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A Simple and Modified Method Development of Vancomycin Using High Performance Liquid Chromatography

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Abstract

Vancomycin is well known as a prominent member of the glycopeptide class of antibiotics. In this paper, a rapid resolution method using high performance liquid chromatography is employed to identify the presence of Vancomycin. A robust method is established and validated for the simultaneous quantification of glycopeptides antibiotic like Vancomycin and applied for their pharmaceutical research. This method is simple, modified, user and environment friendly with new techniques and all sample preparations are performed in excellent manner. The method showed good sensitivity with 2 µg/mL limit of quantification (LOQ) and the calibration curve is linear in the range of 2-500 µg/mL concentration range. The within- run and between- run precision obtained is less than 2 and 4% respectively. The method is proposed to be applied in an experimental micro particulate carrier based oral study design. The results can proved to be specific, linear, accurate and sensitive and the available data can provide valuable information regarding the analysis and pharmacokinetics of Vancomycin after the oral administration.

Keywords: Vancomycin; Glycopeptide; High performance liquid chromatography; Antibiotics

Introduction

Vancomycin (VCM) is a glycopeptide antibiotic which is widely employed in the treatment of serious infections by Gram positive bacteria [1,2]. It was chosen for therapy of infections in patients allergic to β-lactam antibiotics. This antibiotic acts by preventing the peptidoglycan synthesis of bacterial cell wall [3]. Monitoring VCM in the biological fluids and in pharmaceutical products is of importance to prevent side effects in patients under treatment and to achieve optimum therapeutic concentrations [4]. Moreover, antibiotics including VCM have been found in the aquatic environment such as waste and polluted water resources. This compound can play a role in the maintenance or extension of antibiotic resistance bacteria, finally resulting in hazards to human health [5,6]. Therefore, screening VCM both in biological and environmental studies seems to be very important. Several different approaches in liquid chromatography have been developed to satisfy the demand for fast analysis without compromising separation efficiency and resolution. The main efforts have been focused on performing separations at high performance liquid chromatography [7].

The aim of our study is to develop a modified HPLC method for measuring total VCM concentrations with acceptable runtimes. The current method is easier to carry out the qualitative and quantitative analysis of vancomycin *in vivo*, by using new improved mobile phases in very short time. In addition, the clinical impact of developed method is planned to be investigated with the new oral formulation in our lab for validating the outcomes. This study might play a very significant role in certain therapeutic effects and warrant further study in the case of oral micro particulate based oral study design.

Experiment

Chemicals and materials

VCM was provided by Concord Biotech Limited. HPLC grade acetonitrile and Formic acid were obtained from RFCL limited. Water was purified by a Milli-Q system (Millipore, Billerica, MA, USA). All the used chemical reagents were of analytical grade and were used as received.

Instruments and chromatographic conditions

Chromatographic experiments were performed on Shimadzu UFLC system (SPD-M20A) equipped with a binary pump (model LC-20AD) with a diode array detector (Gro-Zimmern, Germany). The separation was carried out on Nucleosil 120 C_{18} 5 µm column at 35°C with a flow rate of 1 mL/min and Injection volume was 25 µL. Mobile phase was a mixture of Acetonitrile (A): Water, pH 2.0 (B) and Formic acid was used to adjust the pH of water. For data processing, a Class VP Data system (Shimadzu, Duisburg, Germany) was used. Binary pump was used for carrying two different solvents.

Preparation of standards, calibration standard and test samples

Standard stock solutions were prepared in ultra pure water. The stock solution was further diluted with water in formation of the following standard solutions, with the concentration of 2-20, 40-300 and 10-500 µg/mL, respectively. All the freshly prepared solutions were used for analysis. The chromatograms of different standard solutions are shown in Figure 1. The chromatograms were identified in linear relationship by their retention times and a response height with respect to concentration for the different calibration ranges which are put up in Figure 2.

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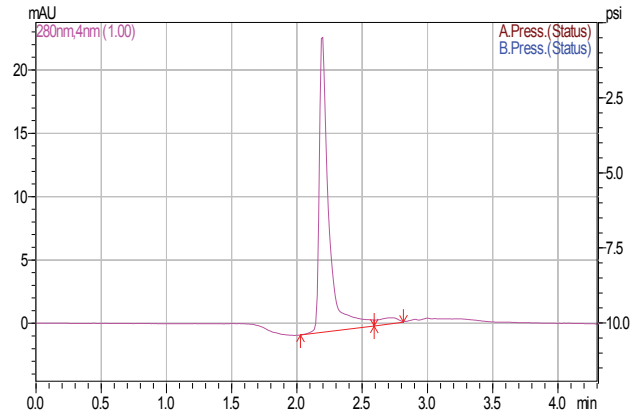

Figure 1: Chromatogram of VCM standard solution.

