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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF NEOMYCIN SULPHATE, POLYMYXIN B SULFATE AND PRAMOXINE HCL IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Neomycin Sulphate, Polymyxin B Sulfate and Pramoxine HCL in Cream dosage form. Chromatogram was run through Denali 150 x 4.6 mm, 5µ. Mobile phase containing Buffer and Acetonitrie in the ratio of 52:48 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA buffer at pH 2.2. Temperature was maintained at 30°C. Optimized wavelength for Neomycin, Polymyxin and Pramoxine was 225 nm. Retention time of Neomycin, Polymyxin and Pramoxine were found to be 2.591 min, 3.255 min and 4.308 min respectively. % RSD of system precision for Neomycin, Polymyxin and Pramoxine were and found to be 0.8, 0.4 and 0.6 respectively. % RSD of method precision for Neomycin, Polymyxin and Pramoxine were found to be 1.0, 0.2 and 0.5 respectively. % recovery was obtained as 99.16 %, 98.85 % and 99.83 % for Neomycin, Polymyxin and Pramoxine respectively. LOD, LOQ values are obtained from regression equations of Neomycin, Polymyxin and Pramoxine were 0.59 ppm, 0.29 ppm, 0.20 ppm, 1.80 ppm, 0.86 ppm, and 0.61 ppm respectively. Regression equation of Neomycin was 7443.x + 3015, Polymyxine was 1864.x + 185 and Pramoxine was 7584.x + 18450.

INTRODUCTION:

Neomycin Sulphate [(2RS, 3S, 4S, 5R) - 5 - Amino - 2 - (amino methyl) - 6 - {(2R, 3S, 4R, 5S) - 5 - [(1R, 2R, 5R, 6R) - 3, 5 - diamino - 2 - ((2R, 3S, 4R, 5S) - 3 - amino - 6 - (amino methyl) - 4, 5 - dihydroxy tetra hydro - 2H - pyran - 2 - yloxy) - 6 - hydroxycyclohexyloxy] - 4 - hydroxy - 2 - (hydroxyl methyl) tetra hydro furan - 3 – yloxy} tetra hydro - 2H - pyran - 3, 4 - diol]. A component of neomycin is produced by Streptomyces fradiae. Neomycin is a bactericidal aminoglycoside antibiotic that binds to the 30S ribosome of susceptible organisms. Binding interferes with mRNA binding and acceptor tRNA sites and results in the production of non-functional or toxic peptides. Neomycin is used as a preventive measure for hepatic encephalopathy and hyper cholesterolemia. By killing bacteria

in the intestinal tract, it keeps ammonia levels low and prevents hepatic encephalopathy, especially prior to GI surgery. It works as an antibiotic that is active against streptomycin-resistant bacteria, including in the case of tuberculosis organisms. It has also been used to treat small intestinal bacterial over growth.

Polymyxin B Sulfate [N - {4 - amino - 1 - { [1 - [[4 - amino - 1 - oxo - 1 - [[6, 9, 18 - tris (2 - amino ethyl) - 15 - benzyl - 3 - (1 - hydroxy ethyl) - 12 - (2 - methyl propyl) - 2, 5, 8, 11, 14, 17, 20 - heptaoxo - 1, 4, 7, 10, 13, 16, 19 - heptazacyclotricos - 21 - yl] amino] butan - 2 - yl] amino] - 3 - hydroxy - 1 - oxobutan - 2 - yl] amino} - 1 - oxobutan - 2 - yl} - 6 - methyl octanamide; sulfuric acid]. Polymyxin B sulfate is a mixture of polymyxins B1 and B2, obtained from Bacillus polymyxa strains, with antimicrobial activity. They are basic polypeptides of about eight amino acids and have cationic detergent action on cell membranes. Polymyxin B is used for infections with gram-negative organisms, but may be neurotoxic and nephrotoxic. All gram-positive bacteria, fungi, and the gram-negative cocci, N. gonorrhea and N. menigitidis, are resistant. It is appropriate for treatment of infections of the urinary tract, meninges, and blood stream, caused by susceptible strains of Pseudomonas aeruginosa.

Pramoxine HCL [4 - [3 - (4 - butoxyphenoxy) propyl] morpholine hydrochloride]. Pramocaine (also known as pramoxine or pramoxine HCI) is a topical anesthetic and antipruritic. It is used for many dermatological and anorectal/anogenital conditions including minor cuts/burns, insect bites, hives/rashes due to poison ivy exposure, hemorrhoids and other anorectal/anogenital disorders. Pramocaine is available by itself and in combination with other medications in various topical preparations. It works by preventing ionic fluctuations needed for neuron membrane depolarization and action potential propagation.

The main aim of the in-process investigation is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for cost effective estimation of Neomycin Sulphate, Polymyxin B Sulfate and Pramoxine HCL in combination.

The objectives is to develop analytical method: Selecting the HPLC separation mode, Selecting/optimizing the mobile phase, Selecting column for analysis, Selecting the appropriate detector system, Selecting appropriate gradient/isocratic medium, Selecting appropriate flow rate, temperature and Ph.

The objectives are to validate the method development: Selectivity (Specificity), Precision, Accuracy, Linearity and Range, LOD and LOQ, Robustness and Ruggedness.

Materials and Instruments:

The following materials were used either AR/LR grade or the best possible pharma grade available as supplied by the manufacturer or supplier without further purification or investigation.

Drug Samples: Were obtained from Spectrum pharma research solutions pvt.ltd.

Chemicals and Solvents Used:

- Water- HPLC grade,
- Acetonitrile-HPLC grade
- Triethyl amine – AR grade
- Potassium dihydrogen orthophosphate - AR grade
- Ortho phosphoric acid – AR grade
- All the above chemicals and solvents are from Rankem

Instruments:

- Electronics Balance - Denver
- BVK enterprises, India, pH meter

- BVK enterprises, Ultrasonicator
- Labindia UV double beam spectrophotometer with UV wins 5
- Waters HPLC 2695 series with quaternary pumps, Photo Diode Array detector and auto sampler integrated with empower 2 software

Sample Processing:

Diluents: Based up on the solubility of the drug diluents was selected, initially dissolved in methanol & diluted with acetonitrile and water.

Preparation of Standard stock solutions: Precisely weighed 17.5 mg of Neomycin, 15 mg of Polymyxin and 50 mg of Pramoxine and transferred to three 50 ml graduated flasks separately. 10 ml of CH_3OH solution was added to flasks and sonicated for 15 mins. Flasks were finished up with water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3.

Preparation of Standard working solutions (100% solution): 1ml from every stock solution was pipped out and taken into 10 ml graduated flasks and finished up with water methanol.

Preparation of sample stock solutions: Take 28.3 gm of ointment (NEOSPORIN PLUS CREAM OTC) transferred into a 10 ml graduated flask, add 5 ml of methanol, stir for 40 minutes on magnetic stirrer and finished up to mark with methanol and then it was centrifuged for 20 min. Then the buoyant liquid was possessed & filtered using 0.45 µm filters using (Millipore, Milford, PVDF) (350 ppm & 300 ppm & 1000 ppm).

