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Highly accurate and New approach for quantification of Gramicidin in medication by RP-HPLC

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ABSTRACT

A significant Reverse Phase-High performance Liquid Chromatography technique was developed for a more accurate, unique and quick economical method was developed for the analysis of Gramicidin in medication dosage forms. The separation of this drug Gramicidin was done by using the X-Bridge phenyl column as a stationary phase, and a mixture of acetonitrile + buffer in 50:50 v/v ratio was used as a movable phase. The buffer used in this method was Octane sulphonic acid of pH-2.5 adjusted with OPA. The maximum absorbance of eluents was observed at 235 nm. A specific flow rate (1 ml/minute) was maintained throughout the runtime of 8 min. The selected drug is eluted at 2.49 minutes. The selected drug obeys Beer Lambert's law in the concentration range of 0.5-7.5 μg/ml of Gramicidin. The percentage of recovery was found to be within the acceptable limit. The selected approach was corroborated with ICH standard ground rules, and the results of parameters like method precision, accuracy, ruggedness, robustness, and degradation studies were found to be within the allowable limit. Thus, the present method was successfully applied for the simultaneous analysis of Gramicidin in routine industrial work.

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INTRODUCTION

Gramicidin is an antimicrobial drug is also known as polypeptide antibiotics is used for various infections of both surface and inner body, including some common flues and cold. Also used in surgeries of bytes and chemotherapeutic medications (Davies and Davies, 2010; Hughes and Karlén, 2014).

Gramicidin D is a polypeptide ionophoric antibiotic is a specific combination of 80% Gramicidin A, 5% of Gramicidin B and 15% of Gramicidin C, respectively. The peptide antibiotics have a great advantage like lower side effects, higher resistance towards the microorganisms because these are naturally occurring with improved antibacterial activity rather than conventional antibiotics. They have a broad range of activities like directly attacks on target bacteria, fungi and on other viruses (juan Zhang and Gallo, 2016).

Gramicidin is a linear peptide that has existed in to six different forms along with their three isoforms like Gramicidin-A, B, & C. It is isolated from soil bacteria brevibacillus brevis, and it consists of 15 amino acids (Shida *et al.*, 1996; Kessler *et al.*, 2004; Hertweck, 2011) except Gramicidin-S, which is a cyclic peptide (Duquesne *et al.*, 2007). The most effective Antimicrobial Peptide (AMP) are Gramicidin-D, and S forms with high destructive mechanism towards bacteria's of gram-negative & gram-positive and few

fungi types are an advanced alternative remedy of antibiotics in near future usage (Shylakhovenko, 2003; Tong *et al.*, 2015; Wang *et al.*, 2012).

The peptide-based antibiotics are reticence on various proteins, DNA, cells and RNA for their electro chemical changes. For moderate improvement in the efficacy of Gramicidin is also used in combination of tyrocidine and antiseptics because it has great antibacterial activity shown on microorganism have antibiotically resistance (Palm *et al.*, 2018; Bosscha, 2004). It was used in eye infections of animals such as horses as eye drops for the treat infection. Their respective structures are shown below in Figure 1.

Few authors are reported some natural antibiotics extracted and characterized by various natural resources (Chandrashekhara *et al.*, 2010).

MATERIALS AND METHODS

Chemicals and reagents

Analytical grade Acetonitrile, Orthophosphoric acid and Octane sulphonic acid were procured from Merck India Pvt. Ltd, India. APIs of Gramicidin as reference standards are procured from Dr. Reddy's laboratory, Hyderabad.

Instrumentation

A High-performance Liquid Chromatography instrument of make Water Alliance e-2695 connected quaternary pump operated with less pressure with vacuum gas de-escalation. The effective PDA detector-2996 was used to detect the drug samples with software Empower-2.0 is used for the analysis of the data.

Preparation of buffer solution

2.5 gms of Octane sulphonic acid was dissolved in 1 lt of HPLC grade water and adjust its pH-2.5 with 0.1% OPA and filter through 0.45 μ filter paper.

Preparation of movable phase

Mix acetonitrile and buffer in 50:50 ratio and filter through 0.45 μ filter paper.

Diluent

The movable phase was used as diluents.

Preparation of Standard stock Gramicidin solution

The standard aqueous solution of Gramicidin was prepared by dissolving 5 mg of the compound into a 100 ml volumetric flask by dissolving 70 ml of diluents are homogenized by sonication for about 30 minutes and made up to the mark with triple distilled water. From this stock solution, we took 5 ml into a 50 ml volumetric flask and make up to the mark with diluents.

ANALYSIS OF RESULTS

The Gramicidin drug was separated by using a phenyl column has X-Bridge with specific dimensions (150 mm x 4.6 mm; 3.5 μ), and the room temperature was maintained with a flow velocity of 1 ml/minute of an inserted dose is 10 μl. The standard chromatogram of the Gramicidin is shown in Figure 2. The predicted wavelengths are in the region of 235 nm, and the observed results are shown in Table 1.

