Asian Journal of Pharmaceutical Education and Research
Vol -9, Issue-3, July-September 2020
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

Development and Validation of Ninhydrin Based Colorimetric Spectrophotometric Assay for Determination of Gentamicin in Pharmaceutical Formulation

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Received 29 April 2020; Revised 5 May 2020; Accepted 15 May 2020, Available online 15 July 2020

Cite this article as: Mishra SP, Jain AP. Development and Validation of Ninhydrin Based Colorimetric Spectrophotometric Assay for Determination of Gentamicin in Pharmaceutical Formulation. Asian Journal of Pharmaceutical Education and Research. 2020; 9(3): 50-55.
https://dx.doi.org/10.38164/AJPER/9.3.2020.50-55

ABSTRACT

A simple, accurate, cheap and quick spectrophotometric method was developed for the estimation of gentamicin in pharmaceutical pure and dosage forms. The method was based on the ninhydrin reaction with primary and secondary amines present in the gentamicin. This reaction produces a purple colour. The absorption of the gentamicin-ninhydrin mixtures at 324 nm had a linear relationship with the gentamicin concentration ranging from 10-100 µg/ml. The %RSD was less than 2%, showing high degree of precision of the proposed method. The methods were satisfactory applied for the determination of drugs in both bulk and pharmaceutical dosage forms. The colorimetric gentamicin assay reported herein is of great practical value because it is reproducible, sensitive, simple and extremely inexpensive.

Keywords: Gentamicin, Ninhydrin, Spectrophotometry, Pharmaceutical dosage forms

INTRODUCTION

Gentamicin is an antibiotic belongs to the family of aminoglycosides that was discovered in 1963 and was isolated from *Micromonospora purpurea.* It is readily soluble in water due to the presence of hydroxyl groups on its chemical structure, but practically insoluble in organic solvents [1]. The narrow therapeutic range possesses by gentamicin has made the antibiotic to be one of the heated debate topics amongst the physicians, whether it should not be used at all or merely that it should not be used extensively [2]. Ototoxicity and nephrotoxicity have been reported widely to be the main concern whenever the aminoglycosides are administered to the patients [1-4]. This concern has become the driving factor for many researchers to develop a method that can minimize the bioavailability of aminoglycoside systemically but at the same time maximize the therapeutic effect only where it is needed. The use of

antibiotic-containing poly (methyl methacrylate) (PMMA) was introduced by Buchholz et al 1970 [5]. The main advantage of these treatment methods as compared with conventional therapy is that they provide high concentrations of antibiotics [6] in a limited blood circulation area and also in the infected bone. This delivery method may reduce the side effects that results from systemic administration of antibiotics. The selection of antibiotics for use in the PMMA cements should be made based on the antibiotic stability characteristics at both body temperature and the highest temperature reached during the setting of PMMA. In addition the antibiotic should present a high germicide activity in order to allow a low dosage so as to avoid possible modifications in the mechanical properties of the cement. The antibiotic selected for the present work was gentamicin and quantified by using polarization fluorescence immunoassay [7], enzyme-linked immunosorbent assay (ELISA) [8], enzyme-immunoassay (EMIT) [9], fluorescence-immunoassay (TDX) [9], microbiologic [10] and chromatographic methods [11]. However most of these methods lack sensitivity and reproducibility (microbiologic) are uneconomical for multiple samples over protracted time periods. Some methods are time consuming [12]. For drugs that obey the Beer–Lambert Law, spectrophotometric methods of analysis of a single component in solution are usually rapid, sensitive and economical [13]. Since gentamicin poorly absorbs ultraviolet and visible light, an indirect spectrophotometric method is necessary for its assay. Ninhydrin colorimetric reaction is commonly used as a general method for the qualitative identification of several drugs containing amino groups [14]. In the present work a new spectrophotometric procedure for the quantitative analysis of gentamicin using ninhydrin as derivatizing agent was developed and validated.

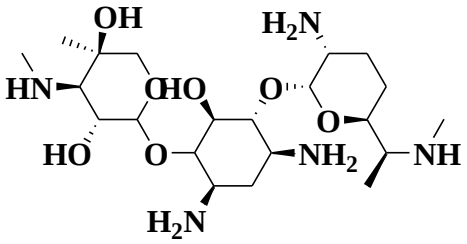

Fig. 1 Chemical structure of Gentamicin

MATERIALS AND METHODS

Chemicals and Reagents

Gentamicin was received as gift sample from Alkem Laboratories Ltd., Indore India. All solvents and reagents were of analytical grade. All the solutions were protected for light and were analyzed on the day of preparations. Triple distilled water was generated in house. Gentaril Injection 80mg /2ml was purchased from local market. Ninhydrin (Qualigens), Disodium hydrogenphosphate and Potassium

dihydrogenphosphate (Qualigens) were purchased. All these were used of AR grade. Distilled water was obtained by Mili Q apparatus by Millipore (Milliford, USA) for whole experimental work.

