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Original Article

Method Development and Validation of Gramicidin, Neomycin and Triamcinolone Acetonide Related Substances and their Impurities Quantification through UPLC

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ABSTRACT

Aim/Background: To develop and validate a sensitive, accurate, simple, precise and cost-effective receptive and understandable method for concurrent evaluation of Gramicidin, Neomycin and Triamcinolone acetonide and their related contaminants via UPLC. **Materials and Methods:** This method includes the separation using a chromatographic Phenyl column (50 mmx2.1 mm, 1.7 µm). A portable stage of 0.1% TEA (Tri-Ethylamine) and acetonitrile in gradient elution mode with 0.5mL/Temperature and minimum flow rate were employed. At 230 nm, UV observations were made. The limitations for Linearity, quantification and recoveries were discovered being within the allowable range. With UPLC, this method was successfully tested and ICH Q2 (R1) recommendations. **Results:** Gramicidin, Neomycin and Triamcinolone acetonide retention times were observed at 9.315 min,3.979 min and 1.697 min respectively. A gradient elution of Triamcinolone acetonide, Neomycin and Gramicidin involves phenyl column that flows from 0.5 mL/min and the column internal temperature were held constant. Mobile phase % TEA likewise acetonitrile was utilised. UV monitoring was brought at 230 nm. **Conclusion:** This study created an entirely new, straightforward, quick, affordable, sensitive and easily accessible UPLC method for the coincident determination of Triamcinolone acetonide, Neomycin and Gramicidin bulky and ointment dose form. This method has the benefits of being less expensive, assessible, reliable, sensitive and reproducible. Under conditions of oxidation, (Neutral, basic and acidic) hydrolysis, photolysis, thermal stress and medicines degradative activities were investigated. The medications were discovered to be solid underheat, unstable, hydrolysis under acid, alkaline and oxidative circumstances. The Final application of the UPLC method to the commercial formulations followed the ICH recommendations.

Keywords: Gramicidin, Neomycin, Triamcinolone acetonide, Development, UPLC.

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INTRODUCTION

Gramicidin, commonly known as gramicidin D, is a combination of ionophoric antibiotics (Gould, 2016), 80%, 5% and 15% of the mixture are, respectively, gramicidin A, B and C. Every gramicidin molecule contains two isoforms, giving the mixture six distinct varieties. *Brevibacillus brevis* soil bacteria can provide them for extraction (Aguirre *et al.*, 2021) Gramicidins are 15 amino acid linear peptides. This contrasts with the associated cyclic peptide gramicidins. Antibiotics known as gramicidins fight gram-positive bacteria (Cash *et al.*, 2010). Like Staphylococcus aureus and Bacillus subtilis (Cash *et al.*, 2010; Cavaletti *et al.*, 2006) but not good enough against gram-negative bacteria like *E. coli*. For sore throat relief and to treat infected wounds, gramicidins are added to lozenges. Numerous antibiotics, such as tyrocidine, are combined with gramicidins likewise antiseptics (Chen *et al.*, 2017). Eye drops for bacterial eye infections also contain gramicidins. They are frequently combined with other antibiotics for drops, such as polymyxin B or neomycin.

Neomycin is an anaminoglycoside (Christos *et al.*, 2021; Flemming and Wuertz, 2019; (Gewaily *et al.*, 2015; Gould, 2016) antibiotic has bactericidal efficacy against certain anaerobic bacteria and gram-negative aerobic bacteria in cases when resistance has not yet developed. Both anaerobic Gram-positive and Gram-negative bacteria are typically resistant to it. Neomycin is available as an eyedrop, cream, ointment and oral formulation. Neomycin is an antibiotic that relates to the category of aminoglycosides,

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which have more than two amino sugars joined using glycosidic linkages. Neomycin is typically applied as a topical medication like Neosporin (neomycin/polymyxin B/bacitracin). The antibiotic may also be used orally, in which case it is typically coupled with other antibiotics. Neomycin has been utilised as a hepatic encephalopathy preventative since it is not absorbed from the digestive tract (Headland and Norling, 2015; Kircheis, 2019; Mohamadi *et al*., 2017; Petersoen and Rogers, 2015). Neomycin reduces hepatic encephalopathy and lowers ammonia levels by killing intestinal bacteria, especially before gastrointestinal surgery.

Synthetic corticosteroid triamcinolone acetonide (Qin *et al*., 2003) medicine used topically to treat different skin disorders (Ran *et al*., 2020; Schlecht *et al*., 2015; Schulz and Jorgensen, 2001) to ease mouth sore discomfort and internally by the procedureists to heal different joint diseases. To treat inflammation, it is additionally administered intralesionally (Tong *et al*., 2015). In several bodily parts, especially the skin (Varma *et al*., 2014). It is utilised to treat allergic rhinitis in nasal spray form (Weingärtner *et al*., 2009). It is a more potent triamcinolone derivative that has an eight times greater potency than prednisone. It is utilised for macular edema associated with uveitis (Wheatley and Togias, 2015).

Experimental

Chemistries and Reagents

Acetonitrile (marked by HPLC), Tri ethyl amine (Mumbai, India's Worli, Merck India Ltd.), provided the water (mark HPLC). APIs of Gramicidin (accuracy 99.8%), Neomycin (accuracy 99.9%) and Triamcinolone acetonide (purity 99.9%) were from Cipla Pharmaceutical Company, Mumbai.