The precision of the method

The High-Performance Liquid Chromatography was operated for about 60 minutes to achieved a standard baseline, and the experiments were performed of about six times by injects the standard solution, shown the results in Table 2.

Method Linearity Results

The linearity results are predicted at a specific range of strengths of the Gramicidin between 0.5-7.5 μg/ml. The other linearity method parameters like Regression equation is y= 34631x+3493, and the other factor like correlation coefficient is 0.9996 was predicted under present experimental conditions for Garmicidin, and the results are mentioned in below Table 3. The linearity calibration curves of Gramicidin are shown in Figure 3.

Analytical precision parameters

Method Repeatability

In order to check the repeatability and precession of the proposed method can be done by studying the experiment of about six separate samples of the same batch were injected and analysed the results of peak area are used in the calculation of average mean values and percentages of RSD results, which are shown in Table 4.

Intermediate precision

To predict the pattern of HPLC chromatogram by analytical factor-like Intermediate Precession shows that the pattern was not deviated while applying significantly other HPLC systems, analyst and other columns. The percentages of Relative Standard Deviations (RSD) are 2% under the present experimental conditions, which supports the proposed method is more ruggedness and results are shown in below.

Accuracy

The samples were injected at three different concentration levels, namely 50%, 100% and 150%. A min-

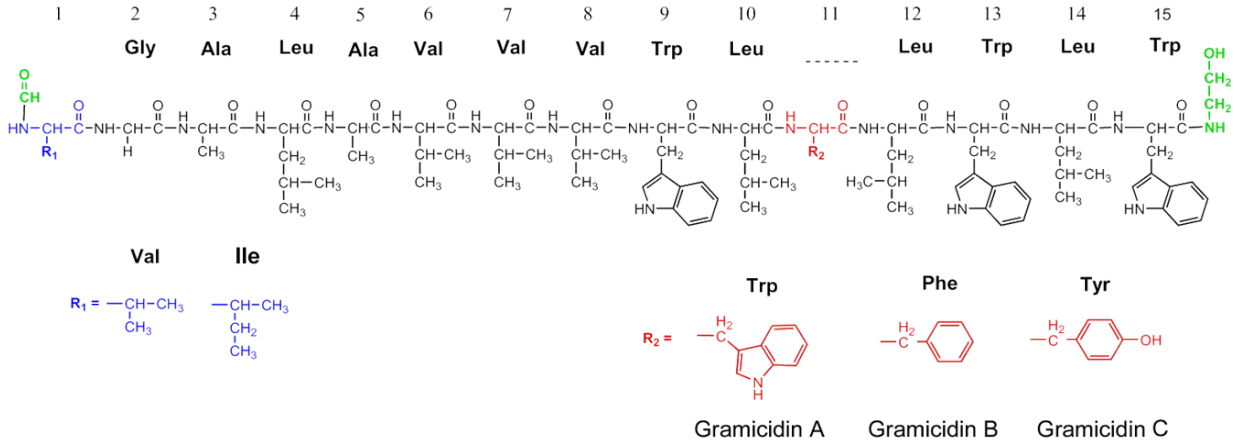

Figure 1: Structures of Garmicidine-A, B, and C

Table 1: The results of the Optimized chromatogram

Stationary phase	X-bridge phenyl (150mmx4.6mm, 3.5 μ)
Mobile phase	ACN: Octane sulphonic acid (50:50)
Flow velocity	1 ml/min
Dose-volume	10 μl
Column temperature	Room temperature
Wavelength	235 nm
Run time	8 min
RT of Gramicidin	2.496 min

Table 2: Results of the system precision

Parameter	Gramicidin
Theoretical plate count	2936
USP Tailing	1.07
USP Resolution	–
Retention time	2.491

Table 3: Results of linearity

Linearity	Gramicidin	
	Conc. (μg/ml)	Area
Linearity-10%	0.5	20947
Linearity-25%	1.25	50404
Linearity-50%	2.5	89176
Linearity-100%	5	178464
Linearity-125%	6.25	221978
Linearity-150%	7.5	260006
Slope	34631.31	
Intercept	3493.56	
CC	0.9996	

Table 4: Results of Method precision and Intermediate precision for Gramicidin

Amount present	Intra-day precision	Inter-day precision
	% RSD	
5	1.79	0.19
50	0.20	0.27
20	0.24	0.65

Table 5: Results of accuracy

Accuracy	Amount of Gramicidin	% Recovery
50%	2.5	99.6
100%	5	101.5
150%	7.5	100.7

Table 6: Robustness of Gramicidin Reports

Parameter	% RSD of Gramicidin
Flow (1.2 ml/min)	0.18
Flow (0.8 ml/min)	1.82
Org phase (55:45)	1.76
Org phase (45:55)	0.71

