Method development and validation of Gentamicin from poly methyl methacrylate (PMMA) bone cement by UV–visible Spectroscopic Method

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ABSTRACT:

To prevent and treat infections of the bones and joints, antibiotic-loaded bone cement, also known as antibiotic-impregnated poly methyl methcrylate (PMMA), was developed. Gentamicin is an antibiotic medication that belongs to the aminoglycoside class. Gentamicin works by inhibiting the synthesis of bacterial proteins, leading to the disruption of bacterial growth and ultimately causing their death. Polymethylmethacrylate (PMMA), also known as bone cement, is used in many types of orthopedic and trauma surgery. Using an orbital shaker, gentamicin is extracted from the bone cement. It is then identified using ascending paper chromatography and compared to both standard and sample gentamicin. It's used in joint replacement surgery, hip arthroplasty, and vertebral augmentation. The phosphate buffer pH 7.4 solvent systems were used to develop the UV spectroscopic method, and it was discovered that the absorbance maxima were found at 202 nm. In order to ensure accuracy, precision, linearity, LOD, LOQ, and reproducibility, the method was validated in accordance with ICH guidelines. Within the 40–120 µg/ml concentration range, the linearity was found to be 0.999.The LOD and LOQ was found to be 13.44 µg/ml and 40.74 µg/m. Hence the developed UV Spectroscopic method was found to be simple, precise, accurate and economical and can be used to for the routine analysis of gentamicin in bulk and pharmaceutical dosage form.

Keywords: Gentamicin,UV, Polymethylmethacrylate (PMMA), Antibiotic

INTRODUCTION

Serious conditions like osteomyelitis and periprosthetic joint infections can cause significant morbidity, necessitate long-term care, and have high recurrence rates. Prolonged osteomyelitis and periprosthetic infections can now be treated with antibiotic-loaded bone cement, also known as antibiotic-impregnated polymethylmethacrylate (PMMA).The antibiotic gentamicin, which is derived from Micromonispora purpurea, is an amino glycoside with a broad spectrum of action. When gentamicin binds to the 16s ribosomal RNA and damages the plasma membrane, it inhibits the synthesis of microbe proteins, which is how it kills bacteria. A broad range of gram positive and gram negative bacteria can be effectively combated by it. Gentamicin comprises three main components, gentamicin C1, C1a, and C2, along with several minor components. It is not a single drug. It is distributed to bodily water and has a hydrophilic nature. Furthermore, glomerular filtration is the kidneys' method of excreting it as the same drug. Factors such as decreased renal function, decreased lean body mass, and rising polypharmacy rates in older adults can all have an impact on the pharmacokinetics of gentamicin. Chemically, it is known as 2-[4,6-diamino-3-[3-amino-6-[1-(methylamino)ethyl].[Oxan-2-yl] [oxy-2-hydroxy cyclo hexyl] oxy-5-methyl-4-(methylamino)3-Oxane-3,5-Diol and it is soluble in water, pyridine, dimethyl formamide, in acidic media with salt formation. PMMA bone cement is utilized in a variety of biomedical applications, such as orthopedic implant fixation, temporary skeletal spacers, and antibiotic-filled beads. Antibiotics are frequently mixed with bone cement to achieve local delivery and reduce post-operative infection. The literature review examined the methods used to analyze gentamicin in various pharmaceutical and Ayurvedic dosage forms, including single and combination dosages, as well as UV-spectrophotometric, HPLC, and a few UV-spectrophotometric, RP-HPLC, and HPTLC methods. According to a literature review, there is no analytical technique for the gentamicin found in polymethyl methacrylate (PMMA) bone cement. This study aims to develop a simple, precise and accurate UV spectroscopic method for the extraction of gentamicin from polymethyl methacrylate (PMMA) bone cement.

