AN IMPROVED METHOD FOR THE IDENTIFICATION AND QUANTIFICATION OF IMPURITIES IN ROXITROMYCIN SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET AND MASS DETECTION

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Erythromycin type macrolide antibiotics are widely used today as effective agents against infections with gram-positive bacteria, gram-negative cocci, mycoplasma and chlamydiae. One of the main disadvantage of erythromycin A (1), which has been the first clinically useful macrolide antibiotic, is the lability of this antibiotic natural product in acidic media. A successful concept to prevent this inactivation is the synthetic transformation of 1 to roxithromycin A (2) which is known to be acid stable and can be applied orally.

Though several articles have been published on the quantitative determination of impurities in macrolide antibiotics (see references in [2] and [3]), the analytical profiling of roxithromycin preparations is still a difficult question requiring an analytical method of high performance; first because the semi-synthetic antibiotic roxithromycin contains impurities deriving from both the fermentation process and the semi-synthetic transformation steps and secondly because nowadays analytical profiling of pharmaceutical drugs is recommended down to a range of 0.1%. The European Pharmacopoeia [1] provides an HPLC method for the quality assurance of roxithromycin preparations, which does not allow an identification of impurity peaks by HPLC-MS, because a non volatile phosphate buffer system is used for elution. This prevents a valuation of roxithromycin samples, which have been obtained by new methods of preparation or purification and contain hitherto unknown impurities.
In this contribution we want to present a new HPLC method using a HyPURITY Elite C18 column for separation, a mixture of acetonitrile and aqueous ammonium acetate solution for elution and an UV detector adjusted to 215 nm for detection. Structural information about the by-products was obtained by comparison with reference substances and by HPLC-MS analysis giving mass spectrometric data of the molecule and fragment ions. A comparison of HPLC-MS and HPLC data revealed the complete information on the impurity profile of the analysed roxithromycin samples and allowed an assignment of mass spectrometric data to HPLC peaks as well as a correlation of peak areas to HPLC-MS peaks. With this new method well in hand we investigated crude and purified samples of industrial produced roxithromycin to demonstrate the impact of the final purification step on the impurity profiles.

<table>
<thead>
<tr>
<th>assignment</th>
<th>m/e</th>
<th>roxithromycin pure (RX-9100105)</th>
<th>roxithromycin crude (RX-9100199)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>R_t</td>
<td>% area</td>
</tr>
<tr>
<td>erythromycin-Z-oxime</td>
<td>749.4</td>
<td>6.24</td>
<td>0.485</td>
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<tr>
<td>erythromycin A</td>
<td>734.5</td>
<td>7.03</td>
<td>0.094</td>
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<td>erythromycin-E-oxime</td>
<td>749.4</td>
<td>7.72</td>
<td>0.152</td>
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<tr>
<td>roxithromycin C</td>
<td>823.5</td>
<td>10.01</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td>837.5</td>
<td>11.23</td>
<td>0.217</td>
</tr>
<tr>
<td>N-desmethyl-roxithromycin</td>
<td>823.5</td>
<td>12.48</td>
<td>0.284</td>
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<tr>
<td>roxithromycin A</td>
<td>837.5</td>
<td>15.42</td>
<td>98.357</td>
</tr>
<tr>
<td>roxithromycin A + second sidechain</td>
<td>925.5</td>
<td>19.04</td>
<td>0.104</td>
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<tr>
<td>roxithromycin A + second sidechain</td>
<td>925.5</td>
<td>22.30</td>
<td>0.093</td>
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<tr>
<td>roxithromycin B</td>
<td>821.5</td>
<td>26.65</td>
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<tr>
<td>roxithromycin A methylated</td>
<td>851.5</td>
<td>28.15</td>
<td>0.160</td>
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<tr>
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<td>843.5</td>
<td>32.26</td>
<td>0.190</td>
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<td></td>
<td>841.5</td>
<td>100.000</td>
<td>99.999</td>
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References: