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Comparative determination of various vancomycin preparations with positive ionization electrospray LC-MS-MS

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1. Introduction

Vancomycin (fig.1) is a glycopeptide antibiotic isolated from both *Streptomyces orientalis* and *Nocardia lucida* (1). It was first introduced for treatment of staphylococcal infections in the 1950s and was quickly relegated to the role of alternate drug, due to high incidence of nephrotoxicity and ototoxicity (2). However, the increasing number of *Staphylococcus* and *Streptococcus* isolates, resistant to various antibiotics, led to rehabilitation of vancomycin. In addition, new generation of preparations was devoid of most impurities and showed fewer side effects (3). Nevertheless, it was generally believed that drug toxicities could be avoided if serum concentrations were kept below 40 mg/l and therapeutic drug monitoring (TDM) was recommended, with a through level of 5 – 10 mg/l (4, 5). Usually TDM of vancomycin is done with immunoassays (FPIA, EMIT). Recently, also an electrospray LC-MS-MS has been introduced after simple plasma precipitation procedures (6, 7).

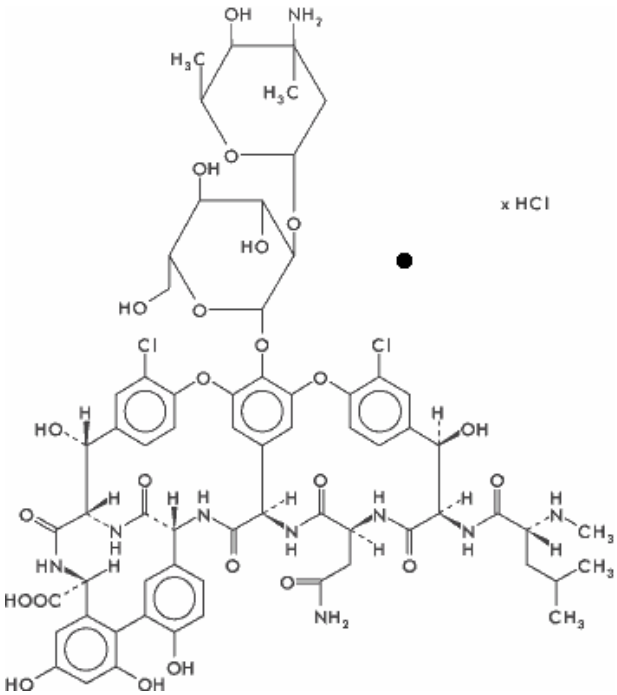


Fig.1. The structure of vancomycin (m.w. 1449)

It has been observed in our hospital that the introduction of the new brand of vancomycin was associated with some incidents of nephrotoxicity among patients. The Department of Hospital Pharmacy requested to perform an analytical study concerning various vancomycin preparations which are currently available. The study was performed using LC-MS-MS technique and the results are presented.

2. Experimental.

2.1. Materials

Following vancomycin preparations were analyzed:

- Vancomycin Hydrochloride USP Abbott (USA) cont. vancomycin hydrochloride equivalent to 1000 mg base per vial
- Vancocin HCl USP Lilly (USA) cont. vancomycin hydrochloride equivalent to 1000 mg base per vial
- Vancomycin hydrochloride DUMEX (Denmark) cont. vancomycin hydrochloride equivalent to 500 mg base per vial
- Vancomycin hydrochloride for Injection BP Faulding (UK) cont. vancomycin hydrochloride equivalent to 1000 mg base per vial
- Vancomycine Merck (Greece) cont. vancomycin hydrochloride equivalent to 500 mg base per vial

From each preparation, stock solutions of 1 mg/ml in acetonitrile-water (20:80) were prepared.

2.2. LC-MS-MS

Following instrumentation was applied: TSQ Quantum triple quadrupole LC-MS-MS (Thermo Finnigan, USA) with quaternary Surveyor pump and Surveyor AS autosampler. Electrospray ion source in positive mode was used. The conditions (spray voltage, sheath and auxiliary gas flow, tube lens voltage, capillary temperature) were optimized for vancomycin using solution of the drug in the mobile phase (acetonitrile – ammonium formate buffer 10 mM, pH 3.0 (20:80), introduced directly to the instrument using syringe infusion (at 5 µl/min) combined through tee-joint with the mobile phase flow at 200 µl/min.

