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DEVELOPMENT OF AN ANALYTICAL METHOD FOR THE SIMULTANEOUS DETERMINATION OF GLYCOPEPTIDES, VANCOMYCIN AND TEICOPLANIN USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED MASS SPECTROMETRY IN HUMAN URINE

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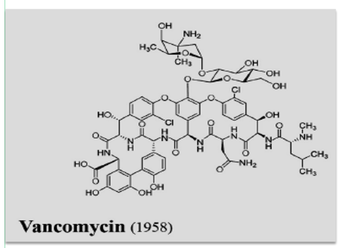
INTRODUCTION

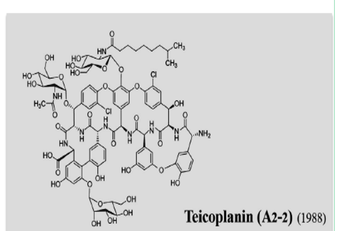
Vancomycin and teicoplanin belong to the glycopeptide class, remaining high on the list of antibiotics for the treatment of hospital infections by resistant bacteria.



Vancomycin (1958)

Vancomycin was isolated from strains of actinomyces *Amycolatopsis orientalis* and had action against *MRSA* and *Gram-positive bacteria.*

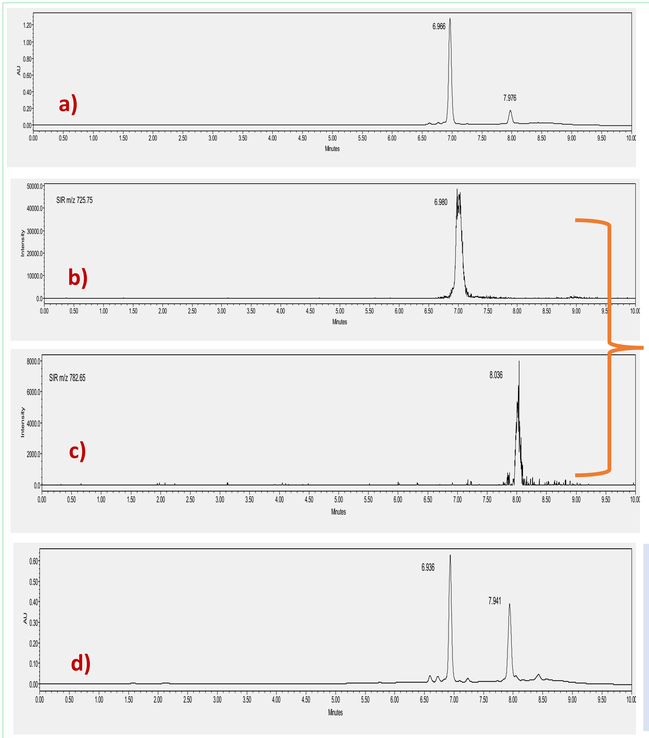
Teicoplanin was produced by *Actinoplanes teichomeceticus* bacteria, is a mixture of six components with A_{2-2} predominating and showed action against aerobic and *anaerobic Gram-positive bacteria.*



Teicoplanin (A2-2) (1988)

METHOD

Development of an optimal HPLC-MS analytical method	- Several chromatographic parameters were examined such as: temperature of column, the mobile phase, the flow rate, elution system, etc. - Selection of the desired ion fragments with the appropriate cone voltage
Validation of the proposed analytical method	Study of: • Linearity • Intra-day and Inter-day precisions • Accuracy • Limits of Detection (LOD) and Quantification (LOQ)
Development of a SPE protocol	Study of the parameters for the optimization of SPE protocol: - the pretreatment of the sample (volume of urine, precipitation of proteins, etc.), the used solvents for the conditioning, washing and elution step, amount and pH of the solvent at each stage of extraction.



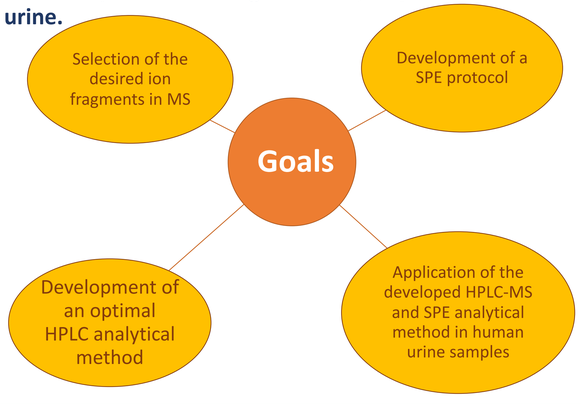
a) Vancomycin and teicoplanin mixture chromatograph with retention times 6.966 and 7.976 min respectively

MS chromatographs of mixture b) vancomycin and c) teicoplanin

d) Application of selected SPE protocol to a sample containing water and vancomycin-teicoplanin mixture and the obtained chromatograph

AIMS

The main aim of the present study was to develop an analytical method using High Performance Liquid Chromatography (HPLC) coupled Mass Spectrometry (MS) for the simultaneous determination of vancomycin and teicoplanin in biological fluids, in particular human urine.



Acknowledgement

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RESULTS

The developed analytical protocol showed a good resolution between vancomycin and teicoplanin with retention times 6.966 and 7.976 min, respectively.

Optimal chromatographic conditions

- ✓ **Mobile phase:** Solvent A: 0.1% TFA in water, Solvent B: 0.1% TFA in methanol
- ✓ **Gradient elution:** 0 min: 95% A - 5% B, 2 min: 95% A - 5% B, 6 min: 50% A - 50% B, 7 min: 95% A - 5% B, 10 min: 95% A - 5% B
- ✓ **Column temperature:** 40°C
- ✓ **Flow rate of mobile phase:** 0,8 mL/min
- ✓ **Ion fragments:** 725.75 (m/z) for vancomycin and 782.65 (m/z) for teicoplanin with a cone voltage of 15 V and 12 V, respectively

Validation results

- ✓ The method indicated **linearity** with $R^2 \geq 0.9990$
- ✓ The **intra-day** and **inter-day precisions** were estimated with maximum coefficient of variation (%CV) values equal to 6.5920%, which are below the 10%
- ✓ **%Recovery** at low and high concentrations of vancomycin and teicoplanin was examined in water samples, ranging from 64 to 121%, showing relatively good recoveries

Limits of detection (LOD) and quantification (LOQ)

Compounds	LOD (mg/L)	LOQ (mg/L)
VAN	0.0252	0.0764
TEIC	0.785	2.4197

CONCLUSION

- ❖ **Successful development and validation of HPLC-MS analytical protocol**
- ❖ **Optimal separation of the two compounds with a novelty system of mobile phase solvents**
- ❖ **Satisfactory recovery rates (64-121%) in samples**

Future research

Further study of urine pretreatment and SPE conditions

Study and other biological fluids such as blood serum and plasma

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