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RP-HPLC Method Development and Validation for Simultaneous Determination of Loteprednol Etabonate and Gatifloxacin in Bulk and its Pharmaceutical Dosage Form

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Abstract: *The simultaneous measurement of loteprednol etabonate (LOTE) and Gatifloxacin (GATI) in bulk and pharmaceutical dose forms was accomplished through the development and validation of a straightforward, quick, accurate, and precise reversed-phase high-performance liquid chromatographic (RP-HPLC) method. A Grace C18 column (4.6 × 250 mm, 5 µm) was used for the chromatographic separation. Acetonitrile and phosphate buffer (65:35, pH 4) were used as the isocratic mobile phase, and the flow rate was set at 1.0 mL/min. The wavelength of detection was 271 nm. It was discovered that the retention durations for GATI and LOTE were 4.21 and 7.13 minutes, respectively. With recovery percentages ranging from 98 to 102%, the approach demonstrated good accuracy and high precision, with relative standard deviation (RSD) values < 2%. The LOTE and GATI had respective limits of detection (LOD) and quantification (LOQ) of 0.981 µg/mL and 2.78 µg/mL and 0.865 µg/mL and 1.68 µg/mL. The technique demonstrated specificity, robustness, and reproducibility after being validated in accordance with ICH criteria. As such, it can be used for routine quality control analysis of LOTE and GATI in combination ophthalmic formulations. Simple, economical, and quick analysis without interference from excipients or degradation products are benefits of the developed method.*

Keywords: Gatifloxacin, Loteprednol etabonate, RP-HPLC, Validation

I. INTRODUCTION

Gatifloxacin (GATI) is a powerful antibiotic belonging to the fourth-generation fluoroquinolone family. Chemically known as 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid, it is widely recognized for its broad-spectrum antibacterial properties[1]. It holds an official place in the Indian Pharmacopoeia(2), which lists HPLC(High-Performance Liquid Chromatography) methods for its measurement. Over the years, researchers have explored a range of analytical techniques to estimate GATI—these include UV spectroscopy[3,4,5,6], HPLC[7,8,9,10,11],HPTLC[12,13], colorimetric methods[14], and spectrofluorimetry[15]both in its pure form and when combined with other drugs.(Figure 1).

On the other hand, Loteprednol Etabonate (LOTE) is a corticosteroid commonly used in eye medications. Its chemical name is quite a mouthful—chloromethyl 17-ethoxycarbonyloxy-11-hydroxy-10,13-dimethyl-3-oxo-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-17-carboxylate[16]—but essentially, it's known for its anti-inflammatory effects. Unlike GATI, LOTE isn't officially listed in any standard pharmacopoeia yet. However, various studies have reported HPLC[17,18,19] methods to estimate LOTE alone, along with its degraded products, and in combination with other drugs.(Figure 2).

Despite the extensive research on both drugs individually, there has been no validated RP-HPLC method available so far for estimating Gatifloxacin and Loteprednol Etabonate together in a single ophthalmic formulation. The present study aims to fill this gap by developing and validating a simple, precise, accurate, robust, and economical RP-HPLC

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method for their simultaneous estimation—following the guidelines set by the International Council for Harmonisation (ICH).

II. MATEIALS AND METHODS

Chemical, reagents and solution

Free samples of Loteprednol Etabonate (LOTE) and Gatifloxacin (GATI) were generously provided by Swapnroop Drugs & Pharmaceuticals, India. For the study, the marketed formulation—Lotegate eye drops—was purchased from a local pharmacy. High-purity HPLC grade Methanol, Acetonitrile, and Water were sourced from Merck Ltd., India, ensuring the reliability of the analysis.

To prepare the stock solutions, 10 mg each of LOTE and GATI were accurately weighed and dissolved in 10 mL of Methanol, creating concentrated standard solutions. These were then diluted further using Methanol to achieve working concentrations of 50 µg/mL for LOTE and 30 µg/mL for GATI.

The wavelength at which the drug mixture showed maximum absorbance (λmax) was identified using a Shimadzu 1800 UV spectrophotometer, scanning across the range of 200 to 400 nm. Methanol was used as the blank solution during this process. The combined solution of LOTE and GATI displayed a peak absorbance at approximately 271 nm.