Preparation of sample working solutions (100% solution): From the filtered liquid 1 ml was pipped out into a 10 ml graduated flask & finished up to 10 ml with solvents. (35 ppm & 30 ppm & 100 ppm).

Preparation of buffer:

0.01 N Na_2HPO_4 Buffer: Take 1.41 gm of Na_2HPO_4 in a 1000 ml of volumetric flask. Add about 900 ml of milli-Q water and degas to sonicate and at last make up the volume with water then PH adjusted to 3 with dil. OPA solution.

0.01 N KH_2PO_4 Buffer: Take 1.41 gm of KH_2PO_4 in a 1000 ml of graduated flask, add about 900 ml of milli-Q water and degenerate to sonic barrier and at last make up the volume with H_2O then pH corrected to 5.4 as well as dil. OPA solution.

0.1 % OPA Buffer: 1 ml of orthophosphoric acid was diluted with 1000 ml of HPLC grade water.

RESULT AND DISCUSSION

System suitability parameters:

The system suitability variables were determined by preparing standard solutions of Neomycin, Polymyxin and Pramoxine and the solutions were injected 6 times and the variables like USP peak tailing, resolution and USP plate count were analyzed. The % RSD for the area of 6 standard injections results should not be > 2%.

Discussion: Plate count of the Neomycin was 3236 ± 60, Polymyxin was 5000 ± 286 and Pramoxine was 4487 ± 66. Tailing factor of Neomycin, Polymyxin and Pramoxine was found to be 1.33 ± 0.26, 1.13 ± 0.08, and 1.32 ± 0.17 respectively. Resolution between Neomycin and Polymyxin was 3.5 and resolution between Polymyxin and Pramoxine was 4.7. As per ICH guide lines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be > 2. All the system acceptable boundaries were passed.

Specificity:

Checking of the interference in the optimized method. We should not found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Discussion: Retention time of Neomycin, Polymyxin and Pramoxine were 2.591 min, 3.255 min and 4.308 min respectively. We did not find any peaks in blank & placebo at retention times of these drugs. So, this technique was said to be unique.

Precision:

Preparation of Standard stock solutions: Precisely weighed 17.5 mg of Neomycin, 15 mg of Polymyxin and 50 mg of Pramoxine and transferred to three 50 ml graduated flasks separately. 10 ml of CH_3OH solution was added to flasks and sonicated for 15 mins. Flasks were finished up with water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3.

Preparation of Standard working solutions (100% solution): 1ml from every stock solution was pipped out and taken into 10 ml graduated flasks and finished up with water methanol.

Preparation of Sample stock solutions: Take 28.3 gm of ointment (NEOSPORIN PLUS CREAM OTC) transferred into a 10 ml volumetric flask, add 5 ml of methanol, stirr for 40 minutes on magnetic stirrer and made up to mark with methanol and then it was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF) (350 ppm & 300 ppm & 1000 ppm).

Preparation of Sample working solutions (100% solution): 1 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluents. (35 ppm & 30 ppm & 100 ppm).

System Precision Discussion: From a solitary volumetric flask of working standard arrangement of six infusions were given and obtained zones were said above. Mean, standard deviation & % RSD were calculated for three drugs. Mean was obtained as 255988, 56306, and 771427, Standard deviation was 2606.7, 87.2, and 3744.5, and % RSD was 1.0 %, 0.2 % and 0.5 % respectively for Neomycin, Polymyxin and Pramoxine. As the limit of method precision was < 2 the system precision was passed in this method.

Intermediate Precision Discussion: Various examining from a sample stock arrangement was done and six working sample arrangements of same focuses were readied, every infusion from each working sample arrangement was given on the following day of the sample readiness and obtained zones were said in the above table. Average ranges, SD and % RSD were calculated for three drugs and obtained as 0.3 %, 0.4 % and 0.8 % respectively for Neomycin, Polymyxin and Pramoxine. As the maximum of intermediate Precision was < 2 the system precision was crossed in this arrangement.

Accuracy:

Preparation of Standard stock solutions: Take 17.5 mg of Neomycin, 15 mg of Polymyxin and 50 mg of Pramoxine and transferred to three 50 ml volumetric flasks separately. 10 ml of methanol was added to flasks and sonicated for 15 mins. Flasks were made up with water and Methanol (50:50) and labeled as standard stock solution 1, 2 and 3.

Preparation of 50% Spiked Solution: 0.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria: The % Recovery for every level should be between 98.0 to 102.0 %

Discussion: Three levels of Accuracy sample were prepared by standard addition method. Triplicate injections were given for each aligned of accuracy and mean % Recovery was obtained as 100.06 %, 100.19 % and 100.08 % for Neomycin, Polymyxin and Pramoxine respectively.

Linearity:

Preparation of Standard stock solutions: Take 17.5 mg of Neomycin, 15 mg of Polymyxin and 50 mg of Pramoxine and transferred to three 50 ml volumetric flasks separately. 10 ml of methanol was added to flasks and sonicated for 15 mins. Flasks were made up with water and Methanol (50:50) and labeled as standard stock solution 1, 2 and 3.

25% Standard solution: 0.25 ml each from three standard stock solutions was pipette out and made up to 10 ml.

50% Standard solution: 0.5 ml each from three standard stock solutions was pipette out and made up to 10 ml.

75% Standard solution: 0.75 ml each from three standard stock solutions was pipette out and made up to 10 ml.

100% Standard solution: 1.0 ml each from three standard stock solutions was pipette out and made up to 10 ml.

125% Standard solution: 1.25 ml each from three standard stock solutions was pipette out and made up to 10 ml.

150% Standard solution: 1.5 ml each from three standard stock solutions was pipette out and made up to 10 ml.

Discussion: 6 concentrations of Neomycin (8.75-52.5 µg/ml), Polymyxin (7.5-45 µg/ml) and Pramoxine (25-150 µg/ml) were injected in a triplicate manner. Avg. ranges were specified above and linearity equations obtained for Neomycin was y = 7443.5x + 3015, Polymyxine was y = 1864.4x + 185.04 and Pramoxine was y = 7584.1x + 18450. Correlation coefficient obtained was 0.999 for all the three drugs.

Sensitivity:

LOD sample Preparation: 0.25 ml each from three standard stock solutions was pipette out and transferred to 3 separate 10 ml volumetric flask and made up with diluents from the above solutions 0.1 ml, 0.1 ml and 0.1 ml of Neomycin, Polymyxin and Pramoxine solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25 ml each from three standard stock solutions was pipette out and transferred to 3 separate 10 ml volumetric flask and made up with diluents from the above solutions 0.3 ml, 0.3 ml and 0.3 ml of Neomycin, Polymyxin and Pramoxine solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluents.

Discussion: The LOD of Neomycin, Polymyxin, Pramoxine was found to be 0.59 µg/ml, 0.29 µg/ml and 0.20 µg/ml respectively and LOQ of Neomycin, Polymyxin, Pramoxine was found to be 1.80 µg/ml, 0.86 µg/ml and 0.61 µg/ml respectively. LOD was NMT 3 µg/ml and LOQ was NMT 10 µg/ml. The system LOD and LOQ was within the limits.

