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The Analytical Method for the Simultaneous Estimation of Kanamycin and Cephalexin will be Developed by RP-HPLC Method by Optimizing the Chromatographic Conditions.

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ABSTRACT

A new simple, selective, rapid, precise and accurate reverse phase HPLC method has been developed for simultaneous estimation of granisetron and dexamethasone. The method was developed using CPS Hypersil CN column (250×4.6 mm I.D.) with a mobile phase consisting of acetonitrile:buffer (100 mM Triethylamine adjusted to pH 3.0 with o-phosphoric acid) in ratio of 25:75 at a flow rate of 2 ml/min. Detection was carried out at 242 nm. The developed method was evaluated for various system suitability parameters and validated for linearity, accuracy, precision, LOD, LOQ as per ICH guidelines. It was also evaluated for bench top stability and freeze/thaw stability. The proposed method can be used for the estimation of these drugs in their combined dosage forms.

Keywords: dexamethasone, granisetron, RP-HPLC, validation

INTRODUCTION

A drug is a substance which may have medicinal, intoxicating, performance enhancing or other effects when taken or put into a human body or the body of another animal and is not considered a food or exclusively a food.
What is considered a drug rather than a food varies between cultures, and distinctionsbetween drugs and foods and between kinds of drug are enshrined in laws which vary between jurisdictions and aim to restrict or prevent drug use. Even within a jurisdiction, however, the status of a substance may be uncertain or contested with respect to both whether it is a drug and how it should be classified if at all. There is no single, precise definition, as there are different meanings in drug control law, government regulations, medicine, and Colloquial usage.
In pharmacology, a drug is "a chemical substance used in the treatment, cure, prevention, or diagnosis of disease or used to otherwise enhance physical or mental well-being. Drugs may be prescribed for a limited duration, or on a regular basis for chronic disorders.
The molecules of drugs are complex, and most of them consist of many hydrogen and carbon atoms, a few oxygen atoms, and one or a few nitrogen atoms. Drugs may also have no nitrogen atoms in it and many may have chlorine atoms in it, such as chloral hydrate.
Recreational drugs are chemical substances that affect the central nervous system, such asopioids or hallucinogens. They may be used for perceived beneficial effects on perception, consciousness, personality, and behavior. Some drugs can cause addiction and/or habituation.
Analytical chemistry[1] is applied throughout industry, medicine, and all the sciences. Consider a few examples. The concentration

of oxygen and of oxygen and of carbon dioxide are determined in millions of blood samples every day and used to diagnose and treat illnesses. Quantities of hydrocarbons, nitrogen oxides, and carbon monoxide present in automobile exhaust gases are measured to assess the effectiveness of smog-control devices. Quantitative measurements of ionized calcium in blood serum help diagnose parathyroid disease in humans. Quantitative determination of nitrogen in foods establishes their protein content and thus their nutritional value. Analysis of steel during its production permits adjustment in the concentration of such elements as carbon, nickel, and ductility. The mercaptan content of household gas supplies is monitored continually to ensure that the gas has a sufficiently noxious odour to warm of dangerous leaks. farmers tailor fertilization and irrigation schedules to meet changing plant needs during the growing season. Gauging these needs from quantitative analyses of the plants and the soil in which they grow.

Quantitative analytical measurements also play a vital role in many research areas in chemistry, biochemistry, biology, geology, physics, and the other sciences.

For example, quantitative analytical measurement of potassium, calcium, and sodium ions in the body fluids of animals permit physiologists to study the role of these ions in nerve signal conduction as well as muscle contraction and relaxation. Chemists unravel the mechanisms of chemical reactions through reaction rate studies. the rate of consumption of reactants or formation of products in a chemical reaction can be calculated from quantitative measurements made at equal time intervals. Material scientists rely heavily on quantitative analysis of crystalline germanium and silicon in their studies of semiconductor device.