HPLC instrumentation and chromatographic conditions

After identifying the maximum absorbance (λmax) of the drug mixture at 271 nm, this wavelength was chosen for further chromatographic analysis. The separation of the two compounds—LOTE and GATI—was carried out using a Grace C18 column (4.6 × 250 mm, 5 µm particle size) in an isocratic system, with detection done through a UV detector.

To ensure accurate results, standard solutions containing both drugs were prepared and analyzed. Several combinations of solvents were tested to find the most suitable mobile phase that could provide clear, stable, and well-resolved peaks for both LOTE and GATI. Before using, each mobile phase was carefully filtered through Whatman No. 42 filter paper to remove any impurities.

The optimal separation was achieved using Acetonitrile: Phosphate buffer (65:35, pH 4 adjusted with ortho-phosphoric acid) at a flow rate of 1.0 mL/min, which provided sharp, symmetrical peaks with reproducible retention times of 7.13 min for LOTE and 4.21 min for GATI (Figures 3-). The selected conditions ensured baseline separation with no interference from excipients or degradation products.

System suitability test

System suitability testing is an essential requirement in pharmacopoeial analysis. It helps ensure that the chromatographic system is performing well enough to deliver reliable and consistent results. To confirm this, data was collected from five repeated injections of the standard solution, allowing assessment of the system's resolution, accuracy, and reproducibility before proceeding with the actual analysis. Table No.1

Analysis of standard laboratory mixture and marketed formulation to see feasibility of the proposed methods preparation of laboratory mixture (standard and sample)

Standard solutions of Loteprednol Etabonate (LOTE) and Gatifloxacin (GATI) were accurately prepared and thoroughly mixed. In the same way, sample solutions were formulated in the laboratory to reflect the same concentration ratio as found in the marketed product. The concentrations of both drugs were then determined by comparing the peak areas of the standard and sample mixtures. The results obtained from the laboratory-prepared mixtures and the commercial formulation were recorded and compared, as presented in Table No. 2.

Method validation

The developed analytical method was thoroughly validated according to ICH guidelines[20], ensuring it met all required parameters such as accuracy, precision, specificity, and robustness.

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Linearity and range:
Following the guidelines of the United States Pharmacopeia (USP), tablet powder equivalent to 80%, 90%, 100%, 110%, and 120% of the labeled amount was accurately weighed, dissolved, and appropriately diluted using the mobile phase. This helped create solutions within the 80% to 120% range of the target test concentration. Chromatograms of these prepared solutions were recorded, and the results showed that both LOTE and GATI demonstrated good linearity across this concentration range, confirming the method's reliability for analyzing varying strengths of the marketed formulation.. The plot showing linearity and range study for LOTE and GATI is shown in the Fig. No. 6 and 7.

Precision
The precision of an analytical method reflects how consistently it produces similar results and is usually expressed as the standard deviation (SD) or relative standard deviation (RSD) of a series of measurements. In this study, precision was evaluated by repeatedly estimating the drug concentrations using the proposed method to ensure its reliability and reproducibility.

Accuracy
It was confirmed through recovery studies carried out using the standard addition method, where known amounts of the drugs were added to the sample to assess how accurately the method could recover the added quantities. This helped verify the accuracy and reliability of the proposed analytical method.

Robustness
To assess the robustness of the method, the chromatographic conditions were intentionally varied to see how small changes might affect the results. Specifically, factors like flow rate and pH were adjusted, and the separation between LOTE and GATI was closely monitored. These variations helped ensure that the method remained reliable and consistent even under slightly altered conditions, as summarized in Table No. 3.

Ruggedness
The studies of ruggedness were carried out under two different conditions-
1) Days (Interday & Intraday)
2) Different Analyst. The summary of result shows in table no 3.

Specificity
Specificity was evaluated by assessing how well the proposed method could distinctly separate the peaks of LOTE and GATI, ensuring there was no interference from other components present in the sample matrix. Mean retention time for
LOTE – 7.127
GATI – 4.213
The values obtained were very similar to those from the standard laboratory mixture, indicating that there was no interference from other components in the sample matrix.

