International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.12 | Issue 4 | Oct - Dec -2023

www.ijpar.com

ISSN: 2320-2831

DOI : https://doi.org/10.61096/ijpar.v12.iss4.2023.574-583

Research

Analytical Method Development And Validation For Simultaneous Estimation Trifluoperazine HCL, Trihexy Phenidylhcl In Combined Pharmaceutical Dosage Form By RP-HPLC

Asiya Begum*, D. Venkata Ramana[1], Udaya Bhanu Sri Koppu[1]

[1]*Department Of Pharmaceutical Analysis, Holy Mary Institute Of Technology And Science (College Of Pharmacy), Keesara - Bogaram - Ghatkesar Rd, Kondapur, Telangana 501301*

*Author for Correspondence: Asiya Begum
Email: asiyahussain193@gmail.com

Published on: 30 Oct 2023

Published by:
DrSriram Publications

Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Trihexyphenidyl HCL and Trifluoperazine HCL, in its pure form as well as in tablet dosage form. Chromatography was carried out on an Altima C18 (4.6mm x 150mm, 5µm) column using a mixture of ACN, Methanol and Phosphate buffer pH-4.6 (10:25:65 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 265nm. The retention time of the Trihexyphenidyl HCL and Trifluoperazine HCL was 2.088, 6.068 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Trihexyphenidyl HCL and 20-100mg/ml of Trifluoperazine HCL. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Trihexyphenidyl HCL, Trifluoperazine HCL, RP-HPLC, Validation, Accuracy.

INTRODUCTION

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

"] *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

Types of Chromatography

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography (CEC).

Table 1: Principles and classification of chromatography

Technique	Stationary Phase	Mobile Phase	Format	Principal sorption mechanism
Paper chromatography (PC)	Paper (cellulose)	Liquid	Planar	Partition (adsorption, ion-exchange, exclusion)
Thin-layer chromatography (TLC)	Silica, cellulose, ion-exchange, resin, controlled porosity solid	Liquid	Planar	Adsorption (partition, ion-exchange, exclusion)
Gas chromatography (GC)				
Gas-liquid chromatography (GLC)	Liquid	Gas	Column	Partition
Gas-solid chromatography (GSC)	Solid	Gas	Column	Adsorption
Liquid Chromatography (LC)				
High Performance Liquid Chromatography (HPLC)	Solid or bonded-phase	Liquid	Column	Modified partition (adsorption)
Size-Exclusion Chromatography (SEC)	Controlled porosity solid	Liquid	Column	Exclusion
Ion-Exchange Chromatography (IEC), Ion Chromatography (IC)	Ion-exchange resin or bonded-phase	Liquid	Column	Ion-exchange
Chiral Chromatography (CC)	Solid chiral Selector	Liquid	Column	Selective adsorption

Methods in chromatography[5]

According to nature of stationary and mobile phase

- Solid- Liquid chromatography
- Liquid-Liquid chromatography
- Gas- Solid chromatography
- Gas -Liquid chromatography

According to principle of separation

A. Adsorption chromatography

- Gas Solid chromatography
- Thin layer chromatography
- Column chromatography
- High performance liquid chromatography
- Affinity phase chromatography
- Hydrophobic Interaction chromatography (HIC)

Partition chromatography

- Gas liquid chromatography
- Paper partition chromatography
- Column partition chromatography

Based on modes of chromatography

- Normal phase chromatography
- Reversed phase chromatography

Other types of chromatography

- Size exclusion chromatography (SEC)
- Gel permeation chromatography
- Gel chromatography
- Gel Filtration
- Gel permeation chromatography
- Ion exchange chromatography
- Chiral chromatography

High Performance Liquid Chromatography (HPLC) [6]

The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

MATERIALS AND METHODS

Trihexyphenidyl HCL Sura labs, Trifluoperazine HCL Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC Merck.

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Trihexyphenidyl HCL and Trifluoperazine HCL working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1 ml of the above Trihexyphenidyl HCL and Trifluoperazine HCL stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Optimized chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and PDA detector 996 model.
Temperature	:	35ºc
Column	:	Altima C18 (4.6×150mm, 5µ)
Buffer	:	Phosphate buffer (pH-4.6)-Dissolve 0.9g of anhydrous dihydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000mL. Adjust thepH 4.6 by using ortho phosphoric acid.
pH	:	4.6
Mobile phase	:	Buffer: Methanol: ACN (65:25:10v/v/v)
Flow rate	:	1ml/min
Wavelength	:	265 nm

Injection volume : 10 µl
Run time : 14 min

Validation
Preparation of mobile phase
Preparation of mobile phase

Accurately measured 650 ml (65%) of Buffer and 250 ml of Methanol (25%) and 100ml (10%) of Acetonitrile were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)
Mobile phase : Buffer: Methanol: ACN (65:25:10v/v/v)
Column : Altima C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 265 nm
Column temp : 38ºC
Injection Volume : 10 µl
Run time : 14 minutes

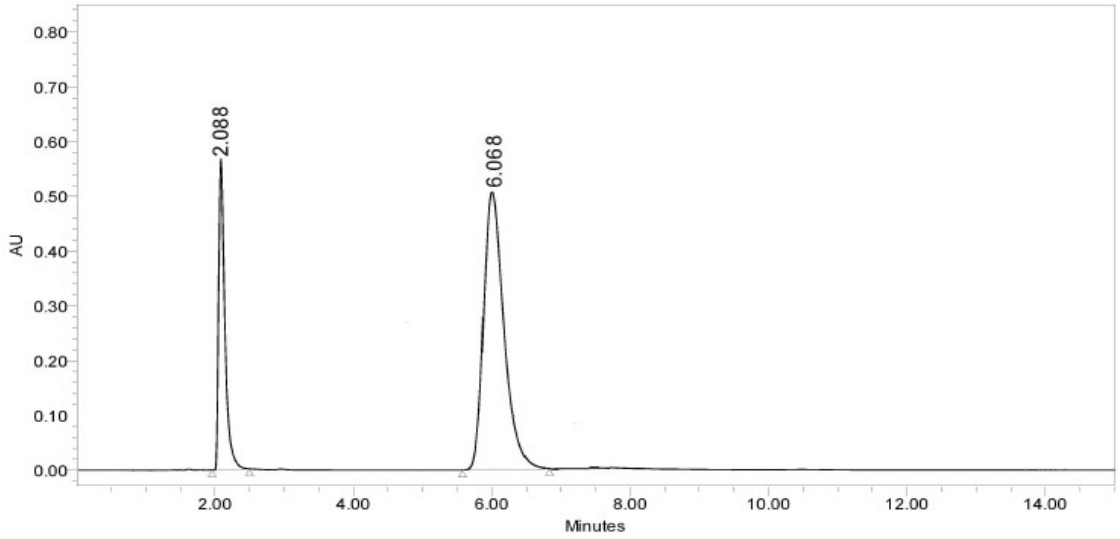


Fig 1: Optimized Chromatogram

Table 2: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Trihexyphenidyl HCL	2.088	3425413	567933		1.0
2	Trifluoperazine HCL	6.068	1629854	517733	2.5	1.1

Optimized Chromatogram (Sample)

