SERVICE CONTRACT

This Service Contract ("Contract") is entered into by and between **M/s Aum Research Labs Pvt Ltd.** and **Swarrnim Startup and Innovation University**, Gandhinagar, Gujarat.
In consideration of the mutual promises and covenants contained herein, the receipt and sufficiency of which are hereby acknowledged, the parties agree to the following terms:

SERVICES

"**The Provider agrees to develop and validate low cost and efficient analytical methods for the APIs and formulations for Aum Research Labs Pvt Ltd..**"

TERMS AND CONDITIONS

This Contract shall commence on **2nd April 2019** and will remain in effect for five years. The contract may be extended for an additional period as mutually agreed by both parties. Should either party wish to terminate the contract, a written notice must be provided 90 days prior to the intended termination date.

PAYMENT

For each completed service, the Provider will submit an invoice as services are delivered. The Client agrees to make payment upon receipt of the invoice, after deducting any applicable TDS (Tax Deducted at Source).

KARTIK * VAKANI

For **Aum Research Labs Pvt Ltd.**

For Swarrnim Startup and Innovation University

Date: 1.7.2022

To,

The Principal,

Swarrnim Science College

Swarrnim Startup and Innovation University

Gandhinagar Gujrat

Subject: Approval for Consultancy Project

Dear Sir/Madam

We are delighted to share the news with you that the consultancy project for which we were exchanging ideas in our earlier meetings has been permitted. The project will proceed as follows:

Project Title:

"Development and Validation of New Analytical Method for the Simultaneous Estimation of Levodropropizine and Chlorpheniramine in Pharmaceutical Dosage Form"

Project Timeline:

The project is expected to be completed within the next 3 to 4 months.

Payment:

A total amount payable after successful completion of Project will be Rs. 5,00,000 plus GST will be made after raising invoice of the same after due TDS.

Should you require any further clarification regarding the project, please feel free to reach out to us. We are excited about this collaboration and look forward to a positive working experience with **Swarrnim Startup and Innovation University**.

Best Regards,

KV

KARTIK VIKANI

For **Aum Research Labs Pvt Ltd.**

Ref.No.swarrnim/RO/SCR/2022/48

Date: 12.10.2022

To,

Aum Research Lab Pvt. Ltd.

Kalol, Gandhinagar

Gujarat.

Subject: - Submission of completion report regarding your shared problem.

Dear Sir/Madam

Please find enclosed herewith all data related to the problem shared by your prestigious company the details are as follows:

Project title: "**Development and Validation of New Analytical Method for the Simultaneous Estimation of Levodropropizine and Chlorpheniramine in Pharmaceutical Dosage Form**"

Date of assigning problem: 01.07.2022

Date of completion: 09.10.2022

Name of person: Ms. Toral Solanki

We are thankful for providing the opportunity to support you and the profession. We will always ready to solve such problems with our best effort.

For any technical support please contact person who has completed the project, the name is Ms. Toral Solanki.

Thanking you.

Patel

Registrar

Swarrnim Startup & Innovation University
Bhoyan Rathod, Gandhinagar

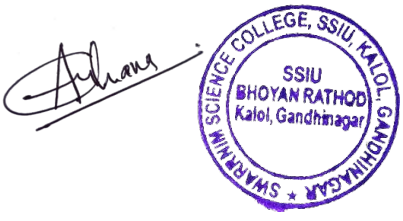
Development and Validation of New Analytical Method for the Simultaneous Estimation of Levodropropizine and Chlorpheniramine in Pharmaceutical Dosage Form

Research Project Report Submission to

Aum Research Lab

Submitted by:

Principal Investigator: Ms. Toral Solanki, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat



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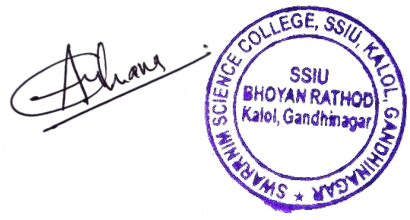

- **Declaration**

I, Ms. Toral Solanki, Principal Investigator of the project titled " **Development and Validation of New Analytical Method for the Simultaneous Estimation of Levodropropizine and Chlorpheniramine in Pharmaceutical Dosage Form** ", certify that the project work has been carried out as per the terms and conditions of the University Grants Commission.