III. RESULT

A simple, reliable, and reproducible RP-HPLC method was successfully developed and validated in accordance with ICH guidelines for the simultaneous estimation of Loteprednol Etabonate (LOTE) and Gatifloxacin (GATI). The chromatographic separation was carried out using a Grace C18 column (4.6 × 250 mm, 5 µm) with a detection wavelength set at 271 nm. An isocratic mobile phase consisting of acetonitrile and phosphate buffer (65:35, pH adjusted to 4.0) was used at a flow rate of 1.0 mL/min. Under these optimized conditions, the retention times were recorded as 7.13 minutes for LOTE and 4.21 minutes for GATI, indicating clear and efficient separation of both compounds. Linearity studies demonstrated excellent performance, as confirmed by a strong correlation between peak area and concentration, with correlation coefficients (R^2) of 0.9997 for Loteprednol Etabonate (LOTE) and 0.999 for

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Gatifloxacin (GATI). Precision tests revealed outstanding reproducibility, with relative standard deviation (RSD) values of ≤ 0.3% for both intra-day and inter-day measurements. Accuracy was validated through recovery studies, showing mean recovery rates between 100.33% and 100.34%, with RSD values not exceeding 0.8%. The method also proved to be highly specific, with no interference observed from excipients or degradation products. Additionally, the method remained robust even when subjected to small variations in parameters such as wavelength, flow rate, and mobile phase composition.

IV. DISCUSSION

Nowadays, fixed-dose drug combinations are commonly used in clinical practice to enhance therapeutic effectiveness and simplify treatment regimens. As a result, there is a growing need for analysts to develop suitable and reliable methods for their accurate analysis. One such combination—Loteprednol Etabonate and Gatifloxacin—has recently become available in the market as an ophthalmic dosage form. This combination is used to treat a range of eye conditions, and its proper analysis is essential to ensure quality, safety, and efficacy.
The literature reported that following methods of analysis for the individual drugs.

Loteprednol Etabonate

Analytical methods reported for Loteprednol Etabonate include High-Performance Liquid Chromatographic (HPLC) methods when used in combination with other drugs, as well as visible spectrophotometric methods. These techniques have been utilized for quantifying the drug in various formulations and assessing its stability and compatibility with other compounds

Gatifloxacin

High-Performance Liquid Chromatographic (HPLC) methods have been well-documented for the analysis of Gatifloxacin (GATI) as a single component. Additionally, stereoselective analyses of GATI in human plasma have been reported, primarily aimed at understanding its pharmacokinetic behavior. However, despite the availability of individual methods, no validated method has yet been documented for the simultaneous estimation of Gatifloxacin and Loteprednol Etabonate (LOTE) in combined dosage forms.
he chromatographic separation was successfully carried out using a Grace C18 column (4.6 × 250 mm, 5 µm) with a detection wavelength set at 271 nm. An isocratic mobile phase consisting of acetonitrile and phosphate buffer in a 65:35 ratio (pH adjusted to 4.0 using ortho-phosphoric acid) was used, flowing at a rate of 1.0 mL/min. Under these conditions, the retention times were observed to be 7.13 minutes for Loteprednol Etabonate (LOTE) and 4.21 minutes for Gatifloxacin (GATI), as shown in Figures 8 to 10.
Loteprednol Etabonate (LOTE), a corticosteroid, and Gatifloxacin (GATI), a fluoroquinolone antibiotic, are often combined in ophthalmic solutions like Lotegate Eye Drops. This combination works synergistically to treat a variety of eye conditions, including inflammation, infections, post-surgical complications, and allergic conjunctivitis.
The analytical method developed for their simultaneous estimation is straightforward, sensitive, reliable, and cost-effective. It was thoroughly validated in accordance with ICH guidelines to ensure its suitability for routine quality control and analysis.

- Accuracy (recovery: 98–102%),
- Precision (RSD < 2%),
- Specificity (no matrix interference),
- Linearity (5–50 µg/mL for LOTE; 3–30 µg/mL for GATI; $R^2 > 0.999$),
- Robustness (stable under variable conditions),
- LOD/LOQ (0.865–2.78 µg/mL).