Instrumentation

Empower version 2's chromatographic software was employed. Agilent 1290 Infinity II LC System PDA detector and quaternary pump and utilise 2.0 software were used.

UPLC Requirements

During the chromatographic process, a column of phenyl (50 mm x 2.1 mm, 1.7 µm) with ambient temperature a gradient elution containing 0.1% TEA and the flow rate of acetonitrile was employed as the 0.5 mL/min with injection quantity of 5 µL worked for UPLC. The gradient programme, Table 1 demonstrates.

Standard Preparation of Stock Solution

Accurately weighed out 100 mg of Triamcinolone acetonide, 250 mg of Neomycin and 250 mg of Gramicidin into a 100 mL container sonicated with 70 mL of diluent and the flask is dissolved and made up to the mark with diluent.

Impurity Stock Solution Preparation

Weighed out accurately 5 mg each of TAA Imp-A, TAA Imp-C, TAA Imp-D, Neo Imp-A, Neo Imp-C, Neo Imp-D, Gra Imp-B, Gra Imp-C and 10 mg each of TAA imp-B, Neo Imp-B, Gra Imp-A, Gra Imp-Dinto a 100 mL container and 70 mL diluent is sonicated and dissolved with diluent and made up to the mark.

Making of the standard solution

5 mL of reference stock is taken and 5 mL of impurity is added to stock 50 mL capacity flask, with appropriate use of diluents.

Solution Sample Preparation

The sample solution was prepared through dissolution of 1 of the Triamcinolone acetonide, Neomycin and Gramicidin sample is added to 10 mL of volumetric flask. 7 mL of diluent is added and ultrasonication is carried out 15 min and diluents are centrifuged for 20 min, then diluted up to 10 mL mark with diluents. Lastly, 0.45 filter paper is used to filter the solution. LC column is made with a syringe before injecting.

Validation of Method

By analysing the parameters, the systematic UPLC method was proven with system appropriateness, linearity, accuracy, detection and quantification limits and robustness etc., resulting in the discovery that the outcomes fell within the ICH criteria acceptable range.

System Appropriateness

Using the parameters, we evaluated the system's performance like USP plate count, USP tailing and percentage of related deviation.

Accuracy and linearity

Standard answers to equations for studying linearity with Triamcinolone acetonide, Neomycin and Gramicidin at various dilution levels (25%, 50%, 75%, 100%, 125% and 150%). Three distinct dilution levels-50%, 100% and 150%-were investigated for accuracy. Last but not least, recovery and RSD rates were calculated.

Precision

Three different types of precision exist.

System Accuracy

Reference Standard Solution of Triamcinolone acetonide, Neomycin and Gramicidin % RSD was calculated after six injections.

Method Precision

Six individual determinations of Triamcinolone acetonide (100 µg/mL), Neomycin (250 µg/mL) and Gramicidin (250 µg/mL)

spiked with impurities were injected and calculated % recovery and % RSD.

Intermediate Precision

Six individual determinations of Triamcinolone acetonide (100 µg/mL), Neomycin (250 µg/mL) and Gramicidin (250 µg/mL) spiked with impurities were injected in different days. Then calculated % recovery and % RSD.

Robustness

This method was investigated by altering flowing of ± 10% and phase organic often %.

Stress Deterioration

The peaks found in the forced deterioration preparations chromatograms are not affected by stress degradation. Learnings on stress degradation were carried out in accordance with ICH guidelines Q1 (A) R2. The resolution between the peaks must be at least 1.0 in order for the deterioration peaks to be separated from one another and for the principle peak form to pass.

Acid Discoloration

In acid degradation,1g of sample is taken and transferred into a 10 mL volumetric flask, 7 mL of diluent is added and sonicated to dissolve for 20 min and centrifuged for 20 min. 1 mL of 1N HCl is added into a 10 mL volumetric flask, heated on a water bath at 60°C for 30 min. Allowed to cool to room temperature and neutralized with 1 mL of 1N NaOH. Then made upto the mark with diluents, filtered and injected into UPLC system.

Degradation by Alkalies

In alkali degradation,1 g of sample is taken and transferred into a 10 mL volumetric flask, 7 mL of diluents are added and sonicated to dissolve for 20 min and centrifuged for 20 min. 1 mL of 1 N NaOH add in to a 10 mL volumetric flask heated on a water bath at 60°C for 30 min. It is allowed to cool to room temperature and neutralized with 1 mL of 1N HCl. Then made up to the mark with diluents, filtered and injected into UPLC system.

Degradation of Peroxide

In peroxide degradation,1 g of sample is taken and transmitted into a 10 mL volumetric flask, 7 mL of diluents are added and ultrasonicated to dissolve for 20 min and centrifuged for 20 min. 1 mL of 30% hydrogen peroxide into a 10 mL volumetric flask heated on a water bath at 60°C for 30 min. Confirmed to cool to room temperature. Then build up to the mark with diluents, filtered and injected into UPLC system.

Degradation Reduction

In reduction degradation, 1 g of sample is conveyed into a 10 mL vacuum flask, 7 mL of diluents are added and accelerated to dissolve for 20 min and centrifuged for 20 min. 1 mL of 10% sodium bisulphate solution into a 10 mL thermos flask heated on a water bath at 60°C for 30 min. Approved to cool to room temperature. Then made up to the mark with diluents, filtered and injected into UPLC system.