Robustness:

Small deliberate changes in method like mobile phase ratio, temperature and flow rate are made but there was no recognized change in the result according to ICH Guide lines within the ranges. Robustness conditions like flow minus (0.9 ml/min), flow plus (1.1 ml/min), mobile phase minus, mobile phase plus, temperature minus (25 °C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. % RSD was within the limit.

Discussion: Robustness conditions like flow minus (0.9 ml/min), flow plus (1.1 ml/min), MP (-) (60B:40A), MP (+) (50B:50A), TM (-) (25°C) & TM (+) (35°C) was kept up and tests were infused in a duplicate manner. Frame work reasonableness parameters were not much affected and all the parameters were passed. % RSD was within the ranges.

Assay:

Neosporin plus cream otc from Johnson & Johnson Consumer Pvt Ltd, baring the label claim Polymyxin 3.5 mg, Neomycin 3 mg and Pramoxine 10 mg per unit formulation. Assay was performed with the above formulation. Average % Assay for Neomycin, Polymyxin and Pramoxine obtained was 100.40 %, 99.75 % and 100.62 % respectively.

Degradation studies:

Oxidation: To 1 ml of stock solutions of Neomycin, Polymyxin and Pramoxine 1 ml of 20 % hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^{o}C. For HPLC study, the resultant solution was diluted to obtain 35 µg/ml, 30 µg/ml and 100 µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock s solution Neomycin, Polymyxin and Pramoxine, 1 ml of 2N HCL was added and kept for 30 minutes at 60 ^{o}C. The obtained solution was diluted to get 35 µg/ml, 30 µg/ml and 100 µg/ml of all components and 10 µl solutions were injected into the HPLC and the chromatograms were recorded to estimation the stability of test.

Alkali Degradation Studies: To 1 ml of stock solution Neomycin, Polymyxin and Pramoxine, 1 ml of 2N NAOH was added and kept for 30 minutes at 60 ^{o}C. The obtained solution was diluted to get 35 µg/ml, 30 µg/ml and 100 µg/ml of all components and 10 µl were injected into the HPLC and the chromatograms were recorded to estimation the stability of test.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105 ^{o}C for 1 h to study dry heat degradation. For HPLC study, the obtained solution was diluted get 35 µg/ml, 30 µg/ml and 100 µg/ml of all components and 10 µl were injected into the HPLC and the chromatograms were recorded to estimation the stability of the test**.**

Photo Stability studies: The photo chemical stability of the drug was also studied by exposing the 250 µg/ml, 800 µg/ml and 200 µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1 day or 200 Watt hours/m^2 in photo stability chamber for HPLC study, the obtained solution was diluted to get 35 µg/ml, 30 µg/ml and 100 µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to estimation the stability of test.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by kept the drug in water for 6 hrs at a temperature of 60 ^{o}C. For HPLC study, the obtained solution was diluted to get 35 µg/ml, 30 µg/ml and 100 µg/ml of all components and 10 µl were injected into the HPLC and the chromatograms

were recorded to estimation the stability of the test**.**

Discussion: Stability considerations were accomplished as well as the formulation & the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

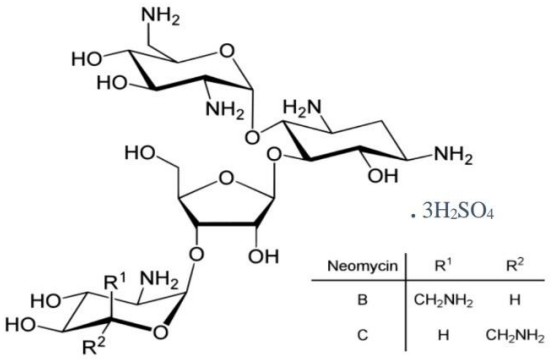

Fig 1. Structure of Neomycin Sulphate

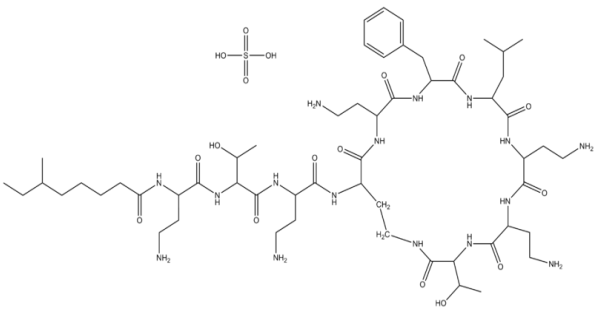

Fig 2. Structure of Polymyxin B Sulfate

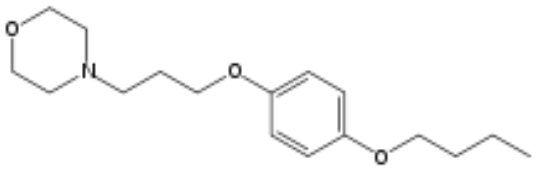

Fig 3. Structure of Pramoxine HCL

Table 1. System suitability parameters for Neomycin, Polymyxin, Pramoxine

S No	Neomycin			Polymyxin			Pramoxine		
Inj	RT(min)	TP	Tailing	RT(min)	TP	Tailing	RT(min)	TP	Tailing
1	2.584	3185	1.30	3.255	5716	1.09	4.282	4511	1.34
2	2.586	3236	1.33	3.256	5000	1.13	4.295	4487	1.32
3	2.591	3259	1.36	3.256	5165	1.22	4.298	4516	1.34
4	2.591	3219	1.30	3.256	5260	1.19	4.303	4584	1.36
5	2.599	3225	1.29	3.257	5628	1.01	4.308	4405	1.36
6	2.600	3362	1.33	3.275	5560	1.04	4.321	4421	1.32

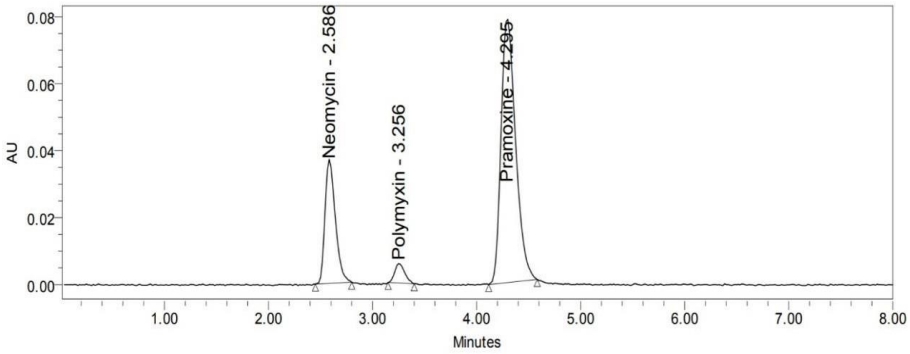

Fig 4. System suitability chromatogram

Specificity chromatograms:

