In recent years the growing interest in drug stability problem has been observed. The stability of pharmaceutical products seems to play an important role from the economical point of view. However, there are not many studies that reported about the stability of drugs past their expiration dates. The objective of the current study was to determine tablet content of expired tablets and tablets with expiry date has not been exceeded. The analyzed tablets contained metoprolol tartrate (50 mg) and propranolol hydrochloride (10 mg), respectively. Content determination was performed using HPLC method with UV detection. The proposed method was validated with regard to linearity, sensitivity, intermediate accuracy and precision. No discrepancies between the results of determination and the declared values range for all the analyzed tablets were observed. The results of performed study might suggest that storage of analyzed batches of tablets over time period exceeding the expiry date given by the manufacturer did not influence their contents.

EXPERIMENTAL

Chemicals

Metoprolol tartrate, propranolol hydrochloride and thymidine were from Sigma-Aldrich Co. All the solvents used were of HPLC grade (J.T. Baker).

The formulated dosage forms of metoprolol tartrate: Metocard 50 mg, tablets (batch no. 30408; exp. date 04.2011; batch no. 20105; exp. date 01.2008; Polpharma, Poland), Metohexal 50 mg, tablets (batch no. 7R5720; exp. date 04.2010; batch no. 44JZ70; exp. date 08.2007; Hexal AG; Poland), and propranolol hydrochloride, Propranolol 10 mg,
tablets (batch no.02ME0108; exp. date 01.2011; batch no. 05ME0305; exp. date 03.2008; batch no.04ME0402; exp. date 04.2005; Polfa Warszawa, Poland) were purchased from the local market.

**Apparatus**

A liquid chromatographic system (Varian, USA) comprised of ProStar 230 pump, ProStar 420 autosampler, ProStar 510 column oven and ProStar 325 LC UV-Vis spectrophotometric detector was used. Galaxie Chromatography Workstation software 1.9.3.2 (Varian, USA) was used for data acquisition, reporting and analysis.

**Method development**

**HPLC method**

The column used for chromatographic separations was Supelcosil TM LC-18-S (250 mm × 2.1 mm, 5 µm). Mobile phase was pumped in gradient mode at a flow rate of 0.3 mL/min at 25°C. The analytical wavelength was set at 275 nm for metoprolol and 290 nm for propranolol, respectively, indicated by the UV spectra for these substances (Fig. 2 and 3). Samples of 125 µL were automatically injected. As buffers, the following solutions were used: buffer A - the 0.1 M ammonium acetic solution (pH adjusted to 4.0 with acetic acid), buffer B - the mixture of buffer A and acetonitrile (ratio 50/50). The concentration of buffer B was risen up to 100% in 20 min.

**Preparation of standard solutions**

Primary stock solution of metoprolol was prepared in ultra pure water at a concentration of 5.0 mg/mL. Primary standard solution was diluted to 1 mg/mL that served as the secondary stock solution, which was further diluted by mobile phase to obtain working standards in the range of 70–150 µg/mL.

Primary stock solution of propranolol was prepared in ultra pure water at a concentration of 1.0 mg/mL. Primary standard solution was diluted to 250 µg/mL that served as the secondary stock solution, which was further diluted by mobile phase to obtain working standards in the range of 10–50 µg/mL.

**Sample preparation**

Twenty tablets, each containing 50 mg of metoprolol tartrate were accurately weighted and

![Figure 1. Chemical structures of metoprolol and propranolol. The chiral carbon is indicated by asterisk (*).](image)

![Figure 2. UV spectra for metoprolol tartrate dissolved in water (---), methanol (- ■ -), 0.1 M HCl (- - -) and 0.1 M ammonium acetic buffer - pH 4.0 (- ● -).](image)

![Figure 3. UV spectra for propranolol hydrochloride dissolved in water (- – -), methanol (- ● -), 0.1 M HCl (- — -) and 0.1 M ammonium acetic buffer - pH 4.0 (- ■ -).](image)
finely powdered. A quantity of powder equivalent to 25.0 mg of metoprolol was weighted and transferred to a 25.0 mL volumetric flask. After shaking with 5.0 mL of methanol (5 min), 20.0 mL of mobile phase was added. 200.0 µL of the filtered solution was filled up to 2.0 mL with the mobile phase, subsequently. Thymidine, used as an internal standard, was added at a concentration of 20 µg/mL to the prepared samples.