Temperature Degradation

In thermal degradation, 2 g sample is held in a petri-dish and exposed to dry heat at 105°C for 6 hr. 1 g of sample is transported into a 10 mL vessel, added 7 mL of diluents and ultrasonicated to dissolve for 20 min and centrifuged for 20 min, then made up to the mark with diluents, filtered and injected into UPLC system.

Degradation by Photolysis

In photolytic degradation, tablets are crushed finely to powder form and 2 g of sample is exposed to photo light UV 200W-hrs and fluorescence light of 1.2 million lux-hours. 1 g of sample is transferred into a 10 mL hip flask, added 7 mL of diluents and sonicated to dissolve for 20 min and centrifuged for 20 min, then made up to the mark with diluents, purified and implanted into UPLC system.

RESULTS AND DISCUSSION

Agradientelution of Triamcinolone acetonide, Neomycin and Gramicidin involves phenyl column that flows from 0.5 mL/min and the column's internal temperature were held constant.

Mobile phase % TEA likewise acetonitrile was utilised. UV monitoring was brought at 230 nm.

System Appropriacy

To establish a stable baseline, the UPLC system was stabilized for 60 min. In accordance with the test procedure, a standard solution

Table 1: Gradient Programme Specifications.

Time (min)	Acetonitrile	Triethylamine (0.1%)
0	50	80
3	50	50
5	80	20
8	80	20
10	20	80
12	20	80

was injected six times into the UPLC apparatus. By analyzing the data and visualizing the results, it was determined that all of the system suitability parameters fell inside acceptable ranges. The standard chromatogram is seen in Figure 1.

Specificity

An aptitude for assess an indicator reaction when there are contaminants is referred to as specificity in analytical methods. Impurities were present during the testing of the specificity of the suggested method for the active component. The injected standard, placebo and blank solutions and chromatograms were recorded by the apparatus. The method was specific and all the peaks were determined to be pure when they were present together and placebo had no effect on the main peak. Figure 2 shows the blank chromatogram.

Linearity

The new test method's linearity was demonstrated by preparing collection of linearity answers that Triamcinolone acetonide, Neomycin and Gramicidin and their related six concentrations of impurities ranging from Triamcinolone acetonide 25-150 g/mL, Neomycin 62.5-375g/mL, 1.25-7.5 g/mL of TAA Imp-A, TAA Imp-C, TAA Imp-D, Neo Imp-A, Neo Imp-C, Neo Imp-D, Gra Imp-B, Gra Imp-C and 2.5-15 µg/mL of TAA Imp-B, Neo Imp-B, Gra Imp-A which covered 25-150% of target pumped into UPLC (Figure 3) throughout the concentration series of the calibration curves, Triamcinolone acetonide, Neomycin and Gramicidin and their related impurities. The linearity values were listed in Table2. Correlation coefficient values were 0.999.

Accuracy

Recovery investigations, which were conducted at three different dilution percentages (50%, 100% and 150%). The assay was carried out after each spike level's three preparations of the test solutions were injected, as per the test method. Table 3 shows the results, which showed that the share observed recovery values were between 98% and 102%.

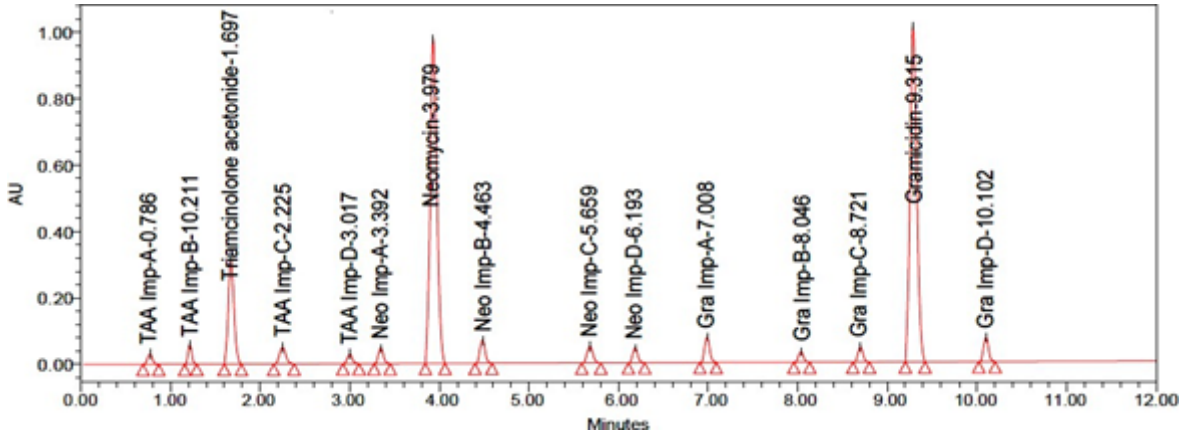


Figure 1: Standard chromatogram.