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This method offers a robust quality-control tool for pharmaceutical analysis of LOTE-GATI combinations.[1]

Based on the findings, it can be concluded that the RP-HPLC technique is well-suited for the simultaneous estimation of Loteprednol Etabonate (LOTE) and Gatifloxacin (GATI) in their combined tablet formulations. The method demonstrated excellent reproducibility and met all key validation parameters—proving to be accurate, precise, specific, sensitive, and reliable.

The analysis showed no interference from excipients, additives, or the sample matrix, confirming the method's specificity. This makes it a strong candidate for routine quality control of combined formulations. Further research involving other pharmaceutical dosage forms could provide additional insights and broaden the applicability of this method.

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Sr. No	Peakarea		Retention Time		Asymmetry		Efficiency	
	LOTE	GATI	LOTE	GATI	LOTE	GATI	LOTE	GATI
1	205678.9	181956.2	7.127	4.211	2.461	2.861	198801.2	166860.3
2	205561.3	181937.1	7.129	4.213	2.465	2.859	198791.5	166899.1
3	205704.7	181996.5	7.131	4.217	2.473	2.876	198709.7	166877.5
4	205631.1	181876.4	7.123	4.219	2.462	2.869	198819.5	166798.2
5	205678.9	181765.9	7.122	4.213	2.463	2.863	198809.8	166901.8
Mean	205650.98	181906.42	7.1264	4.2146	2.4648	2.8656	198786.34	166867.38
±S.D	56.750788	89.695022	0.00384	0.00328	0.00481	0.00691	44.076898	42.217614
C.V	0.0002759	0.0004930	0.00053	0.00077	0.00195	0.00241	0.0002217	0.0002530

Table No.1: Result of System Suitability Study

Table No.2 : Summaryoflaboratorymixtureandmarketedformulationanalysisby RP-HPLC Method

Sr. no.	Sample	Statisticaldata	%Estimation		% Recovery	
			LOTE	GATI	LOTE	GATI
1.	StandardLaboratory mixture	Mean	100.00	100.13	-	-
		S.D.	0.361	0.208	-	-
		C.V.	0.004	0.002	-	-
2.	Lotegate	Mean	101.17	100.47	100.33	100.34
		S.D.	0.306	1.012	0.758	0.482
		C.V.	0.003	0.010	0.008	0.005

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Table no.3: Summary of validation parameter

Sr.No.	Parameter	Value(CV)	
		LOTE	GATI
1	Specificity	No interference	
2	Precision	0.002	0.003
3	Accuracy	0.008	0.005
4	Intraday	0.002	0.003
5	Interday	0.003	0.003
6	DifferentAnalyst	0.001920082	0.004701

Table No.4 : Results and statistical data for Recovery study of LOTE and GATI

Sr. No.	wt. of formulation	Amount of DrugAdded in (µg/ml).		PeakAreaof stand.		PeakAreaof sample		% Recovery	
		LOTE	GATI	LOTE	GATI	LOTE	GATI	LOTE	GATI
1	1010	1	1	205678.9	181956.2	204444.8	181228.4	99.4	99.6
2		1	1			205267.5	182138.2	99.8	100.1
3		1	1			205061.9	182320.1	99.7	100.2
4		2	2			204856.2	181774.2	99.6	99.9
5		2	2			207324.3	182502.1	100.8	100.3
6		2	2			207530.0	182684.0	100.9	100.4
7		3	3			207941.4	182866.0	101.1	100.5
8		3	3			208764.1	183775.8	101.5	101
9		3	3			206090.3	183957.7	100.2	101.1
						Mean		100.33	100.34
						S.D.		0.758	0.482
						C.V		0.008	0.005

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Table No.5 : Summary of Linearity and range study for LOTE and GATI

Table No.1: Result of System Suitability Study .No.	%Labelclaim	Peakarea	
		LOTE	GATI
1	80	164543.12	145564.96
2	90	185111.01	163760.58
3	100	205678.9	181956.2
4	110	224246.79	202151.82
5	120	246814.68	218347.44