Full scan analysis was done in the range 100 > 1500 amu on the 1st quadrupole, the collision energy was set at 5 v.

Chromatographic analysis was performed on Superspher ODS column 125 x 2 mm (Merck). For quantitative MRM analysis, isocratic elution in mobile phase: acetonitrile – ammonium formate buffer 10 mM, pH 3.0 (20:80) at flow rate of 200 µl/min was done. For full-scan profiling of vancomycin preparations, gradient elution was applied, using following profile: 20% acetonitrile in ammonium formate buffer for 1 min, raise to 50% acetonitrile in 5 min, hold 50% acetonitrile for 5 min. The flow rate was 200 µl//min.

3. Results.

Full-scan MS analysis showed the ion m/z 725 amu as the most intensive in all vancomycin preparations (fig.2). This ion corresponds to doubly charged ion of vancomycin. Such finding is quite common for peptide molecules with mass between 1000 and 2000 amu. Small ion m/z 1450 (single-protonated molecular ion) was also visible.

Product scan analysis of m/z 725, performed in the range m/z 100 > 1500 amu in infused vancomycin solution, revealed two main product ions: m/z 1306 and 144 (Fig 3). These transitions have been used for MRM analysis of vancomycin preparations. MRM analysis was done from vancomycin solutions containing 1 ng/µl, the injection volume was 5 µl. The peak of vancomycine was observed at Rt around 1.9 min. Signal intensities for all preparations were similar (Fig. 4 a -4 e).