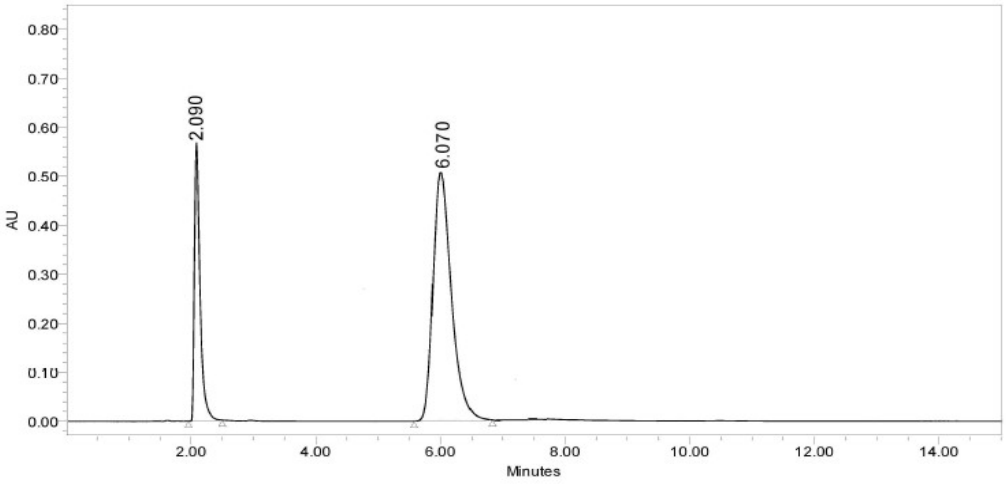


Fig 2: Optimized Chromatogram (Sample)

Table 3: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Trihexyphenidyl HCL	2.090	3468547	567933		1.0
2	Trifluoperazine HCL	6.070	16289441	517733	2.5	1.1

Resolution between two drugs must be not less than 2, Theoretical plates must be not less than 2000., Tailing factor must be not less than 0.9 and not more than 2. It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 4: Peak results for assay standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing
1	Trihexyphenidyl HCL	2.087	3425681	567917		1.0
2	Trifluoperazine HCL	6.067	16235984	517719	2.5	1.1
3	Trihexyphenidyl HCL	2.088	3425413	567933		1.0
4	Trifluoperazine HCL	6.068	16298543	517733	2.5	1.1
5	Trihexyphenidyl HCL	2.088	3465423	567933		1.0
6	Trifluoperazine HCL	6.068	16265213	517733	2.5	1.1

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Sample)

Table 5: Peak results for Assay sample

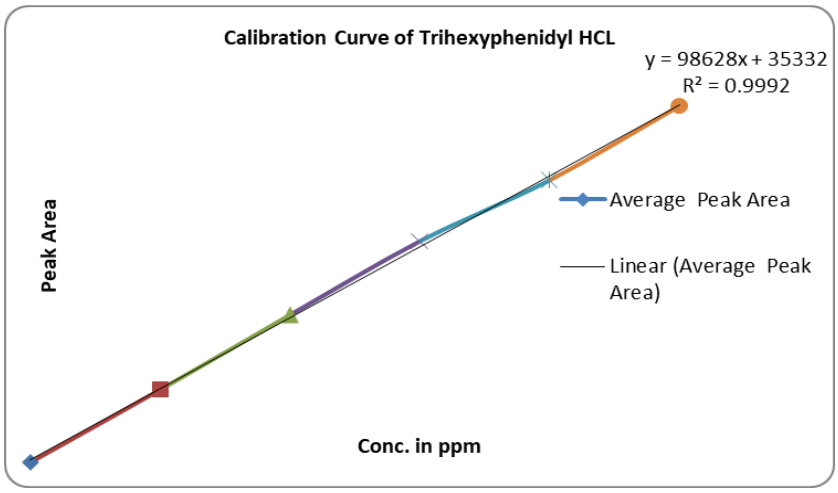
S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate
1	Trihexyphenidyl HCL	2.089	3469821	567917		1.0	6568.0
2	Trifluoperazine HCL	6.069	16259845	517719	2.5	1.1	5359.2
3	Trihexyphenidyl HCL	2.090	3468547	567933		1.0	5565.5

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Trihexyphenidyl HCL and Trifluoperazine HCL in pharmaceutical dosage form was found to be 100.1%.