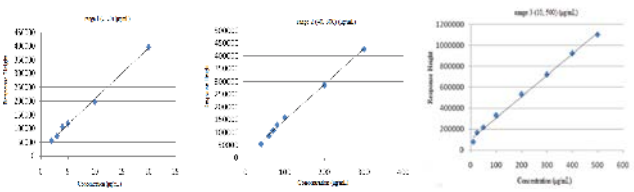

Figure 2: The response height versus concentration curve of VCM.

Results and Discussion

Optimization of chromatographic condition

To obtain reliable chromatographic results and appropriate ionization, several mobile phase systems (acetonitrile-0.2 M sodium sulfate buffer, acetonitrile-10 mM acetate buffer, pH 4.0, acetonitrile-water etc.) were tested and compared [8-19]. The results suggested that acetonitrile-acid aqueous solution was superior to the others. Meanwhile, formic acid was added into the mobile phase for pH adjustment and to improve the peak shape as well as to restrain the peak tailing. This finding was confirmed by the optimal solvent systems containing a mixture of 0.1% formic acid-acetonitrile (A) and water (B). For obtaining the effective separation, gradient elution technique was employed which guarantees high ionization, and minimize the ion suppression.

Optimization of sample preparation

Different methods of sample preparation were tested to select a coherent extraction method for VCM. Different volume ratios (50:50, 80:20, 70:30) of mobile phases were investigated. The results suggested that the volume ratio 50:50 was best among others.

Screening and analysis of optimized samples

The optimized samples were screened and analyzed. The results concluded that the retention time (2.5 min) for the drug was very less as compared to other previously reported methods in literature. The drug was very well separated and identified by their less retention time, with remarkable peak heights.

Method validation

The method was validated with regard to its specificity, linearity, accuracy and sensitivity (within and between days) [20-25].

Linearity and LOQ: Regression equations, linear ranges, correlation of coefficients are shown in Table 1. All calibrations consists excellent linearity with coefficients (r) higher than 0.995. As seen in Table 1 LOQ of three ranges were 2, 40 and 10 µg/mL respectively [26], with accuracy (recovery) between 74.5% and 111% this was absolute for quantification study. The correlation coefficient calculation and the regression analysis were performed without any type of mathematical transformation [27-31].

Extraction recovery: For checking the accuracy of the method, three standard samples with low intermediate and high concentrations of each range were analyzed. The concentrations were calculated from the corresponding calibration standard line (experimental concentrations) and were compared with the theoretical concentrations [32-38]. The extraction recovery was estimated according to Equation 1 and is shown in Table 2.

$$\text{Recovery} = ((C_{exp}/C_{teo}) \times 100) \quad \ldots 1$$

Where C_{exp}=Experimental concentration and

C_{teo}=Theoretical concentration

Assessment of precision, repeatability and reproducibility are listed in Tables 3 and 4, which were calculated on the same day and on 6 different days. Intra and inter- precision (relative standard deviation, RSD) of samples was less than 1.72% and 3.12%, respectively.

Conclusion

The chromatographic system was proved to be a rapid and efficient

Linear range (µg/mL)	Slope	Intercept	Correlation coefficient (r)
2-20	18612	22180	0.996
40-300	1403	8350	0.997
10-500	2036	10654	0.996

Table 1: Regression data and statistical analysis.

Measure conc. (µg/mL)	Accuracy (% Recovery)
2	74.5
10	95.5
20	101
40	75
150	105
300	102
10	111
250	92.4
500	83

Table 2: Recovery Data.

Spiked conc. (µg/mL)	Mean response ± SD	Intra-run (RSD%)
2	57164.4 ± 793.907	1.38
4	74595.83± 1288.45	1.72
10	221093.3 ± 3629.48	1.64
100	158255.2 ± 1668.99	1.05
200	296663 ± 2399.00	0.80
300	428491 ± 1244.61	0.29
400	887793.1 ± 7134.58	0.80
500	1122905 ± 8527.87	0.75

Table 3: Repeatability (Intra-run precision). SD: Standard deviation; RSD: Relative standard deviation.

Spiked conc. (µg/mL)	Mean response ± SD	Inter-run (RSD%)
2	57333.27 ± 1109.148	1.93
4	75966.8 ± 2371.276	3.12
10	221859.6 ± 5534.238	2.49
100	160598.5 ± 4399.284	2.73
200	293177 ± 2511.768	0.85
300	428532.9 ± 2820.084	0.65
400	892793.1 ± 7691.935	0.86
500	1119745 ± 8069.782	0.72

Table 4: Reproducibility (Inter-run precision). SD: Standard deviation; RSD: Relative standard deviation.

for research purpose of VCM formulation in systemic use. The method was established and validated for the simultaneous quantification of the drug and can be applied for their pharmacokinetic research. These works could provide more in- depth knowledge into the active components working *in vivo* with VCM and would be helpful for further investigation regarding the pharmacology and mechanism of VCM. This study would be helpful for explaining the metabolism of drug in rat and human plasma.

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