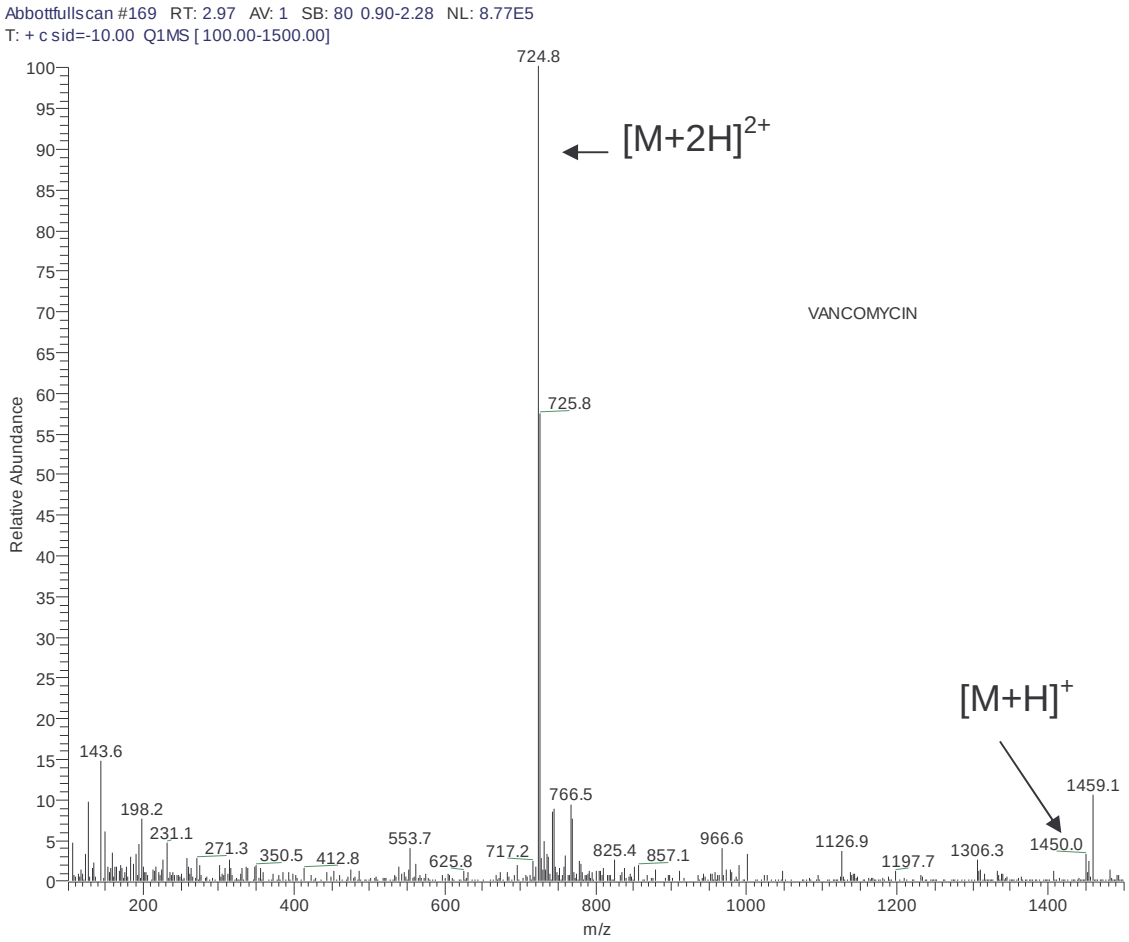


Fig.2. Mass spectrum of vancomycin. Full-scan (100 > 1500 amu) on 1st quadrupole.

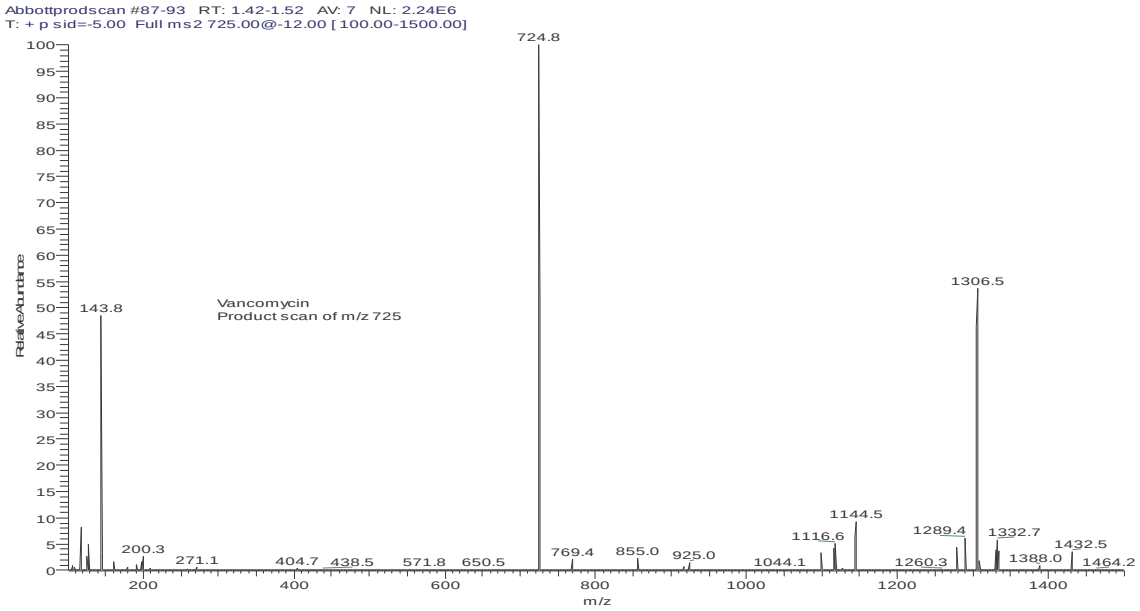


Fig.3. Product scan mass spectrum of m/z 725.