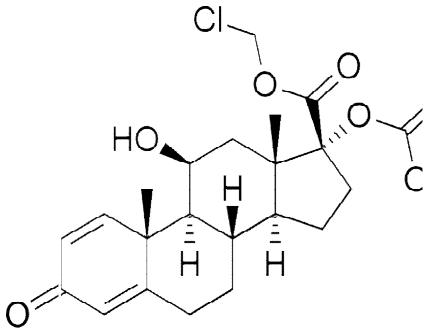

Fig 1: Structure of Loteprednol etabonate

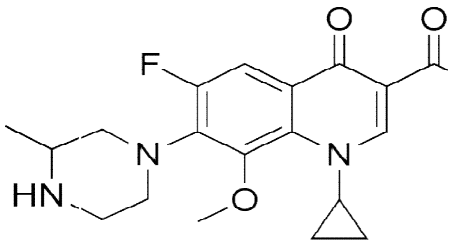

Fig 2: Structure of Gatifloxacin

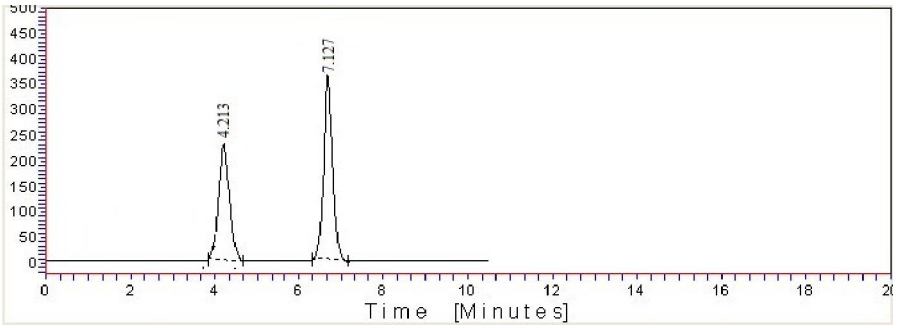

Fig. No.3 Chromatogram obtained by using Acetonitrile: Phosphatebuffer (65:35) pH mobile phase

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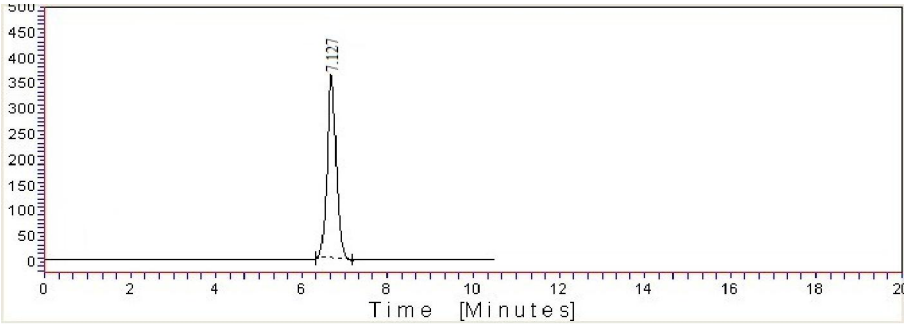

Fig. 4: Chromatogram of Loteprednol etabonate (RT- 7.127)

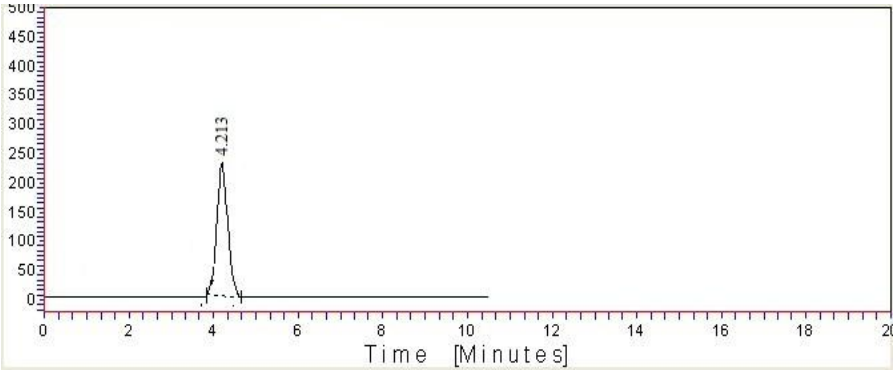

Fig. 5: Chromatogram of Gatifloxacin (RT- 4.213)