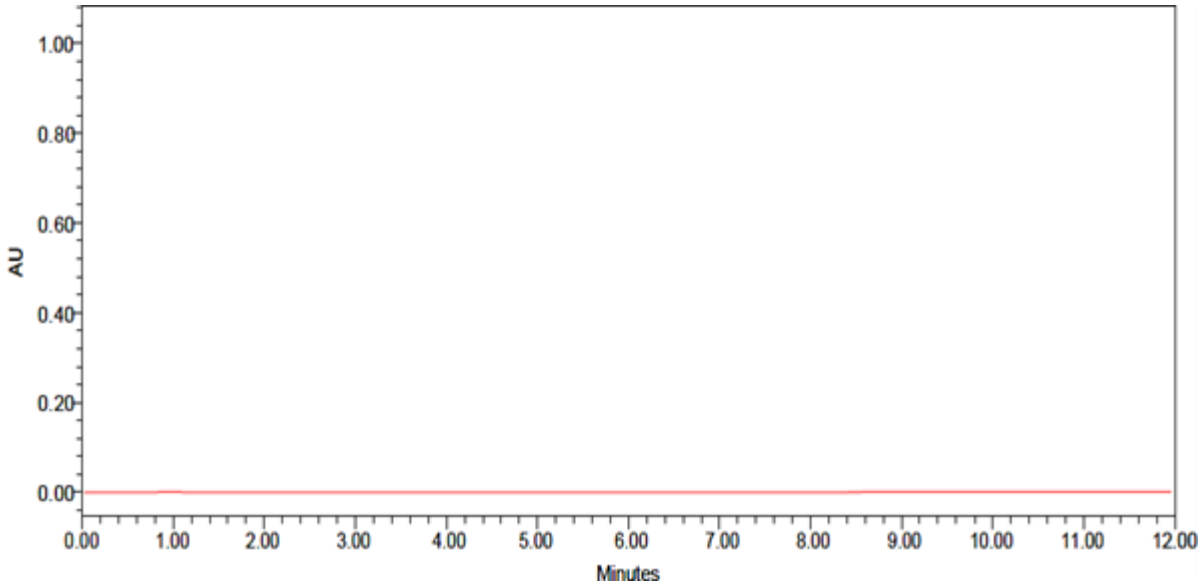


Figure 2: Blank chromatograms.

Table2: UPLC results of Linearity of (A) Triamcinolone acetonide (B) Neomycin (C) Gramicidin.

A

Sl. No.	Triamcinolone acetonide		Impurity-A		Impurity-B		Impurity-C		Impurity-D	
	Conc	Region	Conc	Region	Conc	Region	Conc	Region	Conc	Region
1	25	2606514	1.25	102320	2.50	115274	1.25	98549	1.25	86524
2	50	5298754	2.50	202621	5.00	225402	2.50	196529	2.50	186359
3	75	7720561	3.75	302185	7.50	336528	3.75	288652	3.75	272546
4	100	10502364	5.00	402145	10.00	442547	5.00	392341	5.00	368421
5	125	12874157	6.25	498651	12.50	548248	6.25	485204	6.25	448532
6	150	14889585	7.50	604156	15.00	677814	7.50	585325	7.50	542513
Slope	100582.36		80132.97		44521.93		77859.91		72389.06	
Intercept	155170.96		1226.79		1201.68		396.75		668.89	
CC	0.9992		0.9999		0.9997		0.9999		0.9997	

B

Sl. No.	Neomycin		Impurity-A		Impurity-B		Impurity-C		Impurity-D	
	Conc	Region	Conc	Region	Conc	Region	Conc	Region	Conc	Region
1	62.5	6582431	1.25	165421	2.5	175846	1.25	154725	1.25	154213
2	125.0	13625414	2.50	310254	5.0	342513	2.50	306254	2.50	302510
3	187.5	19254715	3.75	479586	7.5	510230	3.75	456285	3.75	458721
4	250.0	26743892	5.00	621829	10.0	684257	5.00	614251	5.00	605537
5	312.5	32602103	6.25	783021	12.5	847612	6.25	752355	6.25	756924
6	375.0	39154614	7.50	936254	15.0	983652	7.50	909523	7.50	892523
Slope	104355.24		124443.91		66231.89		120909.31		119600.51	
Intercept	142417.29		4244.61		9562.29		2789.07		4416.36	
CC	0.9997		0.9998		0.9995		0.9999		0.9998	

C

Sl. No.	Gramicidin		Impurity-A		Impurity-B		Impurity-C		Impurity-D	
	Conc	Region	Conc	Region	Conc	Region	Conc	Region	Conc	Region
1	62.5	6695642	2.5	175642	1.25	160236	1.25	172354	2.5	178452
2	125.0	14521035	5.0	350213	2.50	318457	2.50	330215	5.0	360451
3	187.5	21302148	7.5	521036	3.75	467451	3.75	502639	7.5	536249
4	250.0	27115496	10.0	701162	5.00	652233	5.00	663854	10.0	721360
5	312.5	34623147	12.5	866523	6.25	782365	6.25	831542	12.5	896325
6	375.0	41052346	15.0	1023602	7.50	943512	7.50	975234	15.0	1044572
Slope	109489.43		68621.67		125959.14		130791.91		70433.87	
Intercept	229276.04		5077.18		2546.64		6078.61		5661.54	
CC	0.9995		0.9998		0.9996		0.9997		0.9996	

Table3: UPLC results of Accuracy of (A) Triamcinolone acetonide (B) Neomycin (C) Gramicidin.

