RADIOSTABILITY OF VANCOMYCIN

DRUQUAR
Drug Quality
&
Registration

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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 isocratic HPLC-UV and the results confirmed with LC-MS, FT-IR and TLC.

Results and discussion

Quantitative assay by HPLC-UV

Radiation conditions: - Mean electron energy on Ta: 10 MeV
- Resulting X electro-magnetic radiation energy: 1.5 MeV
- Irradiation time: 2 hours (25 kGy) and 4 hours (50 kGy)

HPLC Conditions:
- Column: Lichrospher 100 RP 18 (5 µm; 4.0 x 250 mm) (21°C ± 5°C)
- Mobile phase: 1/7/92 %V THF/AcCN/0.4% TEA (pH 3.2 – H_3PO_4)
- Flow: 1.2 mL/min
- UV detection: λ = 280 nm
- Injection volume: 25 µL
- Samples: ± 125 µg/ml (Zn^{2+} treated)

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.

Table 1. Mean assay results of vancomycin (versus untreated control)

Treatment	Drug substance	Drug product
25 kGy	94.5%	95.9%
50 kGy	92.5%	92.6%
2 hrs 50°C	96.5%	97.8%
4 hrs 50°C	98.1%	99.2%

(Standard deviation: ± 2.5%)

Confirmation and investigations with LC-MS, FT-IR ant 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/H_2O/0.2 M NH_4OAc pH 9.0 (30/65/5 %V); flow: 0.15 µL
- UV detection: λ = 285 nm
- Injection volume: 20 µL
- MS detection: LCQ iontrap (Thermo-Finnigan) in positive mode

Forced degradations: 0.1M HCl, 0.5M NaOH and 30% H_2O_2 for 4 hours at 60°C.

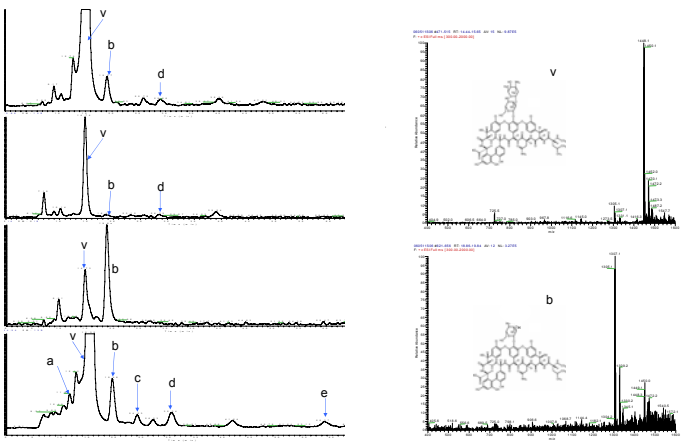

Figure 1: From top to bottom; UV chromatograms of untreated vancomycin(±1.25mg/mL), H_2O_2 treated (0.1mg/mL), HCl treated (0.1mg/mL), and 50kGy irradiated vancomycin(±2mg/mL). Mass spectra of vancomycin peak(v) (vancomycin) and desvancosaminylvancomycin (DESV) peak (b).

Forced degradations:
- **HCl: more than 50% DESV + some aglucovancomycin (not shown)**
- **NaOH: massive dispersed degradation**
- **H_2O_2: 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%**

2) IR

Conditions: - KBr pellets
- Perkin-Elmer 2000 FT-IR
- R_s: 2 cm^{-1} / BG corrected.

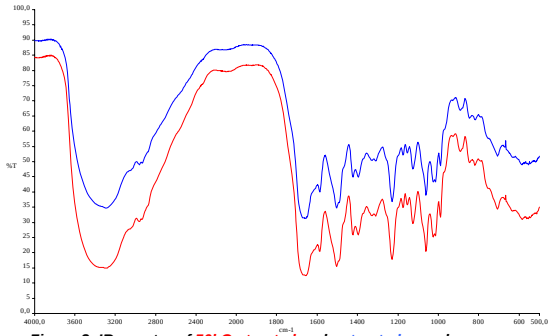

Figure 2: IR spectra of 50kGy treated and untreated sample.

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

3) TLC

Conditions: - Plate: Silicagel $60F_{254}$
- Mobile phase: H_2O/1-Propanol (40/60 %V)
- Concentration: 0.5 mg/ml
- Spot: 20 µL
- Detection: $HClO_4$ 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.