Linearity
Chromatographic data for linearity study
Trihexyphenidyl HCL

Concentration Level (%)	Concentration µg/ml
33.3	10
66.6	20
100	30
133.3	40
166.6	50



Trifluoperazine HCL

Concentration Level (%)	Concentration µg/ml
33	20
66	40
100	60
133	80
166	100

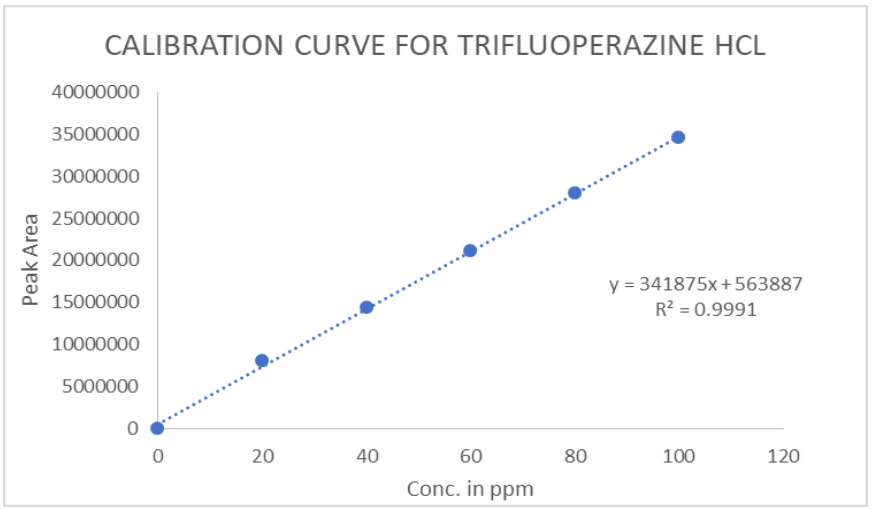


Fig 3: Chromatogram showing linearity level

Repeatability

Table 6: Results of Repeatability for Trihexyphenidyl HCL

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Trihexyphenidyl HCL	2.084	3569412	567917	5568.0	1.0
2	Trihexyphenidyl HCL	2.083	3465125	517719	5359.2	1.1
3	Trihexyphenidyl HCL	2.082	3598154	567933	5565.5	1.0
4	Trihexyphenidyl HCL	2.081	3586491	517733	5355.2	1.1
5	Trihexyphenidyl HCL	2.080	3582694	567917	5568.0	1.0
Mean			3560375			
Std. Dev			54225.61			

%RSD for sample should be NMT 2. The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 7: Results of Repeatability for Trifluoperazine HCL

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Trifluoperazine HCL	2.080	3582264	567917	5568.0	1.0
2	Trifluoperazine HCL	2.081	3586491	517719	5359.2	1.1
3	Trifluoperazine HCL	2.082	3598154	567933	5565.5	1.0
4	Trifluoperazine HCL	2.083	3564125	517733	5355.2	1.1
5	Trifluoperazine HCL	2.084	3569412	562173	5568.0	1.0
Mean			3580089			
Std. Dev			13609.81			
% RSD			0.380153			

%RSD for sample should be NMT 2., The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 8: Results of Intermediate precision day1 for Trihexyphenidyl HCL

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Trihexyphenidyl HCL	2.081	3481579	567917	5568.0	1.0
2	Trihexyphenidyl HCL	2.082	3458121	517719	5359.2	1.1
3	Trihexyphenidyl HCL	2.083	3426581	567933	5565.5	1.0
4	Trihexyphenidyl HCL	2.084	3465712	517733	5355.2	1.1
5	Trihexyphenidyl HCL	2.085	3451476	567917	5568.0	1.0
6	Trihexyphenidyl HCL	2.085	3452106	567514	5359.2	1.1