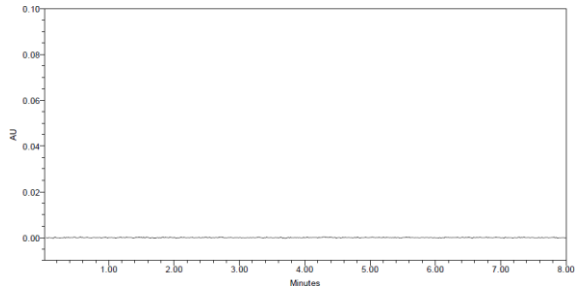

Fig 5. Blank Chromatogram

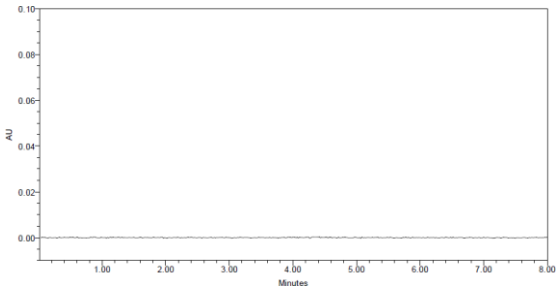

Fig 6. Placebo Chromatogram

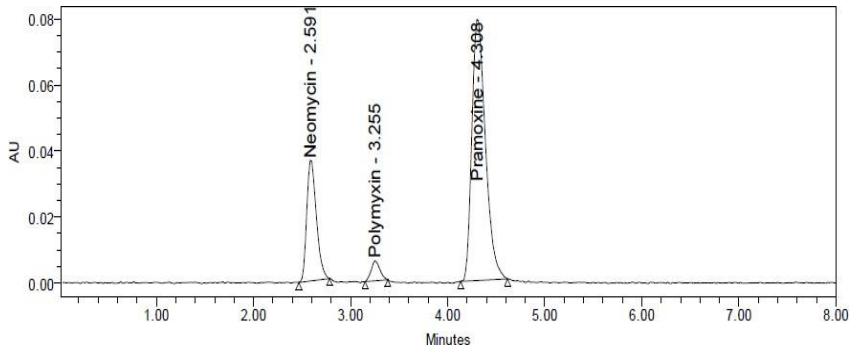

Fig 7. Optimized Chromatogram

Table 2. System precision table of Neomycin, Polymyxin and Pramoxine

S. No	Area of Neomycin	Area of Polymyxin	Area of Pramoxine
1.	259360	56346	765298
2.	252422	56170	774619
3.	256863	56414	768681
4.	258139	56334	771732
5.	254082	56238	774479
6.	255059	56335	773752
Mean	255988	56306	771427
S.D	2606.7	87.2	3744.5
% RSD	1.0	0.2	0.5

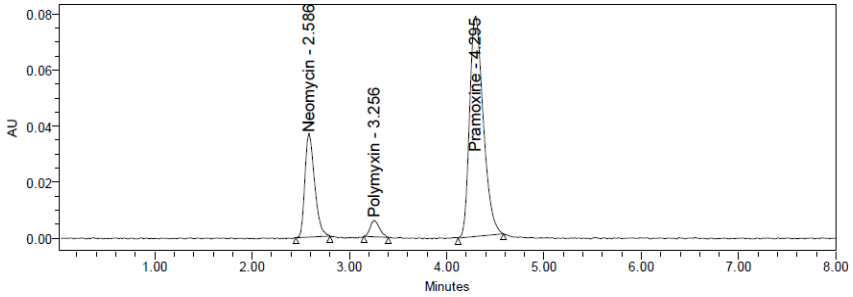

Fig 8. System Precision Chromatogram

Table 3. Intermediate precision table of Neomycin, Polymyxin and Pramoxine

S. No	Area of Neomycin	Area of Polymyxin	Area of Pramoxine
1.	195036	56191	253292
2.	194621	56191	255503
3.	195492	56016	257906
4.	195800	56188	251578
5.	195573	56425	254610
6.	194325	56659	253891
Mean	195141	56278	254463
S.D	581.6	227.4	2143.8
% RSD	0.3	0.4	0.8

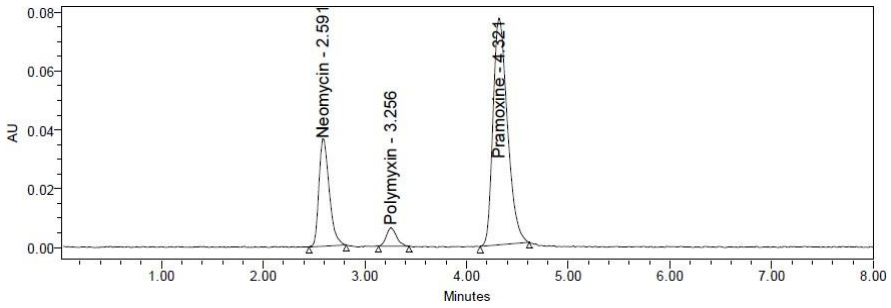

Fig 9. Intermediate Precision Chromatogram

Table 4. Accuracy table of Neomycin

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50%	17.5	17.58	100.44	100.06%
	17.5	17.41	99.50	
	17.5	17.39	99.35	
100%	35	35.27	100.76	
	35	34.96	99.88	
	35	34.93	99.79	
150%	52.5	52.77	100.52	
	52.5	52.17	99.37	
	52.5	52.99	100.94	

Table 5. Accuracy table of Polymyxin

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50%	15	14.90	99.33	100.19%
	15	15.10	100.69	
	15	14.92	99.43	
100%	30	29.81	99.37	
	30	30.00	100.01	
	30	29.99	99.97	
150%	45	45.66	101.47	
	45	44.99	99.98	
	45	45.66	101.46	

Table 6. Accuracy table of Pramoxine

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50%	50	50.22	100.43	100.08%
	50	49.55	99.09	
	50	49.73	99.46	
100%	100	100.89	100.89	
	100	99.59	99.59	
	100	99.44	99.44	
150%	150	150.91	100.61	
	150	149.82	99.88	
	150	151.92	101.28	