Quantitative Analytical Methods

We compute the results of a typical quantitative analysis from two measurements. One is the mass or the volume of sample being analyzed. The second is the measurement of some quantity that is proportional to the amount of analyte in the sample, such as mass, volume, intensity of light, or electrical charge. This second measurement usually completes the analysis, and we classify analytical methods according to the nature of this final measurement. Gravimetricmethods determine the mass of the analyte or some compound chemically related to it. In a volumetric method, the volume of a solution containing sufficient reagent to react completely with the analyte is measured. Electroanalytical methods involve the measurement of such electrical properties as potential, current, resistance, and quantity of electrical charge. Spectroscopic methods are based on measurement of the interaction between electromagnetic radiation by analytes. Finally, a group of miscellaneous methods includes the measurement of such quantities as mass-to – charge ratio of molecules by mass spectrometry, rate of radioactive decay, heat of reaction, rate of reaction, sample thermal conductivity, optical activity, andrefractive index.

Pharmaceutical Analysis plays a very vital role in the quality assurance and quality control of bulk drugs and their formulations. Pharmaceutical analysis is a specialized branch of analytical chemistry, which involves separating, identifying and determining the relative amounts of components in a sample of matter. Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science and electronics etc. Qualitative analysis reveals the chemical identity of the sample. Quantitative analysis establishes the relativeamount of one or more of these species or analytes in numerical terms. Nearly, any physical property or characteristic of a particular element or compound can be made the basis of a methodfor its analytical determination.

E.g.: Spectroscopic technique involves the absorption/ emission of radiant energy in all regions of the electromagnetic spectrum.

Analytical method validation

Validation parameters

- Specificity
- Linearity
- Range
- Accuracy
- Precision
  - Repeatability
  - Intermediate Precision
- Detection Limit
- Quantitation Limit
- Robustness

Specificity

The system suitability for specificity was carried out to determine whether there is any interferenceof any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity

Preparation of stock solution

10 mg of kanamycin and 1mg of cephalexin working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of Level – I (50ppm of kanamycin and 5 ppm of cephalexin)

0.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark withdiluent.

Preparation of Level – II (100ppm of kanamycin and 10ppm of cephalexin)

1 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark withdiluent.

Preparation of Level – III (150ppm of kanamycin and 15ppm of cephalexin)

1.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark withdiluent.

Preparation of Level – IV (200 ppm of kanamycin and 20ppm of cephalexin)

2 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark withdiluent.

Preparation of Level – V (250 ppm of kanamycin and 25ppm of cephalexin).

ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark withdiluent.

Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area)

and the correlation coefficient was calculated. Correlation coefficient should be not less than 0.999.

Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 50µg/ml-250µg/ml and 5µg/ml-25µg/ml of kanamycin and cephalexin respectively.

Accuracy

Preparation of standard stock solution

10mg of kanamycin and 1mg of cephalexin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solutions

For preparation of 50% solution (with respect to target assay concentration)

5mg of kanamycin and 0.5 mg of cephalexin working standard were accurately weighed and transferred into a 10 ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution).Further pipette out 10 ml of the above stock solution into a 100ml volumetric flask andwas diluted up to the mark with diluent.

For preparation of 100% solution (with respect to target assay concentration)

10 mg of kanamycin and 1 mg of cephalexin working standards were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml of above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 150% solution (with respect to target assay concentration)

15 mg of kanamycin and 2 mg of cephalexin working standards into a 10ml clean dry volumetricflask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for kanamycin and cephalexin and calculate the individual % recovery and mean % recovery values. The % recovery for each level should be between 98.0 to 102.0%

Precision

Repeatability Preparation of stock solution

10 mg of kanamycin and 1 mg of cephalexin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipetteout 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

The standard solution was injected for five times and measured the area for all five injectionsin HPLC. The %RSD for the area of five replicate injections was found to be within the specifiedlimits. The % RSD for the area of five standard injections results should not be more than 2.

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method,precision was performed on different days by using different make column of same dimensions. Preparation of stock solution 10 mg of kanamycin and 1mg of cephalexin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.The % RSD for the area of five sample injections results should not be more than 2%.

Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. Thestandard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$LOD = 3.3 \times \sigma - S$$

Where

σ - Standard deviation (SD)S – Slope

Limit of quantification

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$LOD = 10 \times \sigma - S$$

Where

σ - Standard deviation S – Slope

Robustness

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

- The flow rate was varied at 0.4ml/min to 0.6 ml/min.

Standard solution 150 ppm of kanamycin and 15 ppm of cephalexin was prepared and analysed using the varied flow rates along with method flow rate.

- The organic composition in the mobile phase was varied from 65% to75 %standard solution 150 µg/ml of kanamycin and15 µg/ml of cephalexin were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

System suitability

10 mg of kanamycin and 1 mg of cephalexin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of kanamycin and cephalexin from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

VALIDATION REPORT

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The chromatograms are shown in Figs.

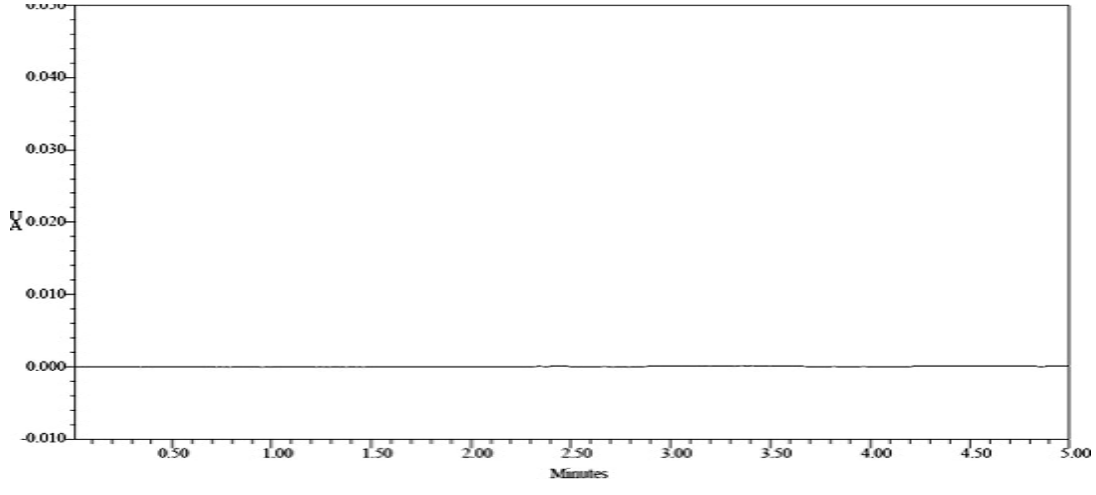


Fig 1: Chromatogram showing blank (mobile phase preparation)