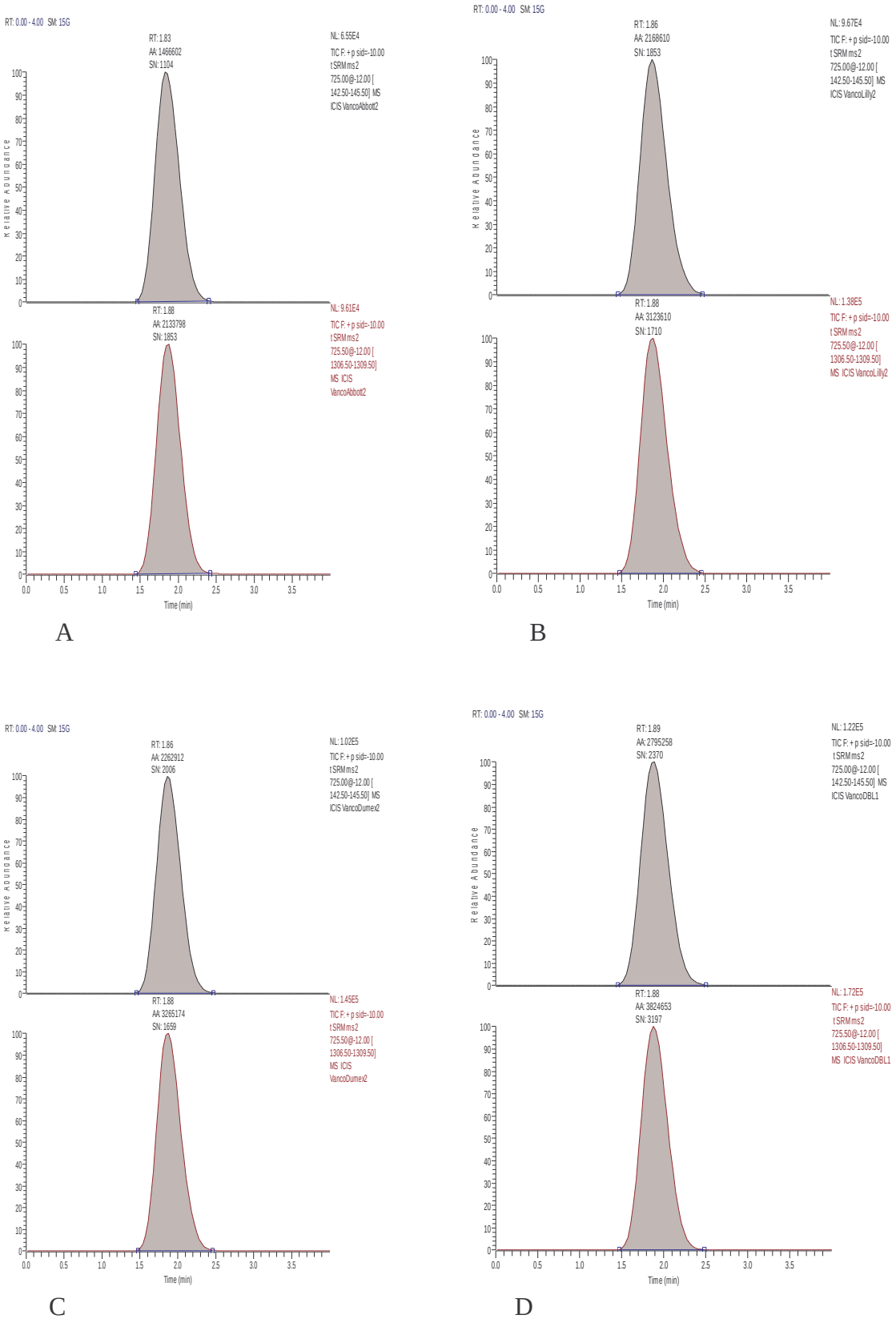
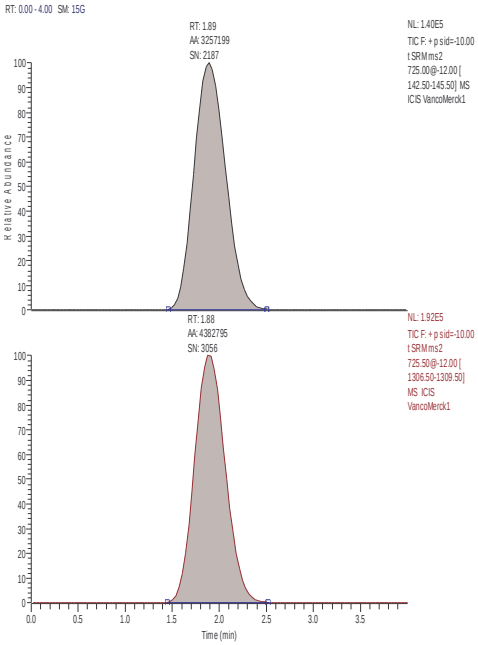


Fig.4 . Mass chromatograms of vancomycin preparations from Abbott (A), Lilly (B), Dumex (C) and Faulding (D). 5 ng amounts were injected.



E

Fig.4 (continued). Mass chromatograms of vancomycin preparations from Merck (E). 5 ng amounts were injected.

Figs. 5, 6, 7, 8, and 9 show mass chromatograms of vancomycin solutions, analyzed in full scan mode in gradient elution conditions.

In all cases very similar chromatograms were observed, without indication for any different peak pattern in particular sample.