Twenty tablets, each containing 10 mg propranolol hydrochloride were accurately weighted and finely powdered. A quantity of powder equivalent to 10.0 mg of propranolol was weighted and transferred to a 25.0 mL volumetric flask. After shaking with 5.0 mL of methanol (5 min), 20.0 mL of mobile phase was added. 200.0 µL of the filtered solution was filled up to 2.0 mL with the mobile phase, subsequently. Thymidine, used as an internal standard, was added at a concentration of 20 µg/mL to the prepared samples.

**Method validation**

HPLC method was validated to determine linearity, range, sensitivity, intermediate accuracy and precision in agreement with the International Conference on Harmonisation (3).

### Table 1. HPLC method validation parameters for determination of metoprolol and propranolol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Metoprolol</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linearity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibration range</td>
<td>70–150 µg/mL</td>
<td>10–50 µg/mL</td>
</tr>
<tr>
<td>Calibration points</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Slope (%RSD)</td>
<td>0.027</td>
<td>0.0067</td>
</tr>
<tr>
<td>Intercept (%RSD)</td>
<td>1.32</td>
<td>0.16</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9995</td>
<td>0.9995</td>
</tr>
<tr>
<td>LOD</td>
<td>0.333 µg/mL</td>
<td>7.22 ng/mL</td>
</tr>
<tr>
<td>LOQ</td>
<td>1.008 µg/mL</td>
<td>21.87 ng/mL</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>80 µg/mL</td>
<td>25 µg/mL</td>
</tr>
<tr>
<td>% RSD</td>
<td>2.00</td>
<td>0.97</td>
</tr>
<tr>
<td>% Recovery</td>
<td>101.32</td>
<td>98.11</td>
</tr>
</tbody>
</table>

*Values indicated inter-day variation expressed as a slope (% RSD) of three calibration curves prepared on three consecutive days

*b Precision expressed as % RSD of four determinations

### Table 2. The average content of metoprolol tartrate and propranolol hydrochloride in tablets.

<table>
<thead>
<tr>
<th>Product</th>
<th>Mean (± SD)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metocard (50 mg; batch no. 30408; exp. date 04.2011)</td>
<td>96.12 (± 0.79)</td>
<td>0.82</td>
</tr>
<tr>
<td>Metocard (50 mg; batch no. 20105; exp. date 01.2008)</td>
<td>93.16 (± 0.54)</td>
<td>0.58</td>
</tr>
<tr>
<td>Metohexal (50 mg; batch no. 7R5720; exp. date 04.2010)</td>
<td>99.28 (± 0.35)</td>
<td>0.36</td>
</tr>
<tr>
<td>Metohexal (50 mg; batch no. 44IZ70; exp. date 08.2007)</td>
<td>94.78 (± 0.29)</td>
<td>0.31</td>
</tr>
<tr>
<td>Propranolol (10 mg; batch no.02ME0108; exp. date 01.2011)</td>
<td>97.20 (± 0.82)</td>
<td>0.84</td>
</tr>
<tr>
<td>Propranolol (10 mg; batch no. 05ME0305; exp. date 03.2008)</td>
<td>94.52 (± 0.75)</td>
<td>0.80</td>
</tr>
<tr>
<td>Propranolol (10 mg; batch no.04ME0402; exp. date 04.2005)</td>
<td>89.52 (± 0.52)</td>
<td>0.58</td>
</tr>
</tbody>
</table>

*a average (%) of three determinations
Five-point calibration curves were constructed over the concentration range of 70–150 µg/mL for metoprolol tartrate and 10–50 µg/mL for propranolol hydrochloride. Characteristic parameters for regression equation \(y = ax + b\) of the HPLC method obtained by least squares treatment of the results was used to confirm the good linearity of method development.

For both metoprolol and propranolol, limit of detection (LOD) and limit of quantitation (LOQ) were determined. LOD and LOQ were estimated using the standard estimation error (\(\delta\)) and the slope of calibration curve (\(a\)) from the following equations:

\[
\text{LOD} = 3.3 \times \frac{\delta}{a} \quad \text{and} \quad \text{LOQ} = 10 \times \frac{\delta}{a}
\]

Precision was determined with 4 replicates of quality control (QC) samples. QC samples were prepared in the mobile phase at three concentrations: 80, 100 and 120 µg/mL for metoprolol and 25, 35 and 45 µg/mL for propranolol, following the same procedure as for calibration standards using different primary stock solutions. The results were expressed as percent relative standard deviation for number of samples (%RSD) and percent recovery. For quality control (QC) samples of metoprolol tartrate, propranolol hydrochloride and for thymidine peak asymmetry calculations were made using the following equation:

Asymmetry factor (AF) = \(W_{5\%}/2 \times W_{1/2}\)

where \(W_{5\%}\) - peak width (mm) measured at 5% peak height, and \(W_{1/2}\) - the width (mm) of the left half of the peak at 5% peak height.