- *%RSD of five different sample solutions should not more than 2.*

Table 9: Results of Intermediate precision day1 for Trifluoperazine HCL

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Trifluoperazine	6.061	15481579	567917	5568.0	1.0
2	Trifluoperazine	6.062	15369852	517719	5359.2	1.1
3	Trifluoperazine	6.063	15248454	567933	5565.5	1.0
4	Trifluoperazine	6.064	15874692	517733	5355.2	1.1
5	Trifluoperazine	6.064	15236547	567933	5568.0	1.0
6	Trifluoperazine	6.064	15217547	567133	5359.2	1.1

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 10: Results of Intermediate precision Day 2 for Trihexyphenidyl HCL

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Trihexyphenidyl	2.081	3481579	567917	5568.0	1.0
2	Trihexyphenidyl	2.082	3458121	517719	5359.2	1.1
3	Trihexyphenidyl	2.083	3426581	567933	5565.5	1.0
4	Trihexyphenidyl	2.084	3465712	517733	5355.2	1.1
5	Trihexyphenidyl	2.085	3451476	567917	5568.0	1.0
6	Trihexyphenidyl	2.085	3452106	567514	5359.2	1.1

- *%RSD of five different sample solutions should not more than 2.*

Table 11: Results of Intermediate precision Day 2 for Trifluoperazine HCL

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Trifluoperazine HCL	6.061	15481579	567917	5568.0	1.0
2	Trifluoperazine HCL	6.062	15369852	517719	5359.2	1.1
3	Trifluoperazine HCL	6.063	15248454	567933	5565.5	1.0
4	Trifluoperazine HCL	6.064	15874692	517733	5355.2	1.1
5	Trifluoperazine HCL	6.064	15236547	567933	5568.0	1.0
6	Trifluoperazine HCL	6.064	15217547	567133	5359.2	1.1

%RSD of five different sample solutions should not more than 2., The %RSD obtained is within the limit, hence the method is rugged.

Accuracy

Table 12: The accuracy results for Trihexyphenidyl HCL

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1543793	15	15.2	101.9	100.9%
100%	3035883	30	30.4	101.4	
150%	4451005	45	44.7	99.4	

Table 13: The accuracy results for Trifluoperazine HCL

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1084420	30	30.07	100.2	99.6%
100%	2096069	60	59.6	99.4	
150%	3112684	90	89.3	99.3	

Robustness

Table 14: Results for Robustness - Trihexyphenidyl HCL

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Flow rate of 1.0 mL/min	3425413	2.088	5568.2	1.0
Flow rate of 0.9 mL/min	3425282	3.111	5922.2	1.2
Flow rate of 1.1 mL/min	3517879	1.880	5868.8	1.2
Less aqueous phase	3175485	3.101	5836.2	1.2
More aqueous phase	3365431	1.881	5282.6	1.1

Table 15: Results for Robustness- Trifluoperazine HCL

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	2029854	6.068	5359.2	1.1
Flow rate of 0.9 mL/min	1738319	7.101	5999.1	1.2
Flow rate of 1.1 mL/min	1638304	5.007	5989.2	1.1
Less aqueous phase	1973724	7.108	5387.2	1.1
More aqueous phase	2102838	5.008	5938.1	1.1

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Trihexyphenidyl HCL and Trifluoperazine HCL in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Trihexyphenidyl hydrochloride is soluble in water. It dissolves in methanol at 50 mg/ml to yield a clear to hazy, colorless solution. It is very slightly soluble in ether and benzene. Chlorpromazine was found to be Soluble in 100% ethanol, methanol or water (50mg/ml), soluble in chloroform; practically insoluble in ether, benzene and soluble in DMSO, and dimethyl formamide. ACN, Methanol and Phosphate buffer pH-4.6 (10:25:65 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Trihexyphenidyl HCL and Trifluoperazine HCL in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Holy Mary Institute Of Technology And Science (College Of Pharmacy), Keesara - Bogaram - Ghatkesar Rd, Kondapur, Telangana , for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

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