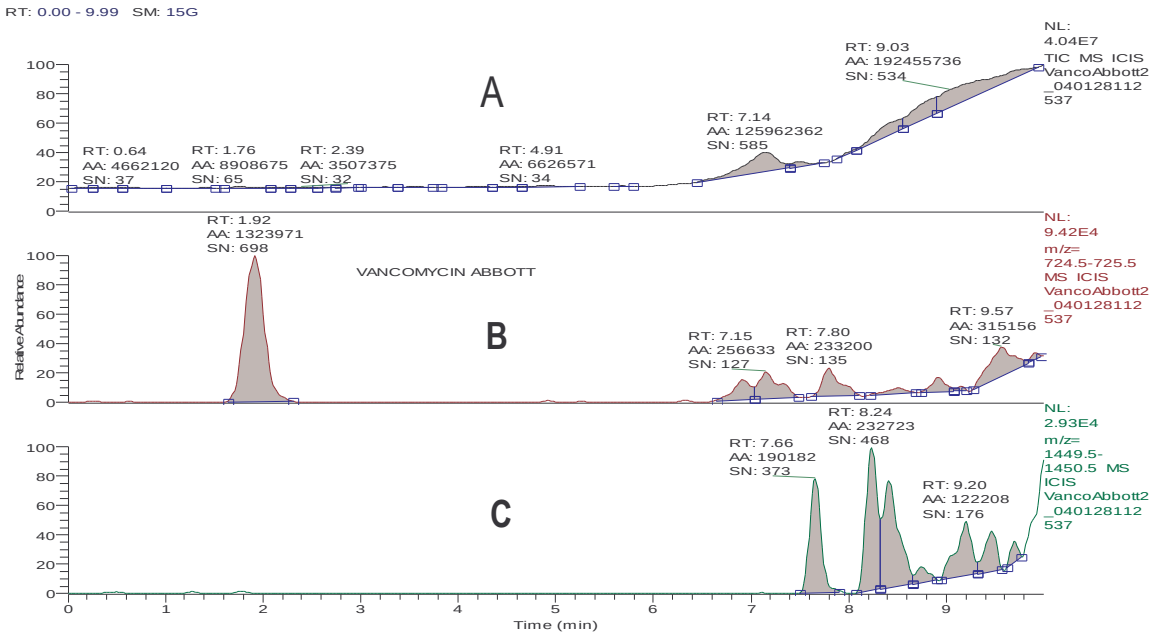


Fig. 5. Full scan LC-MS analysis of vancomycin Abbott. A: TIC trace, B: mass trace m/z 725, C: mass trace m/z 1450.