A

Parameters	TAA	Imp-A	Imp-B	Imp-C	Imp-D
Accuracy[a] (% Recovery)					
50% Mean, % RSD	100.2,0.62	100.1,0.47	100.3,0.64	100.7, 0.51	100.3,1.29
100% Mean, % RSD	100.9,0.37	100.5,1.59	100.9,0.37	99.1,0.35	99.7,0.47
150% Mean, % RSD	99.3,1.24	99.3,0.22	99.9,1.11	100.9,0.27	100.2,0.86

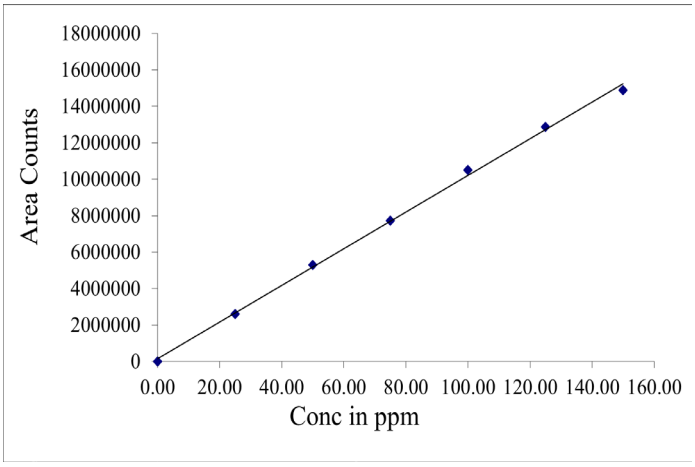
B

Parameters	Neomycin	Imp-A	Imp-B	Imp-C	Imp-D
Accuracy[a] (% Recovery)					
50% Mean, % RSD	99.5,0.46	98.6,0.64	100.5,0.82	99.5,0.52	100.8,1.08
100% Mean, % RSD	99.4,0.84	100.4,1.11	100.4,0.63	99.7,0.37	99.2,0.26
150% Mean, % RSD	98.3,1.22	99.9,0.76	99.1,1.27	100.1,0.66	100.7,0.85

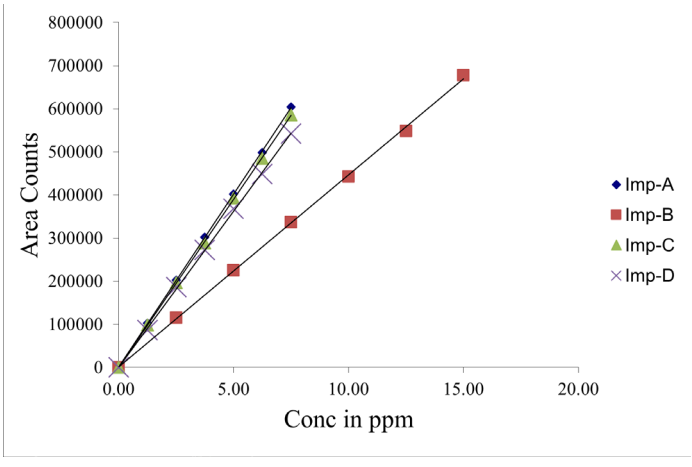
C

Parameters	Gramicidin	Imp-A	Imp-B	Imp-C	Imp-D
Accuracy[a] (% Recovery)					
50% Mean, % RSD	100.2,0.63	100.8,0.69	99.6,0.95	100.2, 0.41	98.8,1.07
100% Mean, % RSD	100.1,0.87	98.7,1.52	100.4,0.46	99.3,0.57	99.2,0.88
150% Mean, % RSD	99.7,1.24	99.2,0.43	99.7,1.38	100.5, 0.86	100.6,0.49

[a]Average of three determinations of each concentration level TAA means Triamcinolone acetonide.