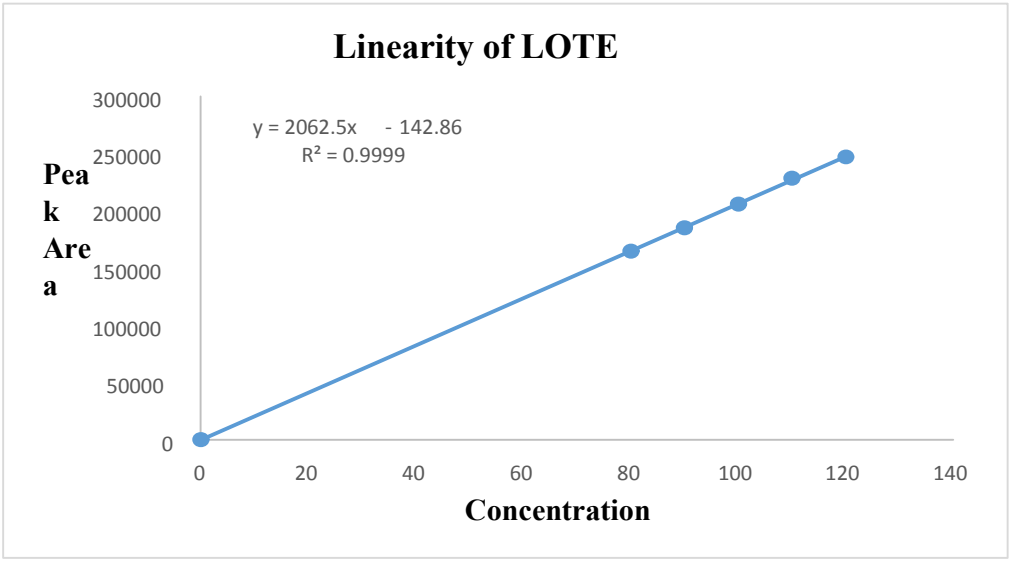

Fig. 6: Plot of linearity and range study for LOTE

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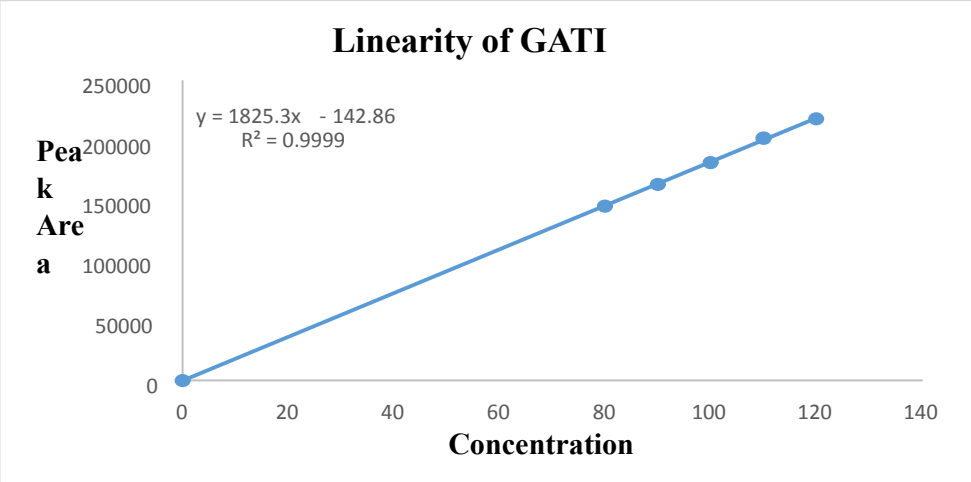

Fig. 7 : Plot of linearity and range study for GATI