and reagents**

Working standards of MET (99.79%) and HCTZ (99.76%) were donated by M/S the Cirex Pharmaceuticals (Hyderabad, India) and CTX Life Sciences Ltd. (Ahmedabad, India), respectively. Betaloc-H\textsuperscript{®} tablets containing MET -50 mg and HCTZ- 12.5 mg were
purchased from local medical shop. HPLC grade acetonitrile was purchased from Merck Specialties Private Ltd. (Mumbai, India). Sodium hydroxide, hydrochloric acid and hydrogen peroxide were of analytical reagent grade from Qualigens Fine Chemicals (Mumbai, India). HPLC grade water was obtained from water purification unit (Elga Ltd., Bucks, England) and was used to prepare all solutions.

**HPLC Instrumentation and conditions**

The HPLC system consisted of a photo-diode array (PDA) detector (MD-2010 plus), Chrompass software, ver. 1.7.403.1, Pump (PU2080), LC Net II/ADC (all from Jasco Corporation, Tokyo, Japan). The separations were achieved on a Kya Tech corporation® HiQ sil C18W (250 mm × 4.6 mm i. d., 10 µm) column and Kya Tech corporation® HiQ sil C18 HS (250 mm × 4.6 mm i. d., 10 µm) both from Kya Tech, Japan. The latter was used for intermediate precision studies. A precision water bath equipped with MV controller (Julabo, Seelbach, Germany) was used to carry out selected reactions in solution. Stability studies were carried out in humidity (KBF720, Binder, Germany) and photo stability (KBWF240, WTC Binder, Germany) chambers both set at 40 ± 1 ºC/75 ±3 % RH. The photostability chamber was equipped with an illumination bank on inside top consisting of a combination of two black light UV lamps (OSRAM L18W/73) and four white fluorescent lamps (OSRAML 18W/20) in accordance with option two of International Conference on Harmonization (ICH) guideline. The samples were placed at a distance of 9 in. from the light bank. Both fluorescent and UV lamps were put on simultaneously. Thermal stability study was carried out in dry air oven (Innovative DTC 96, New Delhi, India). Other equipments used were sonicator (Spectra lab UCB-30), analytical balance (Shimadzu Corporation, Japan).

**Preparation of stock and standard solutions**

Stock solutions at concentrations of 1000 µg mL⁻¹ each of MET and HCTZ were prepared separately in acetonitrile and water (50:50). The stock solutions were protected from light and stored at 4 ºC to avoid degradation. Aliquots of the stock solutions of MET and HCTZ were diluted with mobile phase to yield standard solutions of 100, 200, 400, 600, 800, 1000 µg mL⁻¹ for MET and concentrations of 50, 100, 200, 300, 400, 500 µg mL⁻¹ for HCTZ. Calibration curves reporting peak areas of MET or HCTZ versus drug concentrations were established in the ranges described above.

**Sample preparation for tablet assay**

Twenty tablets each containing 100 mg of metoprolol and 12.5 mg of hydrochlorothiazide were weighed. Powder equivalent to 100 mg of metoprolol was
weighed accurately and taken into 100 mL volumetric flask and volume was adjusted with acetonitrile : water (50 : 50 %v/v) to give the concentration of 1000 µg mL$^{-1}$ of metoprolol and 125 µg mL$^{-1}$ of HCTZ. It was then filtered through 0.2 µm membrane filter (Gelman Science, India) and 20 µL of this solution was injected into HPLC system to obtain chromatogram.

**Forced degradation studies of API and tablets**

Betaloc-H® tablets containing MET and HCTZ were subjected to various forced degradation conditions to effect partial degradation of the drug preferably in 20-80% range$^{22}$. The force degradation studies were performed not only for the drug product, but also for API of both MET and HCTZ, to determine, whether any observed, because of drug properties or due to drug-excipient interactions.

Moreover, the studies provide information about the conditions in, which the drug is unstable so that measures can be taken during formulation to avoid potential instabilities. The stability samples were prepared by dissolving each API or drug product in acetonitrile : water (50 : 50, v/v) and later diluted with distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide or aqueous hydrogen peroxide solution at a concentration of 100 (MET) and 200 (HCTZ) µg mL$^{-1}$, separately.

**Acid hydrolysis**

Solutions for acid degradation studies were prepared in solvent system (acetonitrile : water 50 : 50, v/v) and 1 N hydrochloric acid (50 : 50) and were refluxed at temperature (80°C). It was observed that acid hydrolysis was almost completed within 1 hr of the sample preparation and therefore, the samples were analyzed after this period of time.