A



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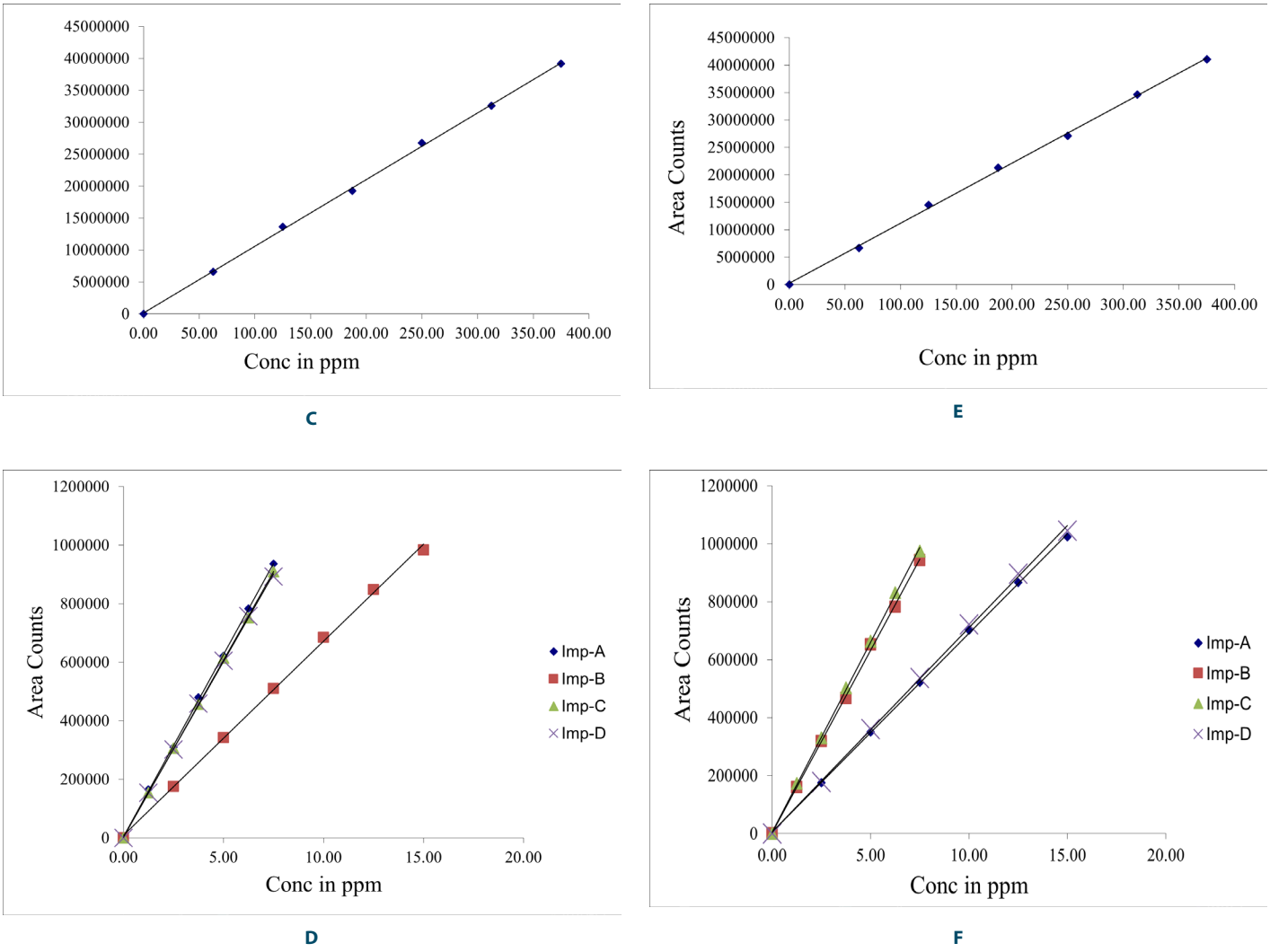


Figure 3: Calibrationplots of (A) Triamcinolone acetonide (B) Triamcinolone acetonide impurities (C) Neomycin (D) Neomycin impurities (E) Gramicidin (F) Gramicidin impurities.

Table 4: UPLC results of Precision of (A) Triamcinolone acetonide (B) Neomycin (C) Gramicidin.

A

Parameters	TAA	Imp-A	Imp-B	Imp-C	Imp-D
		Precision[a] (% RSD)			
System precision	1.26	0.68	0.84	0.96	0.48
Method Precision	0.96	0.75	0.26	1.14	0.72
Intermediate Precision	1.11	0.49	0.85	0.66	0.81

B

Parameters	Neomycin	Imp-A	Imp-B	Imp-C	Imp-D
		Precision[a] (% RSD)			
System Precision	0.92	0.86	0.51	1.26	0.67
Method Precision	1.34	0.74	0.43	1.17	0.76
Intermediate Precision	0.83	0.22	0.96	0.87	0.99

C

Parameters	Gramicidin	Imp-A	Imp-B	Imp-C	Imp-D
Precision[a] (% RSD)					
System Precision	0.84	0.38	0.84	0.49	0.73
Method Precision	0.63	0.26	0.72	1.15	0.64
Intermediate Precision	1.19	0.54	0.67	0.72	0.96

[a]RSD of six determinations of each component.

Table 5: UPLC results of Robustness of (A) Triamcinolone acetonide (B) Neomycin (C) Gramicidin.

A

Parameters	TAA	Imp-A	Imp-B	Imp-C	Imp-D
Precision[a] (% RSD)					
Flow Minus (0.45 mL/min)	0.76	1.64	1.45	0.83	0.71
Flow Plus (0.55 mL/min)	0.48	0.59	0.81	0.61	0.43
OrgPhase (-10%)	0.91	0.85	0.64	0.72	0.53
OrgPhase (+10%)	0.84	0.76	0.43	0.28	0.54

B

Parameters	Neomycin	Imp-A	Imp-B	Imp-C	Imp-D
Precision[a] (% RSD)					
Flow Minus (0.45 mL/min)	1.43	1.11	0.84	0.76	1.05
Flow Plus (0.55 mL/min)	1.14	0.87	0.63	0.82	0.95
OrgPhase (-10%)	0.71	0.43	0.84	0.43	0.79
OrgPhase (+10%)	0.38	0.91	0.72	0.48	0.68

C

Parameters	Gramicidin	Imp-A	Imp-B	Imp-C	Imp-D
Precision[a] (% RSD)					
Flow Minus (0.45 mL/min)	0.85	0.77	0.94	0.15	0.67
Flow Plus (0.55 mL/min)	0.38	0.49	0.76	0.18	0.49
OrgPhase (-10%)	0.98	1.16	0.84	0.73	1.08
OrgPhase (+10%)	1.55	0.89	0.53	0.48	0.97

