In recent years the growing interest in drug stability problem has been observed. However, there are not many studies that reported about the stability of drugs past their expiration dates. If analysis of tablet stability is considered, the most important ones are content determination and dissolution test. Research on the release of medicinal substances is accompanied by a broad range of information concerning the analyzed preparation, i.e. numerous physical and chemical properties of the dosage form, the methods of production. Finally, the changes in dissolution profile might be the result of ageing of the product.

For immediate/conventional-release formulations typically, for quality control purposes, one limit is specified to ensure that most of the active ingredient is released within the present time period and run time usually does not exceed 30, 45 or 60 min. A formulation is in this concern understood as very fast releasing, when at least 80% of the drug substance, corresponding to $Q^* = 75\%$ is dissolved in the above time period (1-3). The comparison of batches during stability tests or establishing the similarity of pharmaceutical dosage forms require to perform 12 dissolution profiles of the compared products with several (e.g. 3) points (2).

The objective of the current study was to perform studies of content determination and dissolution test of expired tablets and tablets which expiry date has not exceeded. The analyzed tablets contained metoprolol tartrate (50 mg) and propranolol hydrochloride (10 mg), respectively (see preceding paper).

EXPERIMENTAL

Chemicals

Metoprolol tartrate and propranolol hydrochloride were from Sigma-Aldrich Co. The formulated dosage forms were the same as described in the preceding paper.

Apparatus

Content determination using spectrophotometric analysis

The spectrophotometric analysis was performed using UV-Vis spectrophotometer Varian Cary 50 Bio, 10 mm quartz cells. Cary WinUV Application software version 02.50(156) (Varian, USA) was used for data acquisition.

Dissolution test

The dissolution test was performed in Cary UV dissolution test system (Varian, USA) comprising of VK method proposed by Moore (6). According to the FDA guidelines, immediate release formulations require to perform 12 dissolution profiles of the compared products with several (e.g. 3) points (2).

The objective of the current study was to perform studies of content determination and dissolution test of expired tablets and tablets which expiry date has not exceeded. The analyzed tablets contained metoprolol tartrate (50 mg) and propranolol hydrochloride (10 mg), respectively (see preceding paper).
Dissolution Apparatus, UV-Vis spectrophotometer 50 Bio, 10 mm quartz cells, VK-810 pump and Full Flow Filters (35 µm). Cary WinUV Dissolution Application software version 02.50 (156) (Varian, USA) was used for data acquisition, reporting and analysis.

Method development
Preparation of standard solutions
Primary stock solution of metoprolol was prepared in ultra pure water to obtain concentration of 1.0 mg/mL. Primary standard solution was further diluted in 0.1 M HCl to obtain working standards in the range of 40-120 µg/mL.

Primary stock solution of propranolol was prepared in ultra pure water to obtain concentration of 1.0 mg/mL. Primary standard solution was further diluted with 0.1M HCl to obtain working standards in the range of 5-30 µg/mL.

Sample preparation
Twenty tablets, each containing 50 mg of metoprolol tartrate were accurately weighed and finely powdered. A quantity of powder equivalent to 25.0 mg metoprolol was weighted and transferred to a 25.0 mL volumetric flask. After shaking with 5.0 ml methanol (5 min), the obtained filtrate in volume of 1.0 ml was filled up to 20.0 ml with 0.1 M HCl.

Twenty tablets, each containing 10 mg propranolol hydrochloride were accurately weighed and finely powdered. A quantity of powder equivalent to 20.0 mg propranolol was weighted and transferred to a 100.0 mL volumetric flask. After shaking with 10.0 mL of methanol (5 min), the obtained filtrate in a volume of 1.0 mL was filled up to 10.0 mL with 0.1 M HCl.

Dissolution test
In the next step, release profiles were examined using dissolution tests for both metoprolol tartrate and propranolol hydrochloride tablets, performed using multibath (n = 6) apparatus with paddles. A dissolution medium was 0.1 M HCl. A paddle speed was 100 rpm and the medium volume was 500 mL. The medium was maintained at 37 ± 0.5°C. The 1-L glass dissolution vessels were covered to minimize evaporation.

