RADIOSTABILITY OF VANCOMYCIN

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Introduction
Vancomycin is a glycopeptide antibiotic, which can be sterilised by radiation treatment. In this study, the drug substance, as well as a controlled-release formulation drug product, were subjected to radiation receiving a Ph.Eur.-recommended sterilisation dose of 25 kGy and an overkill dose of 50 kGy. In addition, non-irradiated samples were also subjected to a 2 and 4 hours heat treatment, mimicking the radiation temperature effects. The treated samples, as well as untreated control samples, were quantitatively assayed with isotropic HPLC-UV and the results confirmed with LC-MS, FT-IR and TLC.

Results and discussion

Quantitative assay by HPLC-UV

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug substance</th>
<th>Drug product</th>
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</thead>
<tbody>
<tr>
<td>25 kGy</td>
<td>94.5%</td>
<td>95.9%</td>
</tr>
<tr>
<td>50 kGy</td>
<td>92.5%</td>
<td>92.6%</td>
</tr>
<tr>
<td>2 hrs 50°C</td>
<td>96.5%</td>
<td>97.8%</td>
</tr>
<tr>
<td>4 hrs 50°C</td>
<td>98.1%</td>
<td>99.2%</td>
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Table 1. Mean assay results of vancomycin (versus untreated control)

A decreasing trend in assay-values is observed. When comparing these results with the Ph. Eur. quality specifications for the drug substance, only the samples treated with an overkill dose of 50kGy are at 93% under-limit.

Confirmation and investigations with LC-MS, FT-IR and TLC

1) LC-MS
Preliminary investigations: no massive degradation-peak found.
Final conditions (Diana et al, 2006):
- Column: Zorbax extended C18 (5 µm, 3.0 x 250 mm) (21°C ± 5°C)
- Mobile phase: MeOH/H2O 0.2 M NH4OAc pH 9.0 (30/65/5 %V); flow: 0.15 µL
- UV detection: λ = 285 nm
- Injection volume: 20 µL
- MS detection: LCG iontrap (Thermo-Finnigan) in positive mode

Forced degradations: 0.1M HCl, 0.5M NaOH and 30% H2O2 for 4 hours at 60°C.

Forced degradations: - HCl more than 50% DESV + some aglucovancomycin (not shown) - NaOH: massive dispersed degradation - H2O2: no major degradation observed

Radiosterilised (overkill 50 kGy): degradation-peaks are observed:
- Peak a (m/z = 1412): ±0.6%
- Peak b (m/z = 1305): ±3.4% (also present in untreated samples at ±1.2%)
- Peak c (m/z = 1463): ±0.8%
- Peak d (m/z = 1428 and 1463): ±1.7% [m/z 1428 peak also present at 0.7% in untreated sample]
- Peak e (m/z = 1428): ±1.0%

No significant changes in the IR spectra are observed, indicative that no major, functional degradant is formed.

2) IR
Conditions: - KBr pellets - Perkin-Elmer 2000 FT-IR - R/2 2 cm-1 BG corrected.

Forced degradations:
- Mobile phase: H2O/1-Propanol (40/60 %V)
- Spot: 20 µL
- Detection: HClO4 20%, 10 min 120°C

No significant degradation spots could be observed for the irradiated samples on the plates. The HCl and NaOH forced degradation experiments samples clearly show major degradation spots.

3) TLC
Conditions: - Plate: Silicagel 60F254 - Mobile phase: H2O/Propanol (40/60 %V) - Concentration: 0.5 mg/ml - Spots: 20 µL - Detection: HClO4, 20%, 10 min 120°C

No significant degradation spots could be observed for the irradiated samples on the plates. The HCl and NaOH forced degradation experiments samples clearly show major degradation spots.

Conclusions
A decreasing trend in HPLC-UV assay-values is observed after radio-sterilisation and some degradation product were detected with LC-MS. No massive degradation of vancomycin as drug substance nor as formulated drug product could be detected, even at the overkill dose of 50kGy: the assay value in the samples treated with an overkill dose of 50kGy are at the under-limit of the quality specifications required by the Ph. Eur. monograph for the drug substance. Several radiation-originated degradation products were detected by LC-UV/MS, accounting for 5.6% of the total related impurities of 7.5%, consistent with the assay loss determined by quantitative HPLC-UV.