Name:

Principal Investigator: Ms. Toral Solanki

Head of Institution: Dr. Hemant Chaube

- **Acknowledgment**

I extend my sincere gratitude to the **Aum Research Labs** for funding this project. I also thank my institution, colleagues, and students who supported and contributed to the successful completion of this project.

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BHOYAN RATHOD
Kalol, Gandhinagar

- **Executive Summary**

This project aims to develop and validate a novel analytical method for the simultaneous estimation of Levodropropizine and Chlorpheniramine in pharmaceutical dosage forms. The research focuses on optimizing chromatographic techniques such as High-Performance Liquid Chromatography (HPLC) or UV-Visible spectrophotometry, ensuring high precision, accuracy, and reliability of the method. The outcomes of this project will provide an effective and cost-efficient solution for pharmaceutical analysis, improving the quality control processes in the industry.

- **Detailed Report**

1. Introduction

Levodropropizine is chemically (-)-(S)-3-(4-Phenyl-1- piperazinyl)-1,2-propanediol. It is the Levo-rotatory (S)- enantiomer of triprolidine. It is a non-opioid agent whose peripheral antitussive action may result from its modulation of sensory neuropeptide levels within the respiratory tract. Levodropropizine is a peripherally acting agent inhibiting the afferent pathways that mediate the generation of the cough reflex. Compared with the racemic drug, levodropropizine maintains the antitussive activity but considerably lower central nervous system depressant actions. Levodropropizine is activated in the bronchopulmonary system as the inhibitor of bronchospasm induced by histamine, serotonin and barquentine.

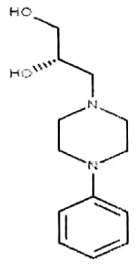


Fig 01: Structure of levodropropazine

Chlorpheniramine maleate is chemically [3-(4- chlorophenyl)-3- (pyridin-2-yl) propyl] dimethylamine is a antihistaminic used in the treatment of allergy. It acts by competing with histamine for H1-receptor sites on effector cells.

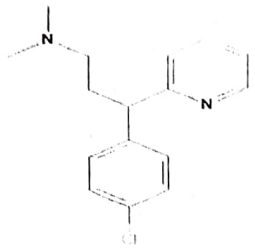


Fig 02: Structure of Chlorphenamine

A combination of Levodropropizine and Chlorpheniramine maleate is used as cough suppressant and also for allergy, itchy throat, common cold, hay fever, watery eyes, and runny nose.

2. Literature Review

Levodropropizine is a peripherally acting antitussive agent used in the treatment of non-productive cough, while Chlorpheniramine maleate is a first-generation antihistamine used for the relief of allergy symptoms. The combination of these two drugs is widely used in cough and cold preparations, providing both cough suppressant and anti-allergic effects.

Several analytical methods have been reported for the individual estimation of Levodropropizine and Chlorpheniramine in various pharmaceutical dosage forms. However, methods for their simultaneous estimation are limited, and few studies provide comprehensive validation in accordance with International Council for Harmonisation (ICH) guidelines.

Various analytical techniques such as HPLC, UV spectrophotometry, and LC-MS have been used to estimate Levodropropizine individually. For example, S. Kumar et al. (2018) developed an HPLC method for Levodropropizine in syrup form, focusing on linearity and specificity.

Chlorpheniramine has been quantified using HPLC, HPTLC, and spectrophotometric methods, either alone or in combination with other antihistamines and decongestants. An RP-HPLC method by Patel et al. (2017) demonstrated accurate results for Chlorpheniramine with high resolution and reproducibility.