The samples of metoprolol tartrate and propranolol hydrochloride were taken at appropriate time intervals at 5, 10, 15, 20, 25, 30, 40 and 60 min and assayed spectrophotometrically at 275 and 290 nm, respectively.

Method validation
Spectrophotometric method of analysis used during dissolution test was validated to determine linearity, range, sensitivity, intermediate accuracy and precision in agreement with the International Conference on Harmonisation recommendations (7).

Five-point calibration curves were constructed over the concentration range of 40–120 µg/mL for metoprolol tartrate and 5–30 µg/mL for propranolol hydrochloride. Characteristic parameters for regression equation (y = ax + b) of the spectrophotometric method, obtained by the least squares treatment of the results, confirmed good linearity of the method.

For both metoprolol and propranolol, the limit of detection (LOD) and limit of quantitation (LOQ) were determined experimentally. Precision was determined with 3 replicates of quality control (QC) samples. QC samples were prepared in mobile phase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Metoprolol</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration range</td>
<td>40–120 µg/mL</td>
<td>5–30 µg/mL</td>
</tr>
<tr>
<td>Calibration points</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Slope (%RSD)</td>
<td>0.013</td>
<td>0.0056</td>
</tr>
<tr>
<td>Intercept (%RSD)</td>
<td>0.43</td>
<td>0.70</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9991</td>
<td>0.9994</td>
</tr>
<tr>
<td>LOD</td>
<td>5 µg/mL</td>
<td>1 µg/mL</td>
</tr>
<tr>
<td>LOQ</td>
<td>15 µg/mL</td>
<td>3 µg/mL</td>
</tr>
</tbody>
</table>

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

\* Precision expressed as % RSD of three determinations
Stability studies of expired tablets of metoprolol tartrate...

Figure 1. Dissolution profiles of Metocard 50 mg.

Figure 2. Dissolution profiles of Metohexal 50 mg.

Figure 3. Dissolution profiles of Propranolol 10 mg.
at three concentrations: 50, 70 and 110 µg/mL for metoprolol and 12, 17 and 22 µg/mL for propranolol, following the same procedure as for calibration standards using different primary stocks. The results were expressed as percent relative standard deviation for number of samples (%RSD) and percent recovery. 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**

Five-point calibration curves were constructed for metoprolol tartrate in the range of 40-120 µg/mL and for propranolol hydrochloride in the range of 5-30 µg/mL. This concentration ranges were selected considering the expected concentrations observed during dissolution test. Standard curve was constructed on three consecutive days and regression parameters, slope, and correlation coefficient were calculated and listed in Table 1.

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>92.74 (±0.56)</td>
<td>0.61</td>
</tr>
<tr>
<td>Metocard (50 mg; batch no. 20105; exp. date 01.2008)</td>
<td>90.30 (±1.39)</td>
<td>1.53</td>
</tr>
<tr>
<td>Metoxeral (50 mg; batch no. 7R5720; exp. date 04.2010)</td>
<td>92.96 (±0.09)</td>
<td>0.10</td>
</tr>
<tr>
<td>Metoxeral (50 mg; batch no. 44JZ70; exp. date 08.2007)</td>
<td>94.29 (±0.09)</td>
<td>0.10</td>
</tr>
<tr>
<td>Propranolol (10 mg; batch no.02ME0108; exp. date 01.2011)</td>
<td>96.66 (±0.83)</td>
<td>0.86</td>
</tr>
<tr>
<td>Propranolol (10 mg; batch no. 05ME0305; exp. date 03.2008)</td>
<td>94.82 (±0.86)</td>
<td>0.91</td>
</tr>
<tr>
<td>Propranolol (10 mg; batch no.04ME0402; exp. date 04.2005)</td>
<td>93.61 (±0.71)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

*average (%) of six determinations

Table 3. The f2 values from comparison of metoprolol and propranolol tablets with different expiry dates.