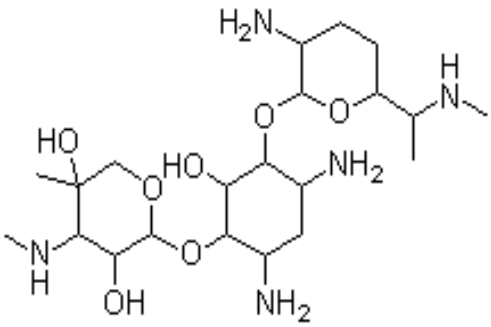

Figure 1: Structure of Gentamicin

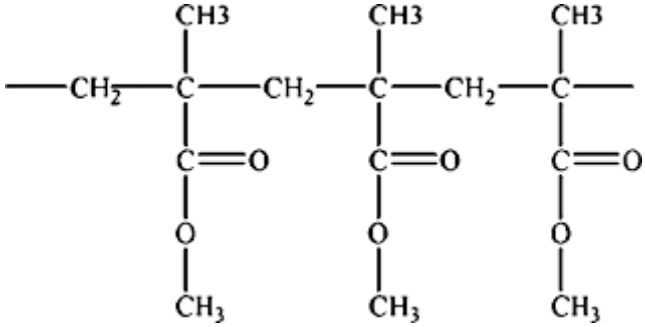

Figure2: Strucuture of PMMA

MATERIALS AND METHODS

Drug samples

Poly (methyl methacrylate) (PMMA)bone cement was obtained from PSG hospital, Peelamedu and the formulation *Gentamicin Injection*20 mg was procured from the local market, manufactured by Tamman Titoe Pharma Pvt Ltd. Chennai.

Reagents and chemicals

Auro Chemicals in Coimbatore provided the milli Q water, potassium dihydrogen orthophosphate, ninhydrin reagent, and all other analytical grade chemicals utilized.

Instrument and apparatus

UV-Spectrophotometer of Shimadzu make and 1800 model having UV probe software were used for analysis.

Table No: UV Spectrophotometer

UV Double beam spectrophotometer	Double beam UV- visiblespectrophotometer (Shimadzu, model- 1650PC, Japan) having two matched quartzcells with 1 cm light path was used for spectral measurements UV probe 2.42software was loaded on to UV-visible -spectrophotometer.
UV system	Shimadzu
UV software	UV Probe
Instrument model number	UV-1650PC
Light source	50W halogen lamp (2000 hrs life) and deuterium lamp
Detector	Silicon photodiode
Sample compartment	Inner dimensions: W110.0 X D230.0 X H105.0 mm.

Method development

Selection of solvent

The process of developing the UV Spectrophotometric method commenced with the identification of the solvent system and the maximum wavelength at which UV light could be absorbed. Gentamicin's solubility was tested through both practical testing and a review of the literature in a variety of solvents. Gentamicin is soluble in phosphate buffer, methanol, and water, according to a survey of current literature. The pH7.4 phosphate buffer was chosen as the solvent to be environmentally friendly.

Selection of Wavelength

The samples were scanned in UV region 200nm-400nm. The λ max of gentamicin hydrochloride was found to be 202nm.

Method validation

The optimized method parameters were validated in accordance with ICH guidelines (ICH guidance Q2A; Q2B) to demonstrate the method's suitability.

Specificity and selectivity

Gentamicin solutions were scanned between 200-400 nm, and the resulting spectra were compared to the blank solvent spectrum to look for any interference at the maximum wavelength at which the solvent could absorb analytes.

Linearity

10 mg of the drug gentamicin was weighed and dissolved in phosphate buffer, which was then transferred into a 10 ml volumetric flask at a concentration of 1000 µg/ml. An aliquot of 0.2, 0.4, 0.6, 0.8, and 1.2 ml was taken from this solution and mixed with phosphate buffer solvent to obtain a concentration in the range of 40-120 µg/ml.

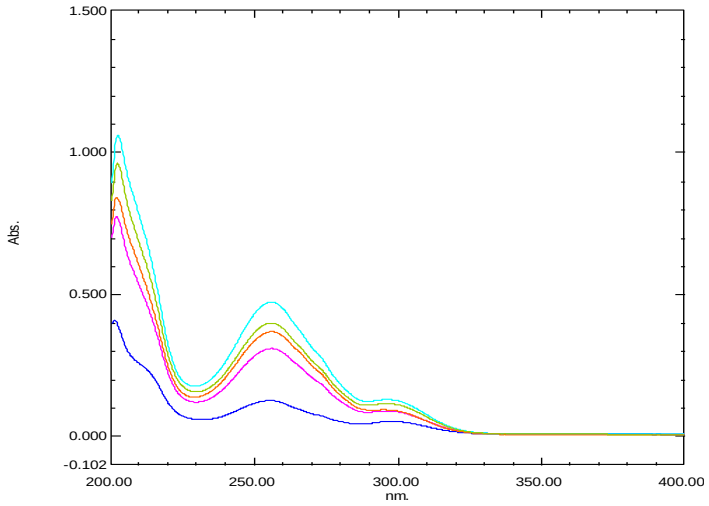

Figure 3: overlain spectra of Gentamicin (40-120 µg/ml)