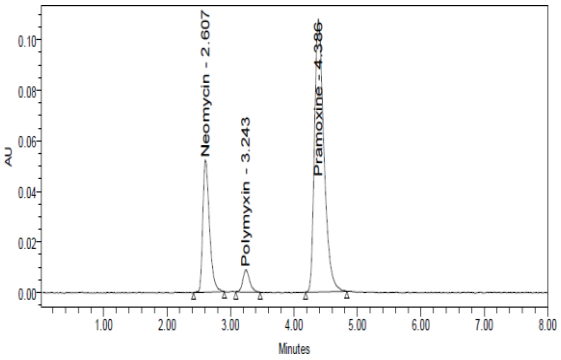

Fig 10. Accuracy 50% Chromatogram

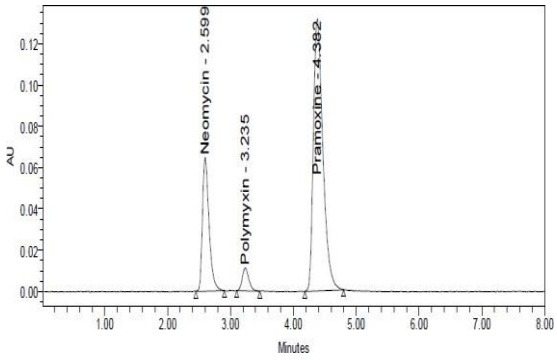

Fig 11. Accuracy 100% Chromatogram

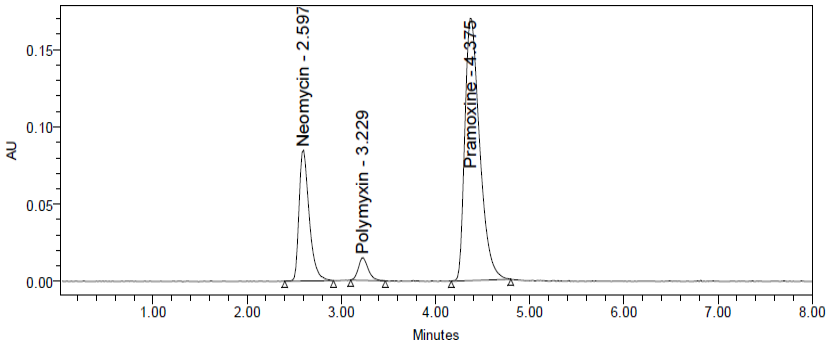

Fig 12. Accuracy 150% Chromatogram

Table 7. Linearity table for Neomycin, Polymyxin and Pramoxine

Neomycin		Polymyxin		Pramoxine	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
8.75	68587	7.5	14376	25	218092
17.5	133334	15	27856	50	405144
26.25	204835	22.5	42482	75	600675
35	259645	30	56089	100	772080
43.75	332818	37.5	70369	125	966328
52.5	389627	45	83770	150	1148488

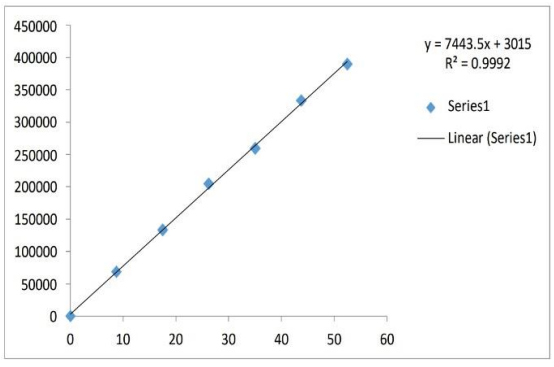

Fig 13. Calibration curve of Neomycin

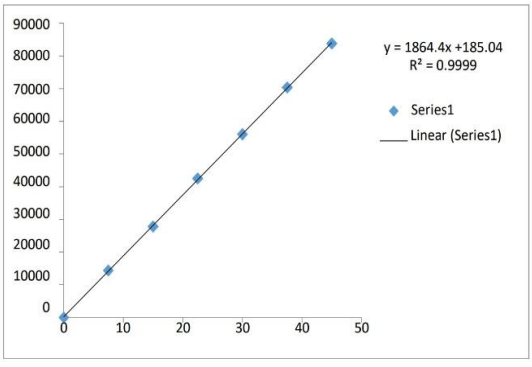

Fig 14. Calibration curve of Polymyxin

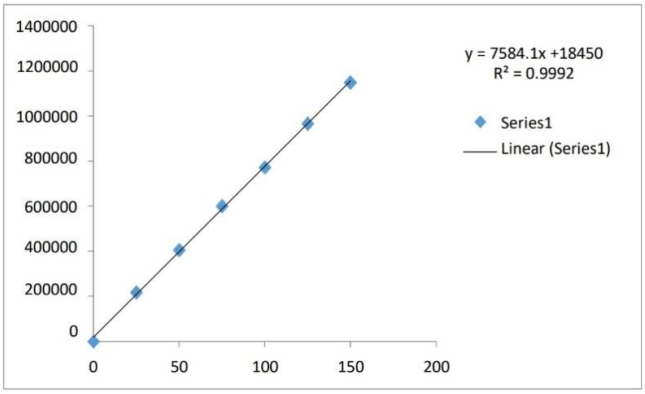

Fig 15. Calibration curve of Pramoxine

Table 8. Sensitivity table of Neomycin, Polymyxin and Pramoxine

Molecule	LOD (µg/ml)	LOQ (µg/ml)
Neomycin	0.59 µg/ml	1.80 µg/ml
Polymyxin	0.29 µg/ml	0.86 µg/ml
Pramoxine	0.20 µg/ml	0.61 µg/ml

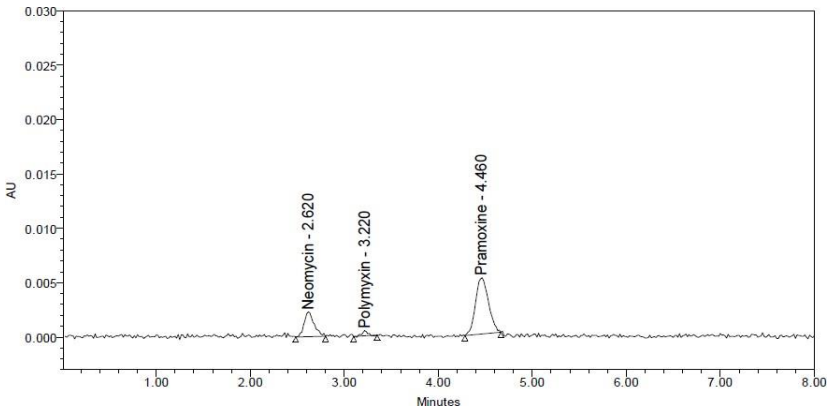

Fig 16. LOD Chromatogram of Standard

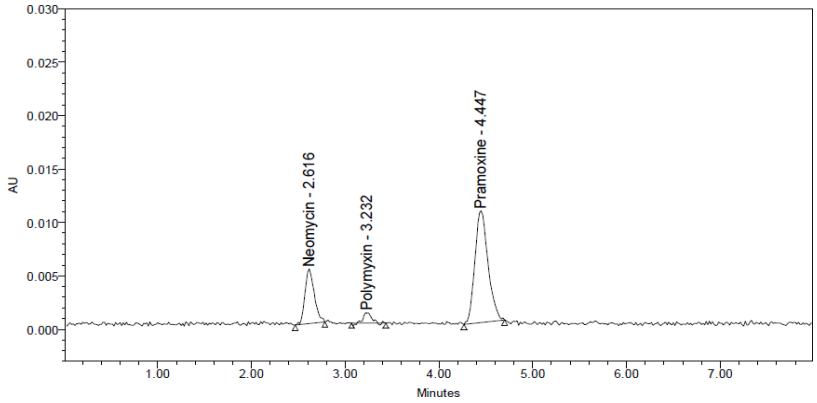

Fig 17. LOQ Chromatogram of Standard

Table 9. Robustness data for Neomycin, Polymyxin and Pramoxine

S.No	Condition	% RSD of Neomycin	% RSD of Polymyxin	% RSD of Pramoxine
1	Flow rate (-) 0.9 ml/min	0.5	0.6	0.9
2	Flow rate (+) 1.1 ml/min	0.4	0.7	0.6
3	Mobile phase (-) 60B:40A	0.7	0.8	0.6
4	Mobile phase (+) 50B:50A	1.3	0.8	0.7
5	Temperature (-) 25°C	1.5	0.3	1.5
6	Temperature (+) 35°C	1.5	0.5	1.5