<table>
<thead>
<tr>
<th>Product 1</th>
<th>Product 2</th>
<th>f2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metocard (50 mg, exp. date 04.2011)</td>
<td>Metocard (50 mg, exp. date 04.2010)</td>
<td>56.55</td>
</tr>
<tr>
<td>Metoxeral (50 mg, exp. date 04.2010)</td>
<td>Metoxeral (50 mg, exp. date 08.2007)</td>
<td>79.53</td>
</tr>
<tr>
<td>Propranolol (10 mg, exp. date 01.2011)</td>
<td>Propranolol (10 mg, exp. date 03.2008)</td>
<td>99.83</td>
</tr>
<tr>
<td>Propranolol (10 mg, exp. date 01.2011)</td>
<td>Propranolol (10 mg, exp. date 04.2005)</td>
<td>99.92</td>
</tr>
</tbody>
</table>

Precision of the method was determined by analyzing quality control (QC) samples at three different concentrations within the calibration range in triplicates. QC samples prepared in blank were dilutions from weightings independent of those used for construction of calibration curves (Table 1). The RSD values were < 0.21 and < 0.62%, and the percent recovery of method was 100 ± 0.87% for metoprolol and 100 ± 3.52% 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 5 µg/mL for metoprolol and 1 µg/mL for propranolol. LOQ as the minimum quantifiable concentration were 15 µg/mL and 3 µg/mL, respectively (Table 1).

No impact of the medium: water, 0.1 M HCl, methanol or acetate buffer on the UV spectra for both metoprolol and propranolol was denoted, as well (preceding paper).

Comparison of quantitative analysis and dissolution profiles

No discrepancies between the results of deter-
Stability studies of expired tablets of metoprolol tartrate... 707

...mination and the declared values range (90–110%) for all the analyzed tablets were observed (Table 2). The estimated spectrophotometrically tablet contents for metoprolol tartrate of all batches were 90.30–94.29% and for propranolol hydrochloride 93.61–96.66%.

Figures 1 and 2 illustrate 1-hour dissolution profiles of two commercial products of metoprolol tartrate (Metocard; Metohexal) with different expire dates. Figure 3 illustrates 1-hour dissolution profiles of propranolol hydrochloride (Propranolol) with different expire dates. The observed dissolution profiles were similar, despite using products which were out of expiry date. This observation was confirmed by calculating $f_2$ values. For all tested products, including those with outdated tablets, $f_2$ was between 50 and 100 (Table 3).

DISCUSSION AND CONCLUSION

The proposed method including spectrophotometric estimation of the tablet content 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. The dissolution profiles, obtained during dissolution tests performed in paddle apparatus and 0.1 M HCl, of both: expired and not expired tablets were found to be similar, as well. This observation was confirmed by calculating $f_2$ values. In recent years, FDA has placed more emphasis on a dissolution profile comparison in the area of post-approval changes and biowaivers. Moore and Flanner proposed a model independent mathematical approach to compare the dissolution profiles using two factors, $f_1$ and $f_2$ (6). The factor $f_1$ is proportional to the average difference between the two profiles, whereas factor $f_2$ is inversely proportional to the average squared difference between the two profiles, with emphasis on the larger difference among all the time-points. The factor $f_2$ – similarity factor, measures the closeness between the two profiles. In dissolution profile comparisons, especially to assure similarity in product performance, regulatory interest is in knowing how similar the two curves are, and to have a measure which is more sensitive to large differences at any particular time point. For this reason, the $f_2$ comparison has been the focus in Agency guidances. When the two profiles are identical, $f_2 = 100$. An average difference of 10% at all measured time points results in a $f_2$ value of 50. FDA has set a public standard of $f_2$ value between 50 and 100 to indicate similarity between two dissolution profiles. In our study, for all tested products, including those with outdated tablets, $f_2$ was between 50 and 100.

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 (8). However, only a few studies have addressed the long-term stability of drug products. The results of our study, including both: tablet content analysis (see also preceding paper) and estimation of dissolution profiles, might suggest that the storage of analyzed batches of tablets containing metoprolol tartrate (Metocard 50 mg, Polpharma, Poland; 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 is worth to note that the batches of tablets used in our study were taken randomly and no data describing the storage conditions were available. Further studies assessing the stability of tablets containing the above drug substances are required.

REFERENCES

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