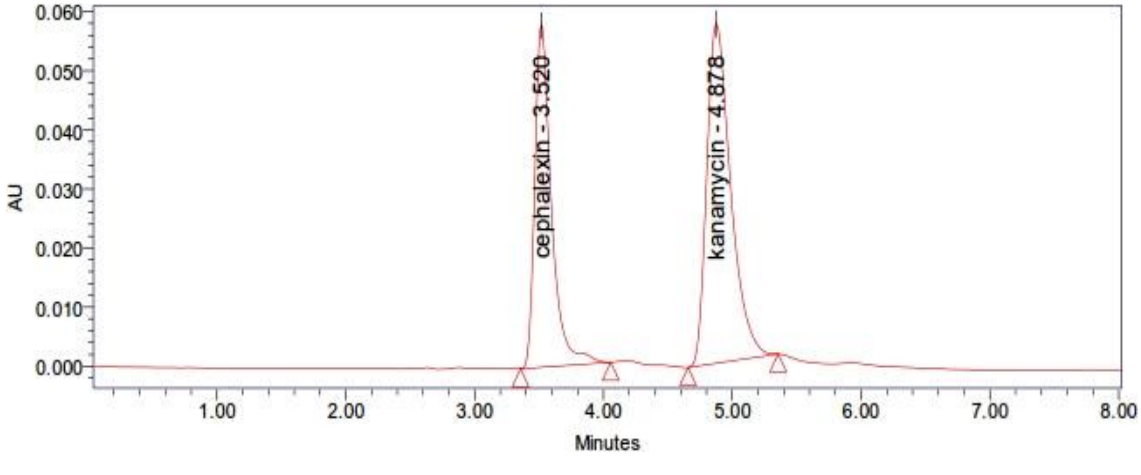


Fig 2: Chromatogram showing standard injection

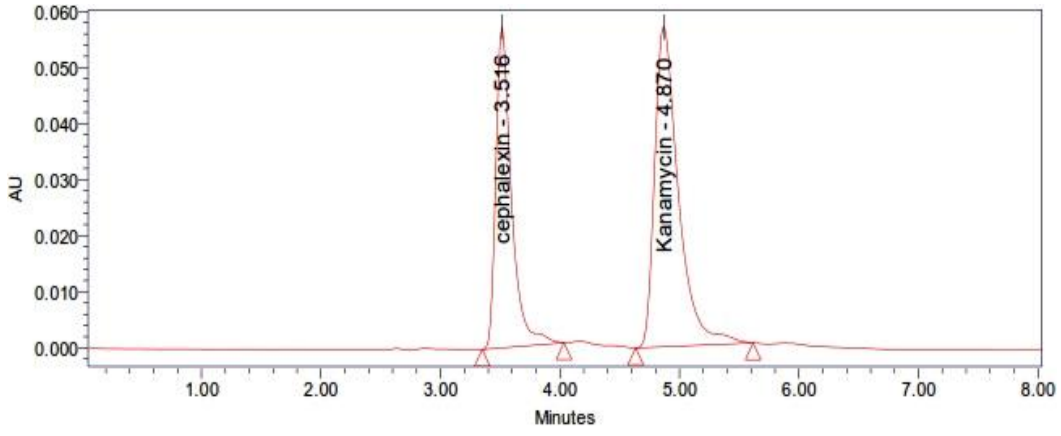
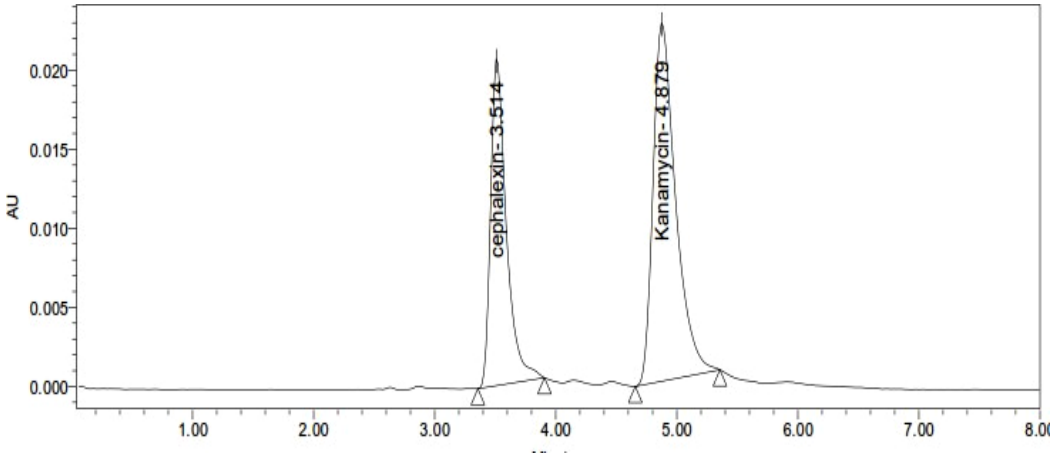