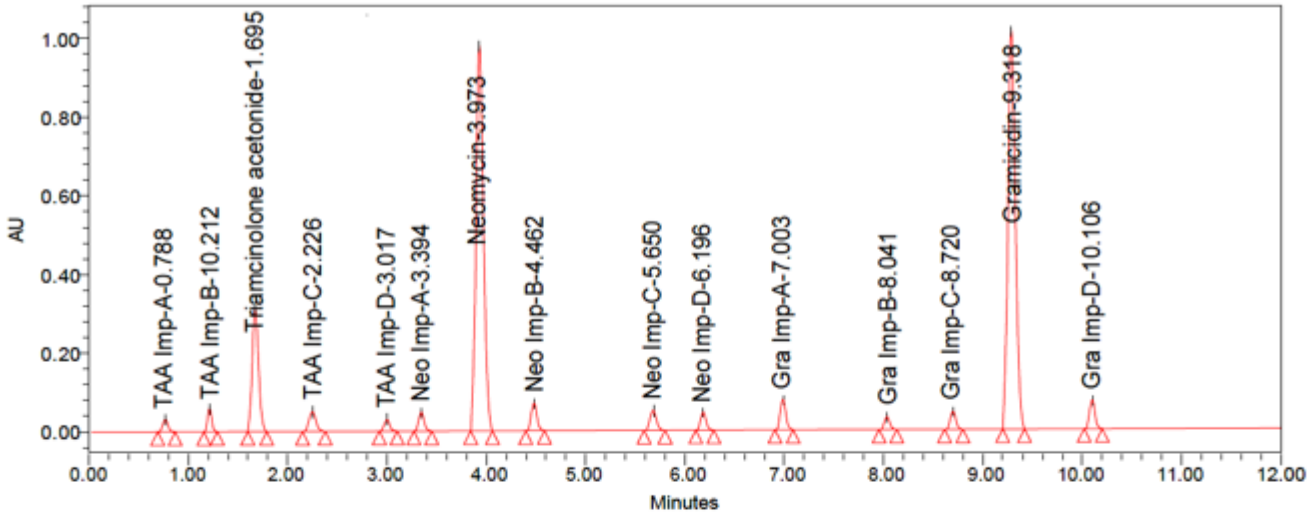
[a]RSD of six determinations of each component.

Precision

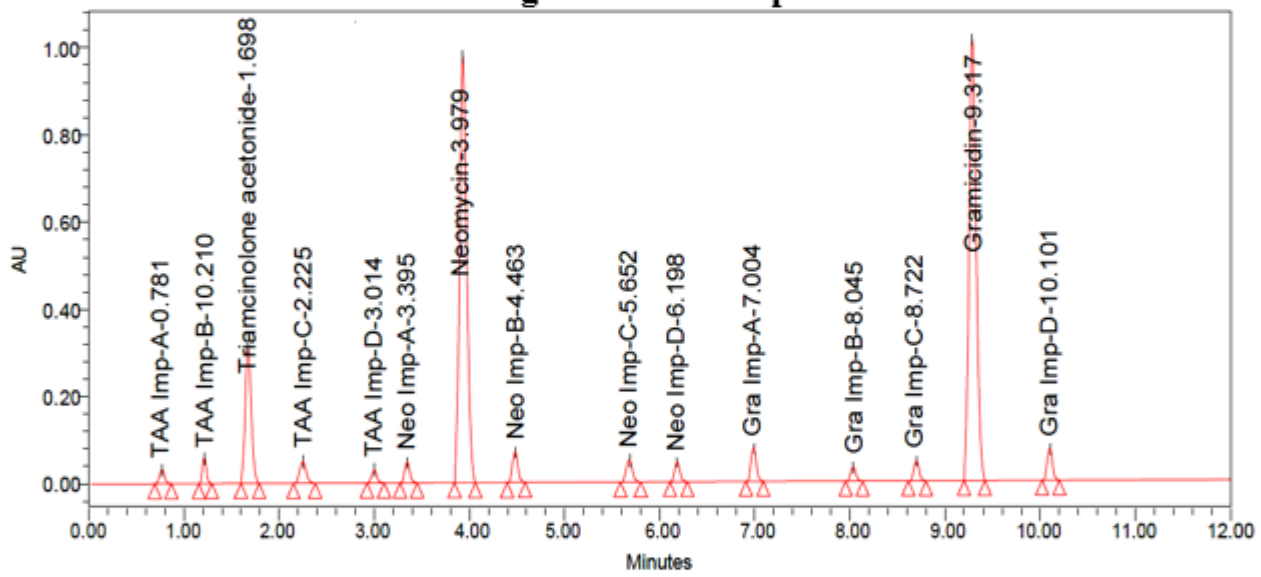
The analysis accuracy was evaluated according to its methodology intermediate variants. In order to compute the intraday studies, six individual example responses equivalent day under identical experimental circumstances. By conducting the analysis on various days, intermediate accuracy of the technique was applied inside the identical lab. The strategy was quite accurate, with RSD values determined to be around 2%. The strategy worked well, as evidenced by the high recovery rates (98 to 102%) of targeted medications. The outcome is furnished in Figure 4. Table 4 shows the results are within acceptable range as per ICH guidelines.