Instrument

In UV-spectrophotometric method, Labindia model- 3000 + series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

Reagents and solutions

Ninhydrin reagent: 1.25% w/v aqueous ninhydrin solutions.

pH 7.4 phosphate buffer: A 50ml aliquot of a 0.2 M monobasic potassium phosphate solution and 39.1 ml of a 0.2 M sodium hydroxide solution were mixed and diluted to 200ml with water.

Preparation of calibration curve

Gentamicin samples were prepared in the buffer solutions pH 7.4 at concentration of l000µg/ml. Assay samples (5ml) were mixed with freshly prepared ninhydrin reagent (0.1ml) and heated in a water bath at 95° C for 5 minutes. The tubes were then cooled in an ice-water bath. The UV-visible spectra over wavelength range of 200-700 nm were measured using the mixture of ninhydrin solution at the appropriate concentration as the blank using UV visible spectrophotometer. Prepared suitable dilution to make different concentration of standard with concentration range of 10-100µg/ml and analyzed for drug content by UV spectrophotometer at a λ_{max} of 429 nm.

Assay of tablet formulation

Two ampoule of injection was taken and average weight of drug was determined. The ampoule was crushed equivalent to 100 mg of drug was transferred to 100ml standard flask. The solution was dissolved in 50 ml of buffer solutions pH 7.4 and made up to volume with of buffer solutions pH 7.4. Assay samples (5ml) were mixed with freshly prepared ninhydrin reagent (0.1ml) and heated in a water bath at 95° C for 5 minutes. The tubes were then cooled in an ice-water bath and filtered through a 0.45µ membrane filter. The filtered solution was diluted suitably and analyzed for drug content by UV spectrophotometer at a λ_{max} of 429 nm.using of ninhydrin solution as blank.

Method Validation

The developed method was validated as per ICH guidelines [15] with respect to linearity, precision, selectivity, recovery, accuracy and stability.

Linearity and construction of calibration curve

Solutions containing 10- 100µg/ml of gentamicin were prepared from standard solution to determine the linearity range. The detection was carried out at 429 nm. Spectrums were recorded and absorbance was

recorded for all the concentrations. A calibration plot of concentration over the absorbance was constructed. The optical characteristics such as Beer's law limits, regression equation and correlation coefficient, mean absorbance value and statistical data of the calibration curve were calculated and results are presented in Table 1.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of clindamycin to pre analysed tablet solutions. The resulting solutions were then reanalyzed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

RESULTS AND DISCUSSION

The proposed spectrophotometric methods are indirect and based on the determination of the gentamicin in marketed formulation using ninhydrin as derivatizing agent. Calibration curves have correlation coefficients (r) 0.999 indicating good linearity over a concentration range of 10-100 μg/ml. The regression characteristics were reported in Table 1.

Table 1: Optical characteristics of the proposed method

Parameters	Results
Wavelength	429nm
Beer's law limit (μg/mL)	10-100
Regression equation (Y=mx+c)	Y=0.010X+0.014
Slope (m)	0.010
Intercept (c)	0.014
Correlation Coefficient (r)	0.998

Table 2: Results of recovery studies on marketed formulations

Recovery level %	% Recovery (Mean±SD)*
80	98.32±0.144
100	97.14±0.132
120	99.21±0.231

*Average of five determination

Table 3: Results of Precision (% R.S.D.)

Parameter	(Mean±SD)*	% RSD
Repeatability	98.30±0.07	0.074
Day to Day	99.38±0.12	0.57
Analyst to Analyst	98.29±0.08	0.083
Reproducibility	99.03±0.21	1.20

*Average of five determination

The accuracy of the methods was determined by investigating the recovery of drugs at concentration levels covering the specified range (five replicates of each concentration) Table 2. The %RSD was less than 2%, showing high degree of precision of the proposed method Table 3. The results of the method lie within the prescribed limit, showing that method is free from interference from excipients.

CONCLUSION

The results of present study demonstrated the developed colorimetric assay can be successfully applied for routine analysis of gentamicin in bulk and pharmaceutical dosages form. The method was validated according to ICH guideline. This method is simple, selective, cost effective and less time consuming can be successfully applied to pharmaceutical formulations and pure drug sample.

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