**Base hydrolysis**

Solutions for base degradation studies were prepared in solvent system and 0.1 N sodium hydroxide (50 : 50 v/v) refluxed at temperature (80°C) and the resultant solutions were analyzed 3 hr after preparation.

**Neutral hydrolysis**

Solutions for neutral degradation studies were prepared in solvent system and water (50 : 50 v/v, ) and the resultant solutions were refluxed at 80 °C for 1 hr. The mixture was then allowed to cool at room temperature, filtered using syringe filters and analyzed.
Oxidation studies

Solutions for use in oxidation studies were prepared in solvent system and 30% hydrogen peroxide (50 : 50, v/v) at room temperature (25 ºC) and the resultant solutions were filtered using syringe filters and analyzed after 24 hr.

Photostability studies

Solutions for photostability studies were prepared in solvent system and the resultant solutions placed in photostability chamber in accelerated conditions in dark and light for five days. The degraded samples were then filtered using syringe filters and analyzed.

Temperature stress studies

Tablets and API in powder forms were exposed to dry heat (50 ºC) in an oven for 20 days. The API and tablet powders were then removed from oven and an aliquot of tablet powder equivalent to weight of one tablet were prepared for analysis as previously described.

RESULTS AND DISCUSSION

HPLC method development

A gradient method was found necessary to optimize the separation of major degradation products formed under various stress conditions. The best resolution was achieved with initial run of acetonitrile–10 mM potassium dihydrogen phosphate of pH 3.0 (17 : 83, v/v) for 3 min, then changed to 55 : 45 (v/v) for next 20 min and finally equilibrated back to 17 : 83 (v/v) from 20 to 25 min. This optimized method employs Kya Tech corporation® HiQ sil C18 W (250 mm × 4.6 mm i. d., 10 µm) for the separation of MET and HCTZ without affecting the stability of these analytes. Using this optimized method, it was possible to separate MET and HCTZ from their degradation products without any interference and thus, the assay can be considered stability-indicating.

Validation of the method

The developed stability-indicating method was validated according to ICH23, 24 guidelines. The validation parameters addressed were linearity, quantitation, accuracy, precision and specificity.
Linearity

Linearity was established over the concentration range of 100–1000 µg mL\(^{-1}\) and 50–500 µg mL\(^{-1}\) for MET (n = 6) and HCTZ (n = 6), respectively. Peak areas (y) of MET or HCTZ were plotted versus their respective concentrations (x) and linear regression analysis performed on the resultant calibration curves. Correlation coefficients (R\(^2\)) were found to be more than 0.999 for both the analytes. Typically, the mean (± SD) of the regression equations were: y = 0.096x (± 0.04512) + 0.1266 (± 0.05064) for MET and y = 0.179x (± 0.14678) – 3.4452(± 1.791504) for HCTZ, respectively.

Accuracy / recovery

Accuracy of the method was determined by adding standard drugs in 50, 100 and 150% levels to both the drug solutions having concentration 100 µg mL\(^{-1}\) and then recovery of the added drug, obtained from the difference between peak areas of unfortified samples and fortified samples, was satisfactory at all tested concentrations (Table 1). The recoveries for MET and HCTZ were found to be 100.06 and 100.08 %, respectively, which were within acceptable ranges of 100 ± 2%

Limit of detection and quantitation

The limit of detection (LOD) and quantitation (LOQ) for both; MET and HCTZ were determined according to ICH guideline Q2B\(^25\). LOD was defined as 3.3 σ/S and LOQ was 10 σ /S based on ‘standard deviation of the response and slope’ of the calibration curve specially constructed in a low region of 0.05 to 1.0% of the target analyte concentration\(^26\). The standard deviation of y-intercepts of the regression lines was used as σ (the standard deviation of the response) and S is the slope of the calibration curve. The LOD and LOQ were estimated as 1.74 and 5.27 µg mL\(^{-1}\) for MET and 33.02 and 100.08 µg mL\(^{-1}\) for HCTZ, respectively.

