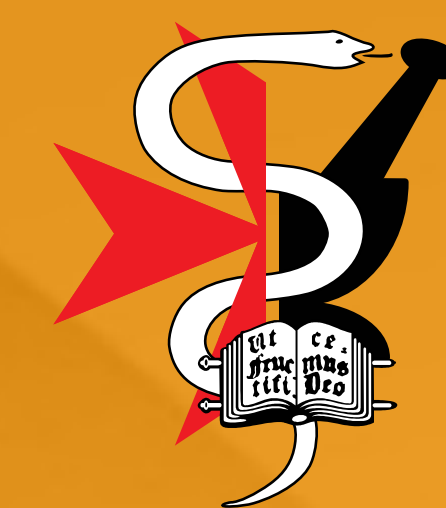
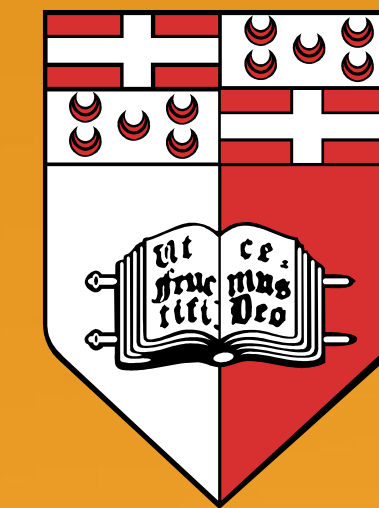


METHOD DEVELOPMENT FOR THE QUANTIFICATION OF CLINDAMYCIN IN HUMAN PLASMA

Janis Vella, Martina Mifsud, Victor Ferrito, Anthony Serracino– Inglott, Lilian M. Azzopardi, Nicolette Sammut Bartolo, Godfrey LaFerla
Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta, Msida, Malta



Department of Pharmacy



University of Malta

INTRODUCTION

Clindamycin, the first lincosamide antibiotic produced is a semi-synthetic derivative of lincomycin. It has various clinical uses as it is active against different bacteria.¹

AIMS

The following work describes the development of a simple alternative High Performance Liquid Chromatographic (HPLC) method used for the quantification of clindamycin in human plasma.

METHOD

Materials and reagents: Reagents used were clindamycin hydrochloride, lincomycin hydrochloride and Phenobarbital powder (Sigma Aldrich), acetonitrile, orthophosphoric acid and analytical grade type 1 water (Fisher scientific) and disodium hydrogen phosphate (Scharlau).

HPLC instrumentation: A Varian® ProStar HPLC unit with a UV-visible detector and a reversed-phase ACE® 5 C18 column (250 x 4.6mm; 5µm particle size) were used.

Method development: Equal volumes of clindamycin and lincomycin standard solutions were mixed and injected to observe separation of peaks using 9 different mobile phases (Table 1). All mobile phases were run at a flow rate of 1ml/min.

Different injection volumes—100, 50, 30, 25 and 10µl of clindamycin and lincomycin were injected consecutively. The detector wavelength was also set at 210, 205, 200 and 195nm. This was done to observe which of the conditions

Mobile phase number	pH	Percentage acetonitrile used	Mobile phase number	pH	Percentage acetonitrile used
1.	2.0	20	6.	3.0	40
2.	2.0	30	7.	6.5	20
3.	2.0	40	8.	6.5	30
4.	3.0	20	9.	6.5	40
5.	3.0	30			

Table 1: Different mobile phases used

gave the best results. A mobile phase with 30% acetonitrile was used.

Analysis of clindamycin in plasma was then performed with lincomycin as the internal standard. Protein precipitation was used to separate the analytes from plasma. Lincomycin was eluting too close to the plasma interferences. The amount of acetonitrile in the mobile phase was changed to try and shift the retention time of lincomycin but this was unsuccessful. Due to this a different internal standard, Phenobarbitone was used. Since it is more hydrophobic than lincomycin, the flow rate was increased to 1.5ml/min.

RESULTS

When selecting the best mobile phase composition, injection volume and wavelength at which to operate the analysis, the best results were given using an injection volume of 30µl with a mobile phase made up of phosphate buffer (pH 3.0) and acetonitrile 70:30 v/v. The best UV absorbance for both compounds was at 195nm.

When using the developed conditions, clindamycin and Phenobarbitone eluted at 4.03 and 7.03 minutes respectively (figure 1). They were well resolved from one another and from any other interferences making analysis and quantification efficient.