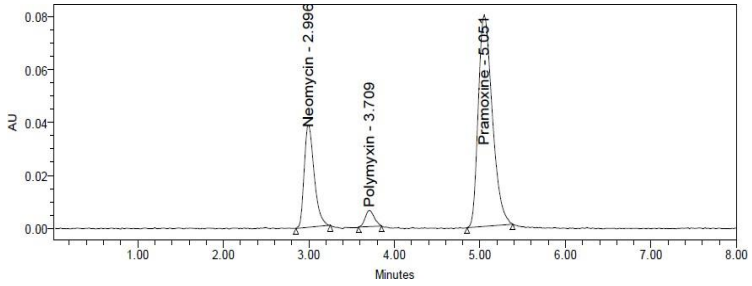

Fig 18. Flow minus chromatogram injection

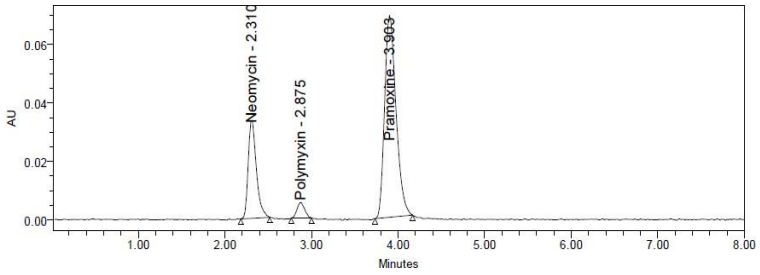

Fig 19. Flow plus chromatogram injection

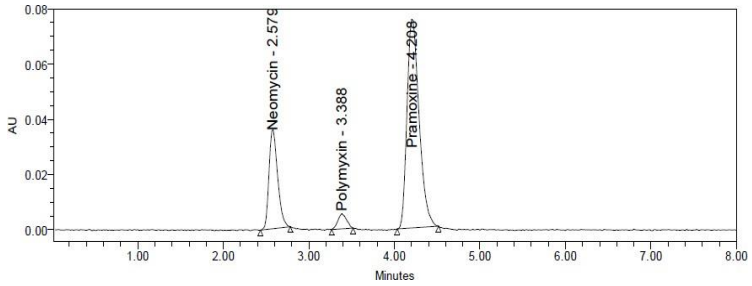

Fig 20. Mobile phase minus chromatogram injection

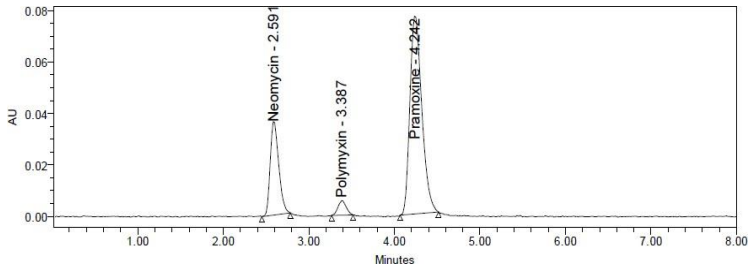

Fig 21. Mobile phase plus chromatogram injection

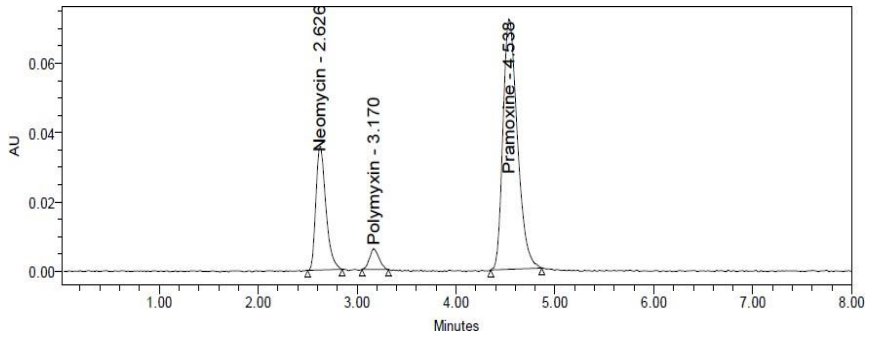

Fig 22. Temperature minus chromatogram injection

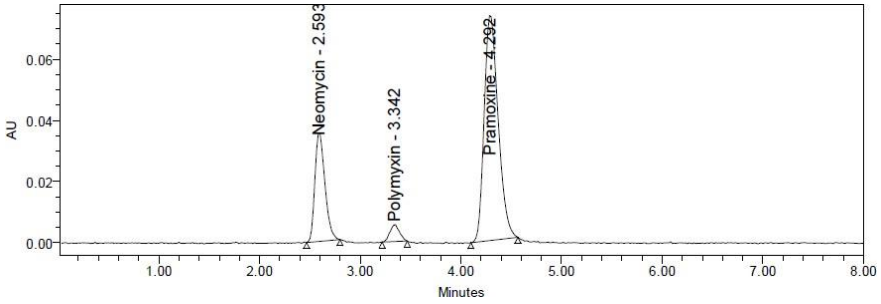

Fig 23. Temperature plus chromatogram injection

Table 10. Assay Data of Neomycin

S.No	Standard area	Sample area	% Assay
1	253292	259360	101.72
2	255503	252422	99.00
3	257906	256863	100.74
4	251578	258139	101.24
5	254610	254082	99.65
6	253891	255059	100.03
Avg	254463	255988	100.40
St dev	2143.8	2606.7	1.022
% RSD	0.8	1.0	1.0

Table 11. Assay Data of Polymyxin

S.No	Standard area	Sample area	% Assay
1	561910	563469	99.82
2	561911	561702	99.51
3	560163	564141	99.94
4	561889	563346	99.80
5	564252	562385	99.63
6	566592	563355	99.80
Avg	562786	563066	99.75
St dev	2273.9	872.3	0.15
% RSD	0.4	0.2	0.2

Table 12. Assay Data of Pramoxine

S.No	Standard area	Sample area	% Assay
1	766274	765298	99.82
2	764890	774619	101.04
3	769879	768681	100.27
4	759968	771732	100.66
5	769223	774479	101.02
6	760447	773752	100.93
Avg	765114	771427	100.62
St dev	4224.9	3744.5	0.488
% RSD	0.6	0.5	0.5