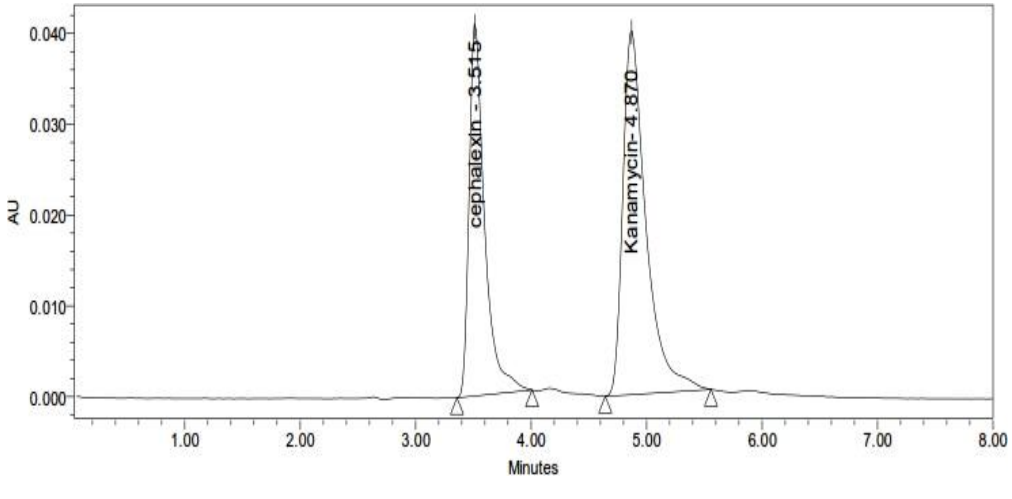
Fig 3: Chromatogram showing sample injection

The specificity test was performed for Kanamycin and cephalexin. It was found that there was nointerference of impurities in retention time of analytical peak.

Linearity

The linearity study was performed for the concentration of 50 ppm to 250 ppm and 5ppm to 25 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The chromatograms are shown in Fig. No.36- 40 and results are tabulated in Table. No.14-15. Calibration graph for CEF and KAN are shown in Figs.