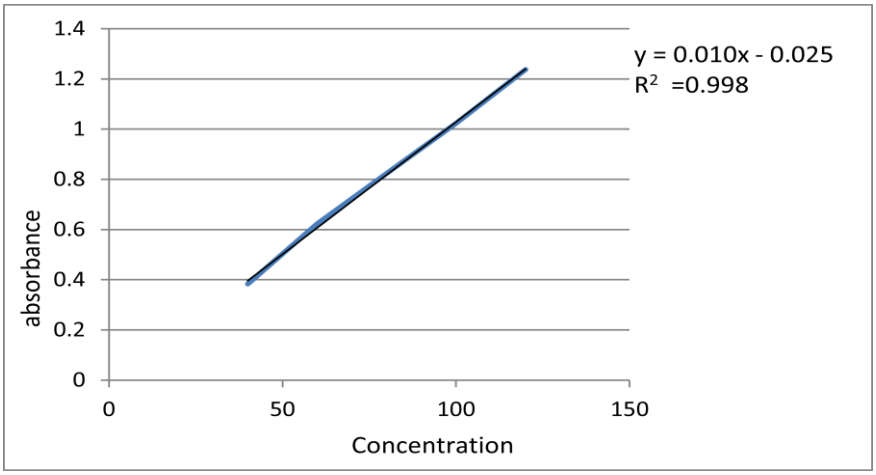

Figure 4: Linearity graph of Gentamicin

Precision

An absorbance measurement was conducted at 202 nm for each of the six replicates of the gentamicin solution. Both intraday and interday precision data, absorbance, and percentage RSD were calculated on the same day at different time intervals and on different days to obtain system precision. Repeatability is the result of the method operating over a short time interval under the same conditions (or) is the % RSD of multiple determinations of a single sample in a single test run (intra-assay precision).

Table No 2: precision data of gentamicin

parameters	Concentration µg/ml	Standard deviation	Mean	%RSD
Intraday precision*	80 µg/ml	0.001538	0.815	1.877
Interday precision*		0.007937	0.821	0.966
Repeatability*		0.000516	0.826	0.062

*Mean of six determinations

Accuracy

The systemic error involved in a method determines its accuracy. It is the degree to which test results produced using that approach resembles the actual value. Three levels of the sample's working concentration—80%, 100%, and 120%—were used to test the method's accuracy. The standard solutions of gentamicin was prepared to levels 80, 100, and 120 percent of the working concentration by adding sample solution to a calculated amount of standard solution. The percentage recovery was computed based on the total amount of drug discovered. Three rounds of this procedure were conducted for each concentration. It was calculated as an RSD percentage. A percentage RSD was computed.

Table No 3: Accuracy studies of gentamicin

Level	% Recovery	%RSD
80	99.1	1.22
100	97.4	0.95
120	98.3	1.65

LOD &LOQ

Limit of detection and quantification was calculated by using statistical calculations using following formulas and %RSD was calculated.

LOD= 3.3* SD/ Slope

LOQ=10*SD /Slope

Where, SD Standard deviation

Table No 4: LOD & LOQ

Drug	**Parameter**	
	LOD µg/mL (202nm)	**LOQ µg/mL (202nm)**
Gentamicin	13.44	40.74

RESULT AND DISCUSSION

The gentamicin was dissolved in Phosphate buffer and the absorbance maxima were found to at the wavelength of 202nm. Solvent spectrum obtained showed no interference of absorbance at maximum wavelengths of Gentamicin showed maximum wavelength at 202. The developed method was validated as per ICH guidelines. Hence method was found to be specific and selective. Standard calibration curve was plotted using concentration vs absorbance's obtained by each linear dilution of analyte. Each concentration showed linear absorbance's range between the concentration ranges of 40-120µg/mL with regression equation of 0.999.From the precsion data, the % RSD calculated were found to be within the acceptance limits,which shows that the method is precise. The accuracy was found to be 98-99.5% which shows accuracy of the method.The LOD and LOQ was found to be 13.44 µg/mL and 40.71 µg/mL respectively.

CONCLUSION

The present research concluded that, newly developed Spectrophotometric technique was found to be simple, specific, selective, linear, precise, and reproducible for development of Gentamicin from Poly (methyl methacrylate) (PMMA) bone cement by UV Spectroscopic method.

ACKNOWLEDGEMENT

The authors would like to thank the Principal at PSG College of Pharmacy affiliated to The Tamilnadu Dr.M.G.R.Medical University, Chennai, India for providing all the facilities to carry out the research project.

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