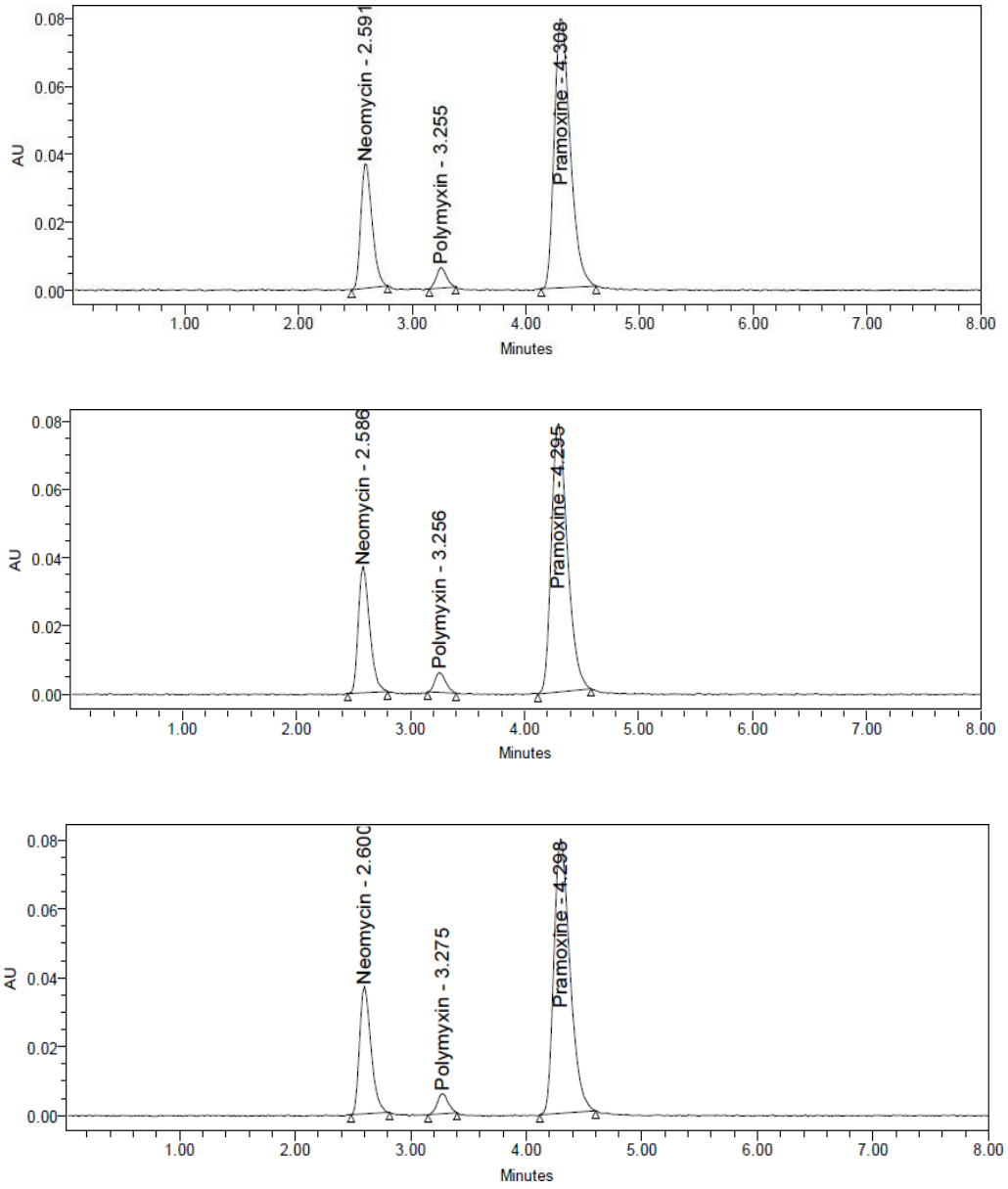

Fig 24. Chromatogram of working standard solution

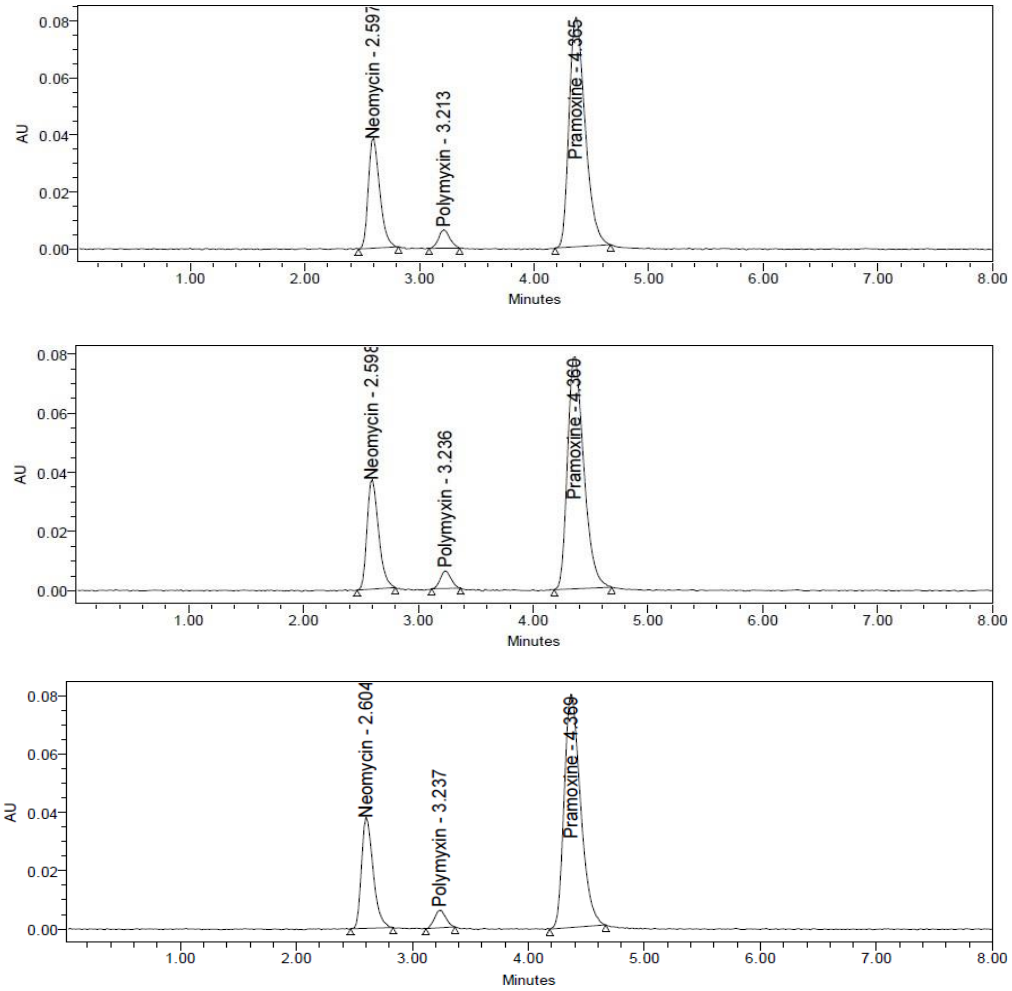

Fig 25. Chromatogram of working sample solution

Table 13. Degradation Data of Neomycin

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.64	0.305	0.381
2	Alkali	3.80	0.239	0.368
3	Oxidation	3.90	0.196	0.374
4	Thermal	2.66	0.184	0.376
5	UV	2.05	0.256	0.376
6	Water	0.95	0.715	0.376