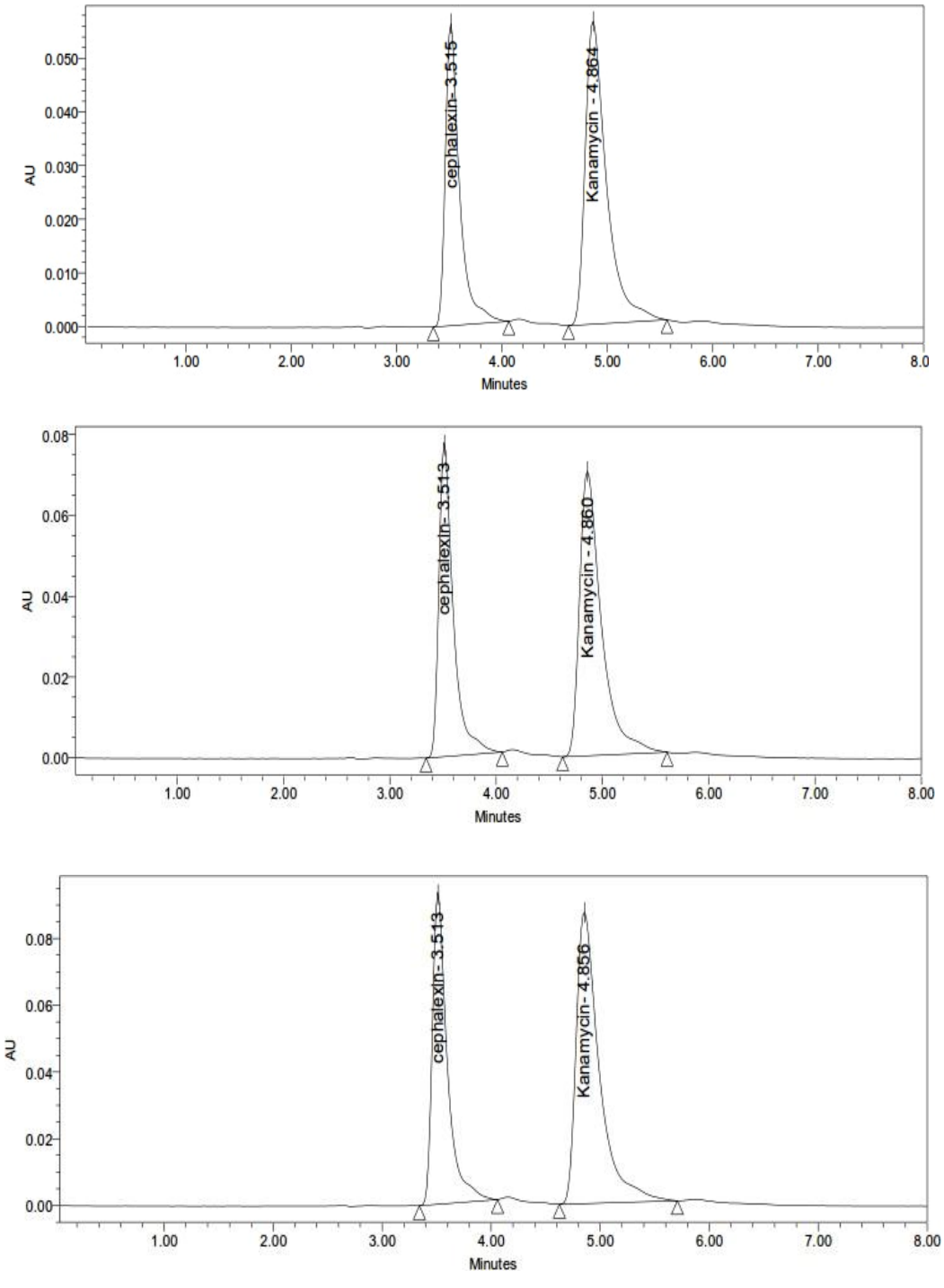
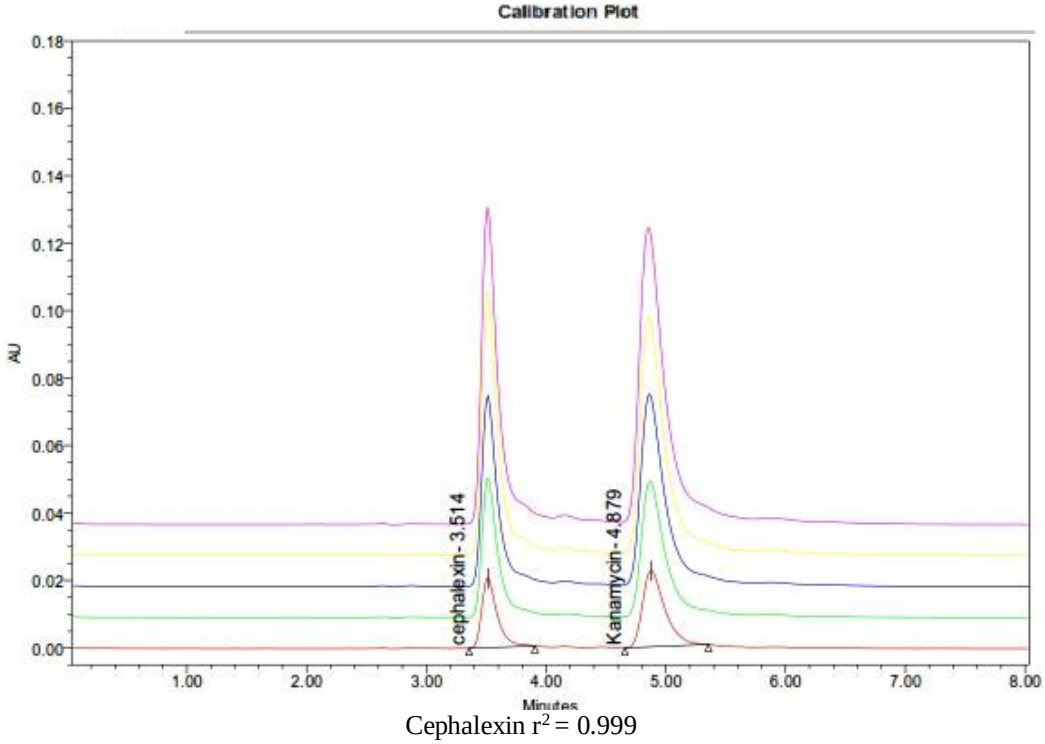