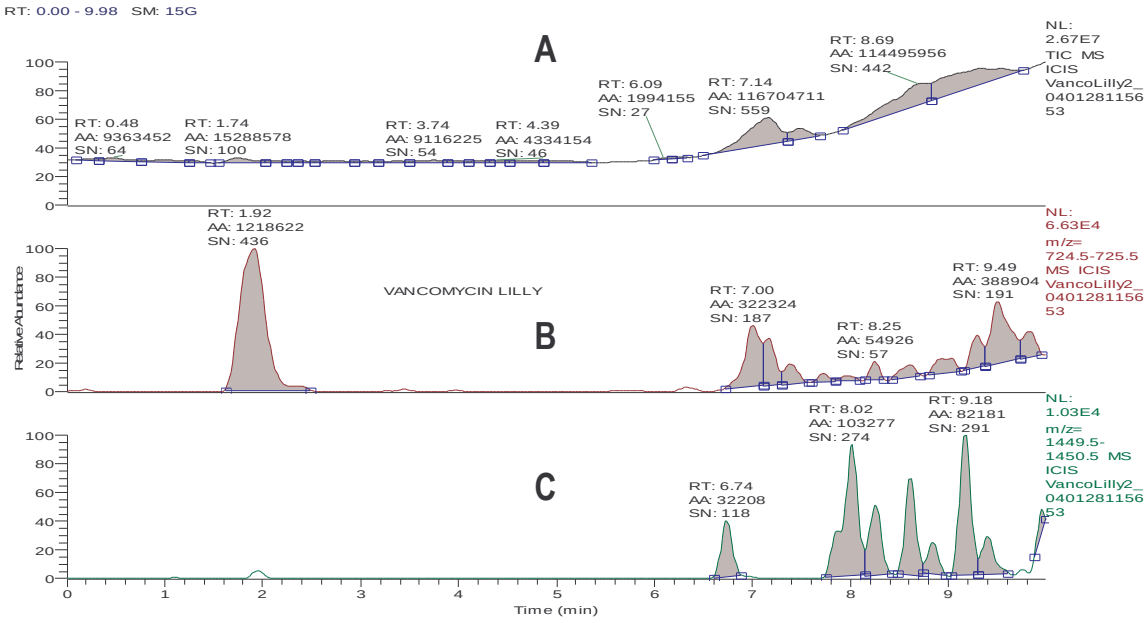


Fig. 6. Full scan LC-MS analysis of vancomycin Lillyt. A: TIC trace, B: mass trace m/z 725, C: mass trace m/z 1450.

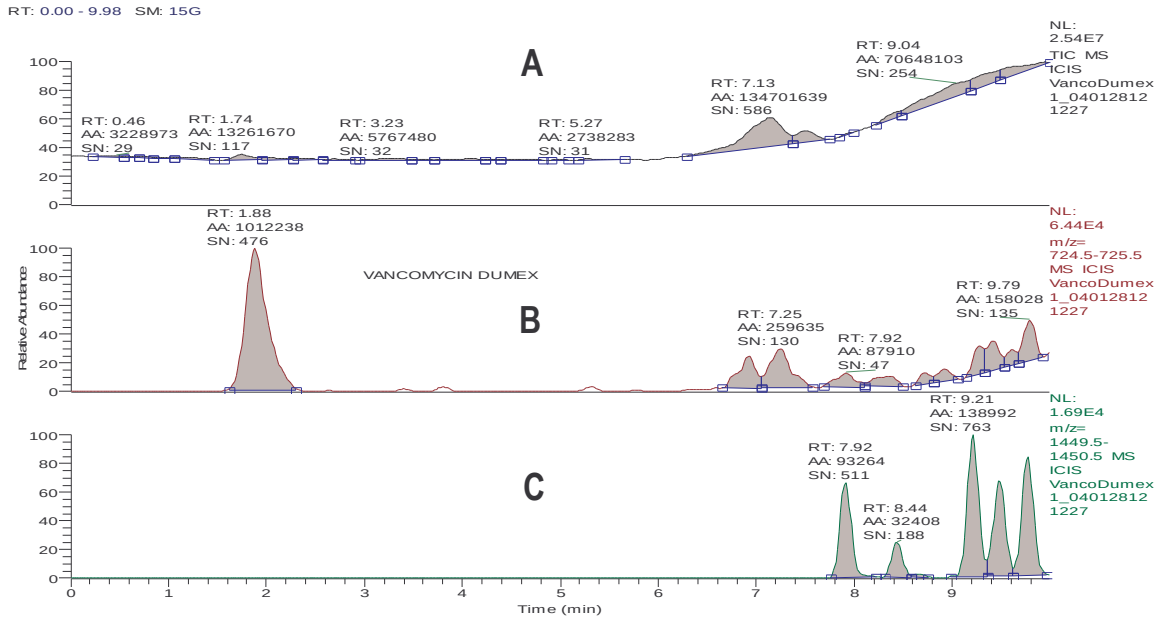


Fig. 7. Full scan LC-MS analysis of vancomycin Dumex. A: TIC trace, B: mass trace m/z 725, C: mass trace m/z 1450.