Table 1: Results of accuracy experiment using proposed method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Range (µg/mL)</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Found ± S. D. (µg/mL), R. S. D. (%)</td>
<td>Found ± S. D. (µg/mL), R. S. D. (%)</td>
</tr>
<tr>
<td>Metoprolol succinate</td>
<td>100</td>
<td>100.2 ± 0.26, 2.5</td>
<td>99.6 ± 0.17, 1.68</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>200.3 ± 0.36, 1.78</td>
<td>199.2 ± 0.15, 0.76</td>
</tr>
</tbody>
</table>
### Table 2: Results of precision experiment using proposed method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Added concentration (µg/mL)</th>
<th>Measured concentration (µg/mL)</th>
<th>% recovery</th>
<th>Mean % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metoprolol succinate</strong></td>
<td>50</td>
<td>50.1</td>
<td>100.2</td>
<td>100.06 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>101.4</td>
<td>100.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>199.2</td>
<td>99.6</td>
<td></td>
</tr>
<tr>
<td><strong>Hydrochlorothiazide</strong></td>
<td>50</td>
<td>50.2</td>
<td>100.4</td>
<td>100.80 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>101.0</td>
<td>101.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>200.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

**Precision**

Six injections, of three different concentrations, were given on the same day (n = 3) and the percent relative standard deviations (% RSD) were calculated to determine intra-
Specificity

Fig. 2: Representative chromatograms of API obtained under stress conditions of: (a) untreated sample; (b) acid hydrolysis (1 N HCL, 80 °C, 1 h); (c) base hydrolysis (0.1 N NaOH, 80 °C, 3 h); (d) neutral hydrolysis (water, 80 °C, 1 h); (e) oxidative degradation (30 % H₂O₂, room temp., 24 h); (f) dry heat degradation ion (50 °C 8 h, daily, 20 days), (g) photolytic degradation ion(photo stability chamber, 5 days) and showing 1 = acidic, 2 = basic, 3, 4 = neutral, 5 = dry heat, 6, 7 = photolytic peaks of MET and a, b = acidic, c = basic, d = neutral, e = photolytic peaks of HCTZ.
The results of forced degradation studies of each drug in the presence of their degradation products indicated a high degree of specificity of this method for both; MET and HCTZ. The degradation products of each of the parent compound was found to be similar for both the Betaloc-H® tablets and API powers assessed. Typical chromatograms obtained following the assay of untreated and stressed samples of both API and tablets are shown in Fig. 2.

**Degradation studies**

Forced degradation studies of both API were carried out under various stress conditions and resultant chromatograms are depicted in Fig. 2. In general, degradation studies were carried out at a concentration of 1 mg/mL of each drug in the solution. MET undergoes 30% decomposition under acidic and basic stress conditions, forming a major acid and base degradant peak at tR = 3.7 (Fig. 2(b) and 2(c)). On other hand, this drug was stable in oxidative condition (Fig. 2(e)) while it shows 20% decomposition in neutral condition, showing major degradant peak at tR = 4.7 and 10.2 (Fig. 2(d)) Thermal degradation shows 30% decomposition of drug, forming degradant peak at tR = 3.7 (Fig. 2(f)) When MET was exposed to photolytic degradation, almost complete degradation was observed (> 90%), with two major degradation products at tR = 3.7 and 6.2 (Fig. 2(g)) In contrast, HCTZ was relatively less stable at all hydrolytic conditions, resulting 60, 40 and 35% degradation under acidic, basic and neutral stress conditions, respectively (Fig. 2(b), 2(c) and 2(d)). In oxidation and thermal conditions, HCTZ was observed stable (Fig. 2(e) and 2(f)), while showing 40% decomposition in photolytic condition, with degradant peak at tR = 6.2 (Fig. 2(g))

**Assay of commercial product**

The validated method was applied to the determination of MET and HCTZ in commercially available Betaloc-H® tablets. Fig. 3. illustrates a typical HPLC chromatogram obtained following the assay of Betaloc-H® tablets. The result of the assays (n = 6) undertaken yielded 99.47% (% RSD = 0.80%) and 101.26% (% RSD = 0.89%) of label claim for MET and HCTZ, respectively. The mean tR of HCTZ and MET were 9.2 and 19.2 min. The results of the assay indicate that the method is selective for the routine analysis of MET and HCTZ with no chromatographic interference from the excipients used.
CONCLUSION

A gradient stability-indicating HPLC-UV method has been developed for the simultaneous estimation of MET and HCTZ in the presence of degradation products. The proposed method is simple, accurate, precise, specific and has the ability to separate the drugs from degradation products. The method is suitable for use in routine analysis of both drugs in bulk API powder or in tablet dosage forms. This method can be applied even to the analysis of stability samples obtained during accelerated stability experiments, as no interference was found with the degradants formed under various stress conditions.

ACKNOWLEDGEMENTS

The authors are grateful to University of Pune for providing grant for this project, to Cirex Pharmaceuticals, Andhra Pradesh for providing gift samples of olmesartan medoximil and hydrochlorothiazide and also to Seth Govind Raghunath Sable College of Pharmacy, Saswad for providing facilities to carry out this work.
REFERENCES


Accepted : 14.04.2009