Fig 4: Hromatograms showing linearity level-1 to level 5 (50ppm-250 ppm)

Table 1: Linearity Results for Cephalaxin

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	471543
2	II	100 ppm	656277
3	III	150 ppm	794999
4	IV	200 ppm	946124
5	V	250 ppm	1002139
		Correlation Coefficient	0.999

OVERLAY DIAGRAM



Cephalexin r^2 = 0.999

Table 2: Linearity Results for Kanamycin

S.No	Linearity Level	Concentration	Area
1	I	5ppm	56472
2	II	10 ppm	73841
3	III	15ppm	92655
4	IV	20ppm	111541
5	V	25ppm	130567
Correlation Coefficient			0.999

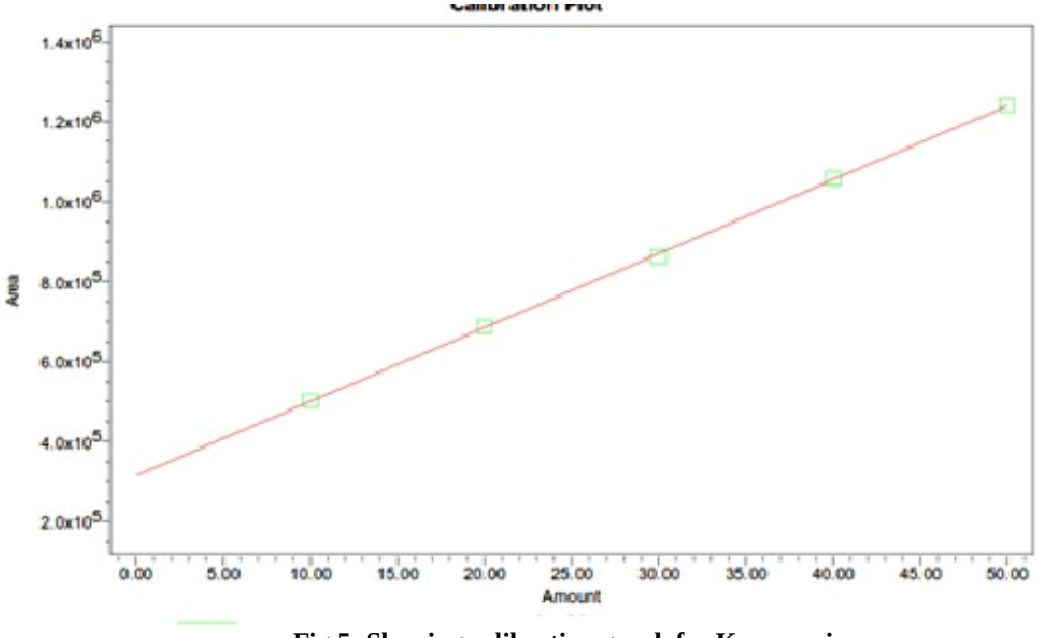


Fig 5: Showing calibration graph for Kanamycin

The linearity study was performed for concentration range of 50.µg-250µg and 5µg-50µg of kanamycin and cephalexin and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999).

SUMMARY AND CONCLUSION

A new method was established for simultaneous estimation of Kanamycin and Cephalexin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Kanamycin and Cephalexin by using SYMMETRY C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphatebuffer (KH_2PO_4 and K_2HPO_4) pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 258nm. The instrument used was Waters HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. Theretention times were found to be 2.403 mins and 3.907 mins. The % purity of Kanamycin and Cephalexin was found to be 100.27% and 99.87% respectively. The system suitability parameters for Kanamycin and Cephalexin such as theoretical plates and tailing factor were found to be 2294, 1.27 and 4891 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Kanamycin and Cephalexin was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.27 and 0.40, % RSD for intermediate precision was 0.27 and 0.94 respectively. The precision study was precise, robust, and repeatable.LOD value was 2.17 and 6.60, and LOQ value was 0.032 and 0.1125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Kanamycin and Cephalexin in API and Pharmaceutical dosage form.

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