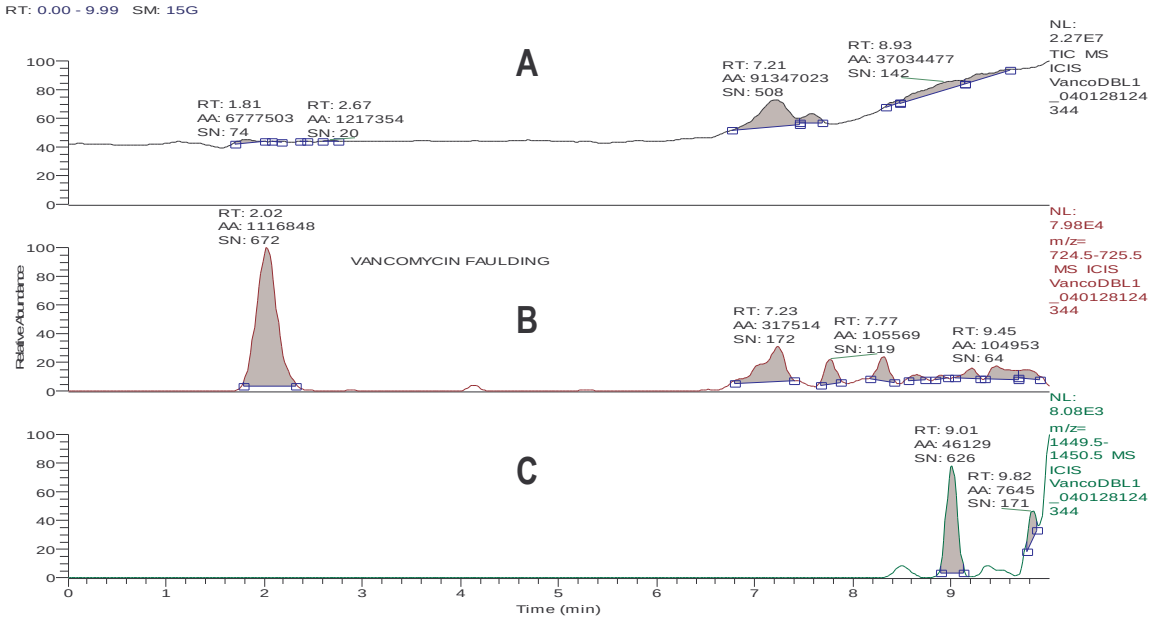


Fig. 8. Full scan LC-MS analysis of vancomycin Faulding. A: TIC trace, B: mass trace m/z 725, C: mass trace m/z 1450.

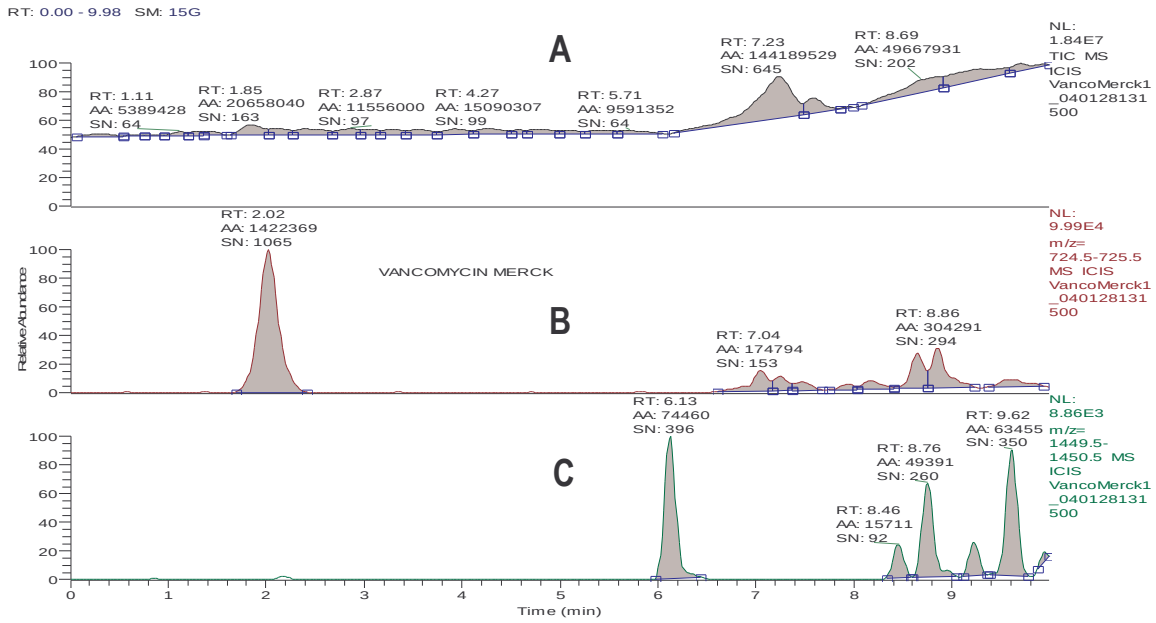


Fig. 9. Full scan LC-MS analysis of vancomycin Merck. A: TIC trace, B: mass trace m/z 725, C: mass trace m/z 1450.

Generally, the study of five vancomycin preparations did not reveal any differences, detectable in LC-MS and LC-MS-MS analysis. Most probably, observed side effects are not related to any particular brand of this drug.

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