Table 14. Degradation Data of Polymyxin

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	7.96	0.810	1.306
2	Alkali	3.91	0.414	0.607
3	Oxidation	2.69	0.423	0.649
4	Thermal	0.89	0.440	0.647
5	UV	2.64	0.426	0.634
6	Water	0.91	0.310	0.496

Table 15. Degradation Data of Pramoxine

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.25	0.355	0.542
2	Alkali	2.32	0.298	0.485
3	Oxidation	1.80	0.467	0.522
4	Thermal	1.00	0.351	0.536
5	UV	0.52	0.334	0.524
6	Water	0.35	0.310	0.497

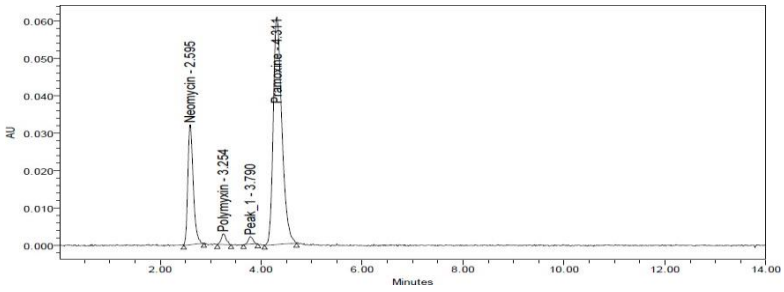

Fig 26. Acid Chromatogram

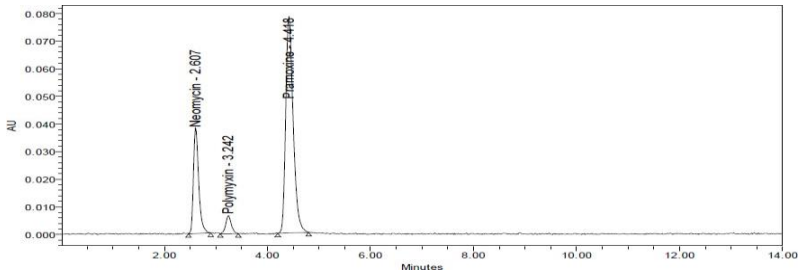

Fig 27. Base Chromatogram

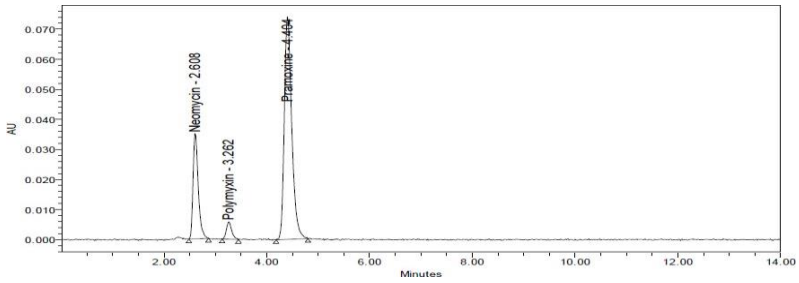

Fig 28. Peroxide Chromatogram

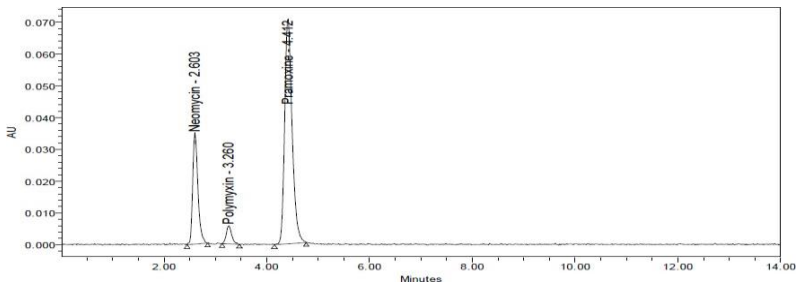

Fig 29. Thermal Chromatogram

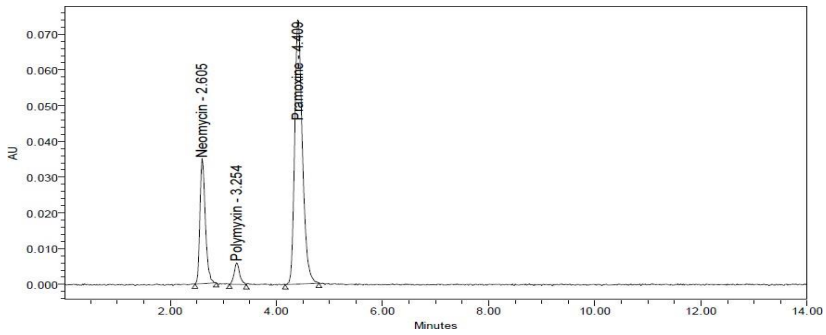

Fig 30. UV Chromatogram

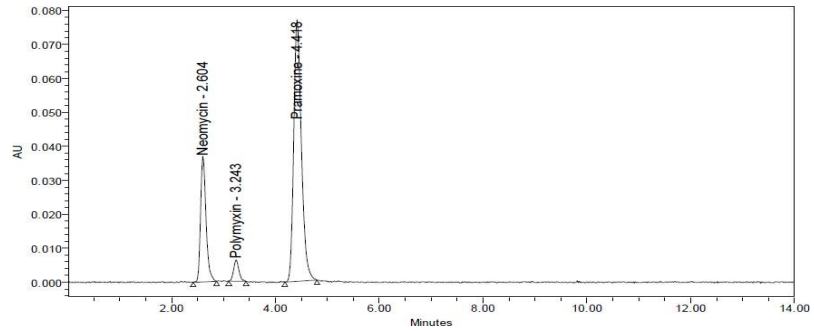

Fig 31. Water Chromatogram

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Neomycin Sulphate, Polymyxin B Sulfate and Pramoxine HCL in Cream dosage form. Retention time of Neomycin, Polymyxin and Pramoxine were found to be 2.591 min, 3.255 min and 4.308 min respectively. % RSD of system precision for Neomycin, Polymyxin and Pramoxine were and found to be 0.8, 0.4 and 0.6 respectively. % RSD of method precision for Neomycin, Polymyxin and Pramoxine were found to be 1.0, 0.2 and 0.5 respectively. % recovery was obtained as 99.16 %, 98.85 % and 99.83 % for Neomycin, Polymyxin and Pramoxine respectively. LOD, LOQ values are obtained from regression equations of Neomycin, Polymyxin and Pramoxine were 0.59 ppm, 0.29 ppm, 0.20 ppm, 1.80 ppm, 1.86 and 0.61 ppm respectively. Regression equation of Neomycin was 7443.x + 3015, Polymyxin was 1864.x + 185 and of Pramoxine was 7584.x + 18450. Retention times are decreased so the method development and design establish was easy, simple and economical. Sparing that can be adopted in regular standard quality control test in pharmaceutical industries.

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