Robustness

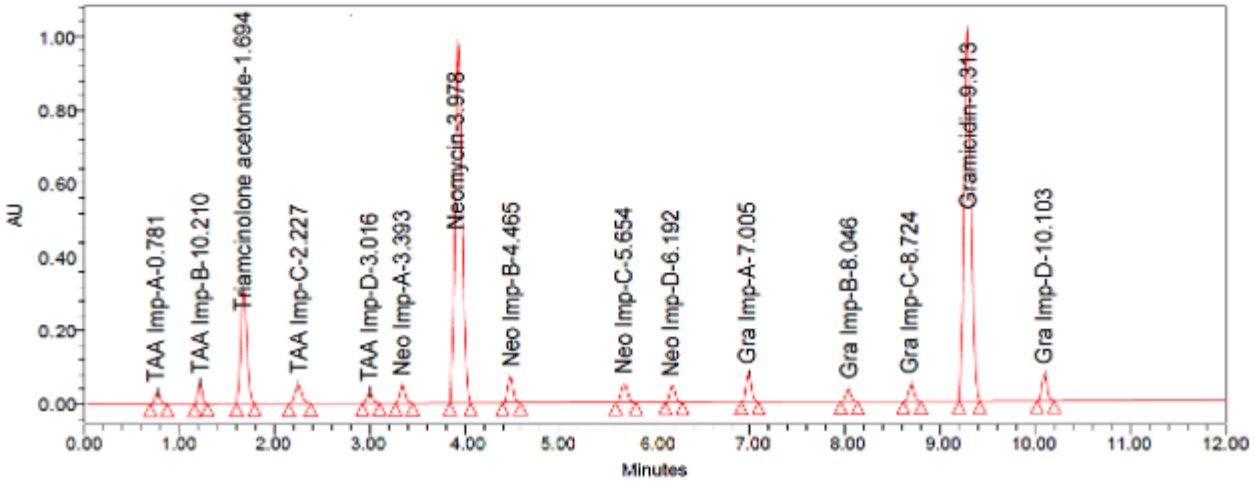
Deliberate modifications to the technique parameters, such as a change in flow, were done in accordance with ICH standards. (±10%) and in the mobile phase, organic content (10%). Therefore, there is no technique capability to remain untouched by system appropriateness. Table 5 Indicates the effectiveness of the strategy was assessed by looking at the effects of the altered



Chromatogram of method precision



System suitability chromatogram



Chromatogram of Intermediate precision

Figure 4: Chromatogram of method precision, System suitability chromatogram and Chromatogram of Intermediate precision.

Table 6: UPLC results of FD of (A) Triamcinolone acetonide (B) Neomycin (C) Gramicidin.

A

Degradation Parameter	%Total impurities of TAA	Mass balance	Purity angle	Purity Threshold
Acid deg	4.74	95.77	0.964	1.745
Alkali deg	4.86	95.83	0.932	1.764
Peroxide deg	4.88	95.16	0.984	1.739
Reduction deg	0.76	98.67	0.965	1.752
Thermal deg	0.88	98.13	0.973	1.776
Photo deg	0.97	98.82	0.925	1.724
Hydrolysis deg	0.64	99.04	0.968	1.763

B

Degradation Parameter	%Total impurities of Neomycin	Mass balance	Purity angle	Purity Threshold
Reduction deg	1.64	98.23	1.574	5.424
Thermal deg	1.11	98.47	1.578	5.438
Photo deg	0.84	98.52	1.516	5.415
Hydrolysis deg	0.51	99.46	1.524	5.449

C

Degradation Parameter	%Total impurities of Gramicidin	Mass balance	Purity angle	Purity Threshold
Acid deg	10.42	90.51	1.968	7.324
Alkali deg	10.37	90.26	1.948	7.316
Peroxide deg	10.66	90.38	1.976	7.354
Reduction deg	2.84	98.32	1.925	7.362
Thermal deg	1.72	98.45	1.949	7.384
Photo deg	0.93	98.37	1.986	7.339
Hydrolysis deg	0.88	99.12	1.994	7.316

parameters for content %, tailing factor and retention time while utilising UPLC. The level of dependability of the outcomes that were acquired by making incremental, intentional changes had demonstrated the effectiveness of the strategy.

Forced Degradation studies

Thermodynamic, forced degradation experiments in basic, acidic, oxidative, photolytic and reductive environments were all conducted in accordance with ICH stability guidelines. Performed employing the illustrative brand name Trim (contains 0.1% of Triamcinolone acetonide, 0.25% Neomycin and 0.25% Gramicidin). Table 6 represents the deterioration of Triamcinolone acetonide, Neomycin and Gramicidin.

CONCLUSION

This study created an entirely new, straightforward, quick, affordable, sensitive and easily accessible UPLC method for the coincident determination of Triamcinolone acetonide, Neomycin and Gramicidin bulky and ointment the dose form. This method has the benefits of being less expensive, assessible, reliable, sensitive and reproducible. Under conditions of oxidation, (Neutral, basic and acidic) hydrolysis, photolysis, thermal stress and medicines degradative activities were investigated. The medications were discovered to be solid under heat, unstable, hydrolysis under acid, alkaline and oxidative circumstances. The final application of the UPLC method to the commercial formulations followed the ICH recommendations.

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CONFLICT OF INTEREST

The writers affirm the absence of any conflicts of interest.

ABBREVIATIONS

UPLC: Ultra-performance liquid chromatography; **ICH:** International Council for Harmonization.

AUTHOR'S CONTRIBUTIONS

Author Madhavi N designed the study, authors Ibrahim Baje S developed and validated the methodology, drafted the first protocol draught of the document and the literature searches. The final manuscript was read by all authors and got their approval.

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