Table 7: Results of stability

Stability	Gramicidin	
	% Lable claim	% Deviation
Initial	100.3	0.2
6 Hrs	99.6	-0.12
12 Hrs	99.1	-0.26
18 Hrs	98.4	-0.37
24 Hrs	97.2	-0.41

Table 8: Results of forced degradation

Stress condition	% of degradation Gramicidin
Acid degradation (1N HCl)	14.2
Alkali degradation (1N NaOH)	12
Peroxide degradation (30% H2O2)	11.7
Hydrolysis degradation (1 ml of HPLC water)	14.5
Thermal degradation (Sample, 70°C, 3 Hrs)	15.5

imum of three injections were given at each level. The recovery results should be not less than 98 percent and not more than 102 percent. The results are shown in Table 5.

Robustness

The robustness of the method was found to be % RSD should be less than 2%. Slightly variations were done in the optimized method parameters like flow rate (±20%), organic content in the mobile phase

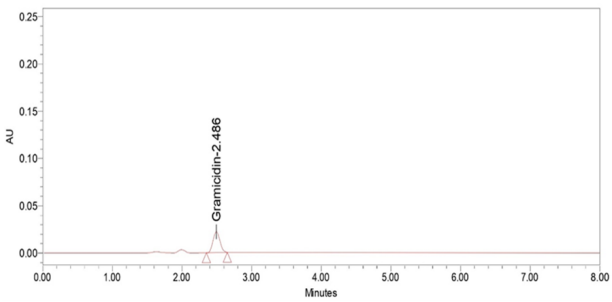

Figure 2: Chromatogram of system precision

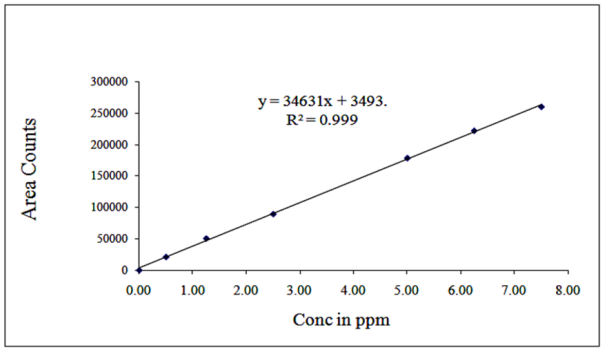

Figure 3: Linearity calibration plots of Gramicidin

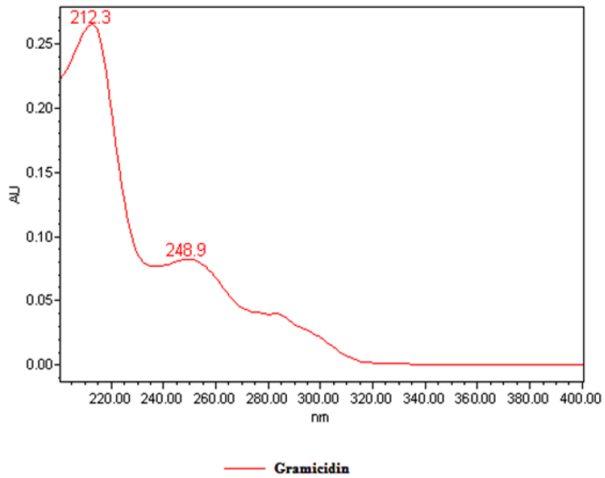

Figure 4: PDA spectrum of Gramicidin

(±10%). The predicted values are given in Table 6.

Stability

By using the sample solution stability of the method was calculated. The sample solution was injected at an initial to 24 hrs at different intervals of time. There is no significant difference in purity. The percentage of deviation results of Gramicidin shown in Table 7.

Forced degradation

The degradation study was carried out according to the ICH requirements include acid, base, peroxide, hydrolysis, thermal degradation. From the chromatograms, it is evident that the selected drugs were stable under the applied stress conditions though the degraded peaks were observed. The results of the forced degradation study were shown in the following Table 8. The forced degradation study of Gramicidin shown in Figure 4 .

CONCLUSIONS

In this method, a novel, quick, economical sensitive, and easily available HPLC method was evolved for the simultaneous estimation of Gramicidin in a bulk and pharmaceutical dosage form. The main advantage of this method is no HPLC methods are reported. In this method, shorter run-time, low price, accessibility, sensitivity, reliability and reproducibility. These properties are important when a large number of samples are to be analysed. The validation of all the parameters like linearity, accuracy, specificity, robustness was done and found to be within the acceptance criteria. The % RSD values were found to be less than 2, i.e., within the acceptance criteria for all the validation parameters. So, the proposed method could be easily applied for the routine analysis and the pharmaceutical formulations of Gramicidin in quality control laboratories without any preliminary separation.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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