To assess inter-day variation, the construction of calibration curve was repeated on three consecutive days. The results were expressed as the means (± SD) of slopes and intercepts.

RESULTS

Validation

The representative chromatograms of blank and extracted samples are shown in Figures 4 and 5. No interfering peaks were observed near the retention time of metoprolol (MET), propranolol (PPN) or thymidine (THYM). The retention times of thymidine, metoprolol and propranolol were approximately 9.7, 16.6 and 20.5 min, respectively. Five-point calibration curves were constructed for 80-120% of the test concentration, i.e. for metoprolol tartrate in the range of 70-150 µg/mL and for propranolol hydrochloride in the range of 10-50 µg/mL.

The standard curve was constructed on three consecutive days and regression parameters, slope and correlation coefficient were calculated and listed in Table 1.

Precision of the method was determined by analyzing quality control (QC) samples at three different concentrations within the calibration range in four determinations. QC samples prepared in blank were dilutions from weightings independent of those used for constructing calibration curves (Table 1). The RSD values were < 2.00 and < 0.97%, and the percent recovery of the method was 100 ± 2.32% for metoprolol and 100 ± 1.89% for propranolol, respectively, indicating that the method was precise and accurate.

LOD values, as the lowest concentrations of the analyte detected by the method, were 0.333 µg/mL for metoprolol and 7.22 ng/mL for propranolol. LOQ as the minimum quantifiable concentration were 1.008 µg/mL and 21.87 ng/mL, respectively (Table 1).

Peak asymmetry values (± SD) for metoprolol tartrate at three concentrations of QC samples: 80, 100, 120 µg/mL were 1.69 ± 0.07, 1.65 ± 0.04 and 1.80 ± 0.06, respectively. Peak asymmetry values (± SD) for propranolol hydrochloride at three concentrations of QC samples: 25, 35 and 45 µg/mL were 1.67 ± 0.07, 1.66 ± 0.04 and 1.82 ± 0.02, respectively. Peak asymmetry value (± SD) for thymidine was 1.29 ± 0.03.
Stability studies of expired tablets of metoprolol tartrate and propranolol hydrochloride

Quantitative analysis

No discrepancies between the results of determination and the declared values range (90–110%) for all the analyzed tablets were observed (Table 2). The estimated tablet contents for metoprolol tartrate of all batches were 93.16–99.28% and for propranolol hydrochloride 89.52–97.20%, respectively.

DISCUSSION AND CONCLUSION

The proposed HPLC method for content determination of tablets is characterized by good linearity, sensitivity, as well as intermediate accuracy and precision. No discrepancies between the results of determination and the declared values range (90–110%) for all the analyzed tablets were observed. It has been shown that many drugs stored under reasonable conditions retain 90% of their potency for at least 5 years after the expiration date on the label, and sometimes much longer. The American Medical Association (AMA) concluded that the actual shelf lives of some products are greater than their labeled expiration dates (4). However, only a few studies have addressed the long-term stability of drug products. One study determined that four products (captopril tablets, fluoxacillin capsules, cefoxitin injection, theophylline tablets) stored under ambient temperature maintained at least 98% of label claim for drug content for 18-170 months past the labeled expiration dates (5). Data from the Department of Defense/FDA Shelf Life Extension Program (SLEP), which tests the stability of drug products past their expiration date, showed that 84% of 1122 lots of 96 different drug products stored in military facilities in their unopened original containers would be expected to remain stable for an average of 57 months after their original expiration date (4, 6). The results of our study might suggest that storage of analyzed batches of tablets containing metoprolol tartrate (Metocard 50 mg, Polpharma, Poland and Metohexal 50 mg, Hexal AG, Poland) or propranolol hydrochloride (Propranolol, 10 mg, Polfa Warszawa, Poland) over time period exceeding the expiry date given by the manufacturer did not influence their contents. It should be noted that the batches of tablets used in the study were taken randomly and no data describing the storage conditions were available. Another point is the choice of drugs used in the performed experiments. Metoprolol and propranolol are substances with well established position on the pharmaceutical market, but the aim of this study was to assess the problem of drug stability over the expiry period declared by the producer.

REFERENCES


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