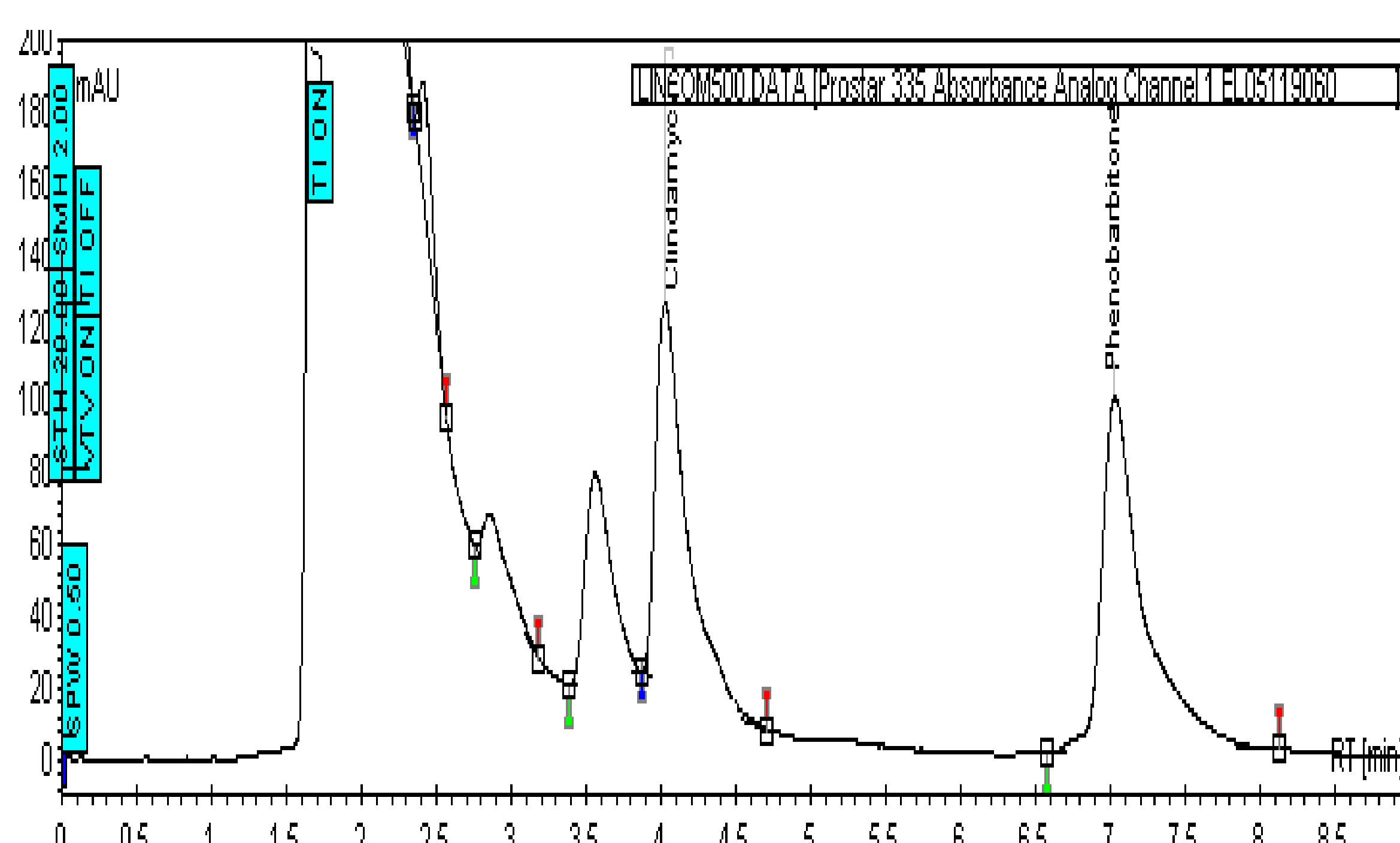


Figure 1: Clindamycin (20µg/ml) and phenobarbitone in plasma

CONCLUSION

The developed method for analysis of clindamycin in plasma is simple and quick to perform. It can be used in a clinical setting to quantify clindamycin in the plasma of patients suffering from diverse conditions. Validation of such a method will offer an alternative method for the efficient determination of such a widely used antibiotic.

Reference(s) 1. Catena E, Perez G, Sadaba B, Azanza JR, Campanero MA. A fast reverse-phase high performance liquid chromatographic tandem mass spectrometry assay for the quantification of clindamycin in plasma and saliva using a rapid resolution package. J Pharm Biomed Anal. 2009. 50(4); 649– 54