Only a few studies have addressed the simultaneous estimation of Levodropropizine and Chlorpheniramine. Some reported methods lacked comprehensive validation or were not

optimized for tablet or syrup dosage forms. Moreover, many of the existing methods use expensive solvents or require long run times, which are not suitable for routine industrial analysis.

3. Objectives

1. To develop a novel analytical method for the simultaneous estimation of Levodropropizine and Chlorpheniramine.
2. To validate the proposed method according to ICH (International Council for Harmonisation) guidelines.
3. To evaluate the method's applicability in pharmaceutical formulations such as tablets and syrups.

4. Methodology

1. Development of Analytical Method:

- Description of the selected analytical technique (e.g., HPLC, UV Spectrophotometry).
- Selection of columns, mobile phase, and other chromatographic parameters.

2. Validation:

- Validation parameters like specificity, linearity, precision, accuracy, robustness, and limit of detection (LOD) and limit of quantification (LOQ).
- Standard solutions of Levodropropizine and Chlorpheniramine were prepared, and calibration curves were plotted for each.

3. Experimental Setup:

- Equipment used (e.g., HPLC system, UV Spectrophotometer).
- Sample preparation (tablet and syrup formulations).

4. Parameters Studied:

- Effect of pH, temperature, and mobile phase composition on retention time and resolution of the two compounds.
- Statistical analysis of the data for reliability and reproducibility.

Linearity

Preparation of Standard stock solutions

Accurately weighed 30mg of Levodropropazine, 2mg of Chlorpheniramine and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution 1and 2. (600µg/ml of Levodropropazine and 40µg/ml of Chlorpheniramine)

25% Standard solution 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Levodropropazine and 1µg/ml of Chlorpheniramine)

50% Standard solution 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (30µg/ml of Levodropropazine and 2µg/ml of Chlorpheniramine)

75% Standard solution 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (45µg/ml of Levodropropazine and 3µg/ml of Chlorpheniramine)

100% Standard solution 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (60µg/ml of Levodropropazine and 4µg/ml of Chlorpheniramine)

125% Standard solution 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Levodropropazine and 5µg/ml of Chlorpheniramine)

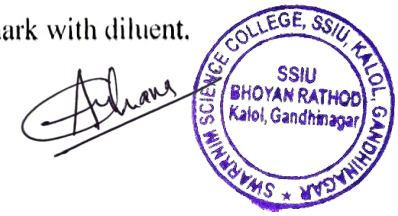
150% Standard solution 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml (90µg/ml of Levodropropazine and 6µg/ml of Chlorpheniramine)

Preparation of Standard stock solutions

Accurately weighed 30mg of Levodropropazine, 2mg of Chlorpheniramine and transferred to individual 50 ml volumetric flasks separately. 3/4 of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution 1and 2. (600µg/ml of Levodropropazine and 40µg/ml of Chlorpheniramine)

Preparation of 50% Spiked Solution

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



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

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

Acceptance Criteria The % Recovery for each level should be between 98.0 to 102.

6.Results and Discussion

Chromatographic conditions:

Mobile phase :0.01N kh2po4: Acetonitrile (60:40)

Flow rate: 1.0ml/min

Column: AscentisC18 (4.6 x 150mm, 5µm)

Detector wave length: 260nm **Column temperature:** 30°C **Injection volume:** 10L

Run time: 10 min

Diluent: Water and Acetonitrile in the ratio 50:50

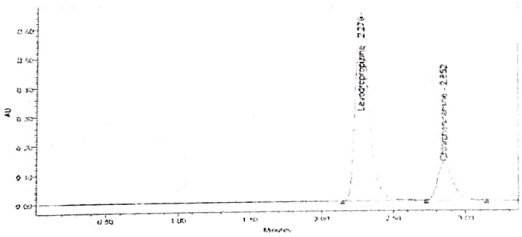


Fig 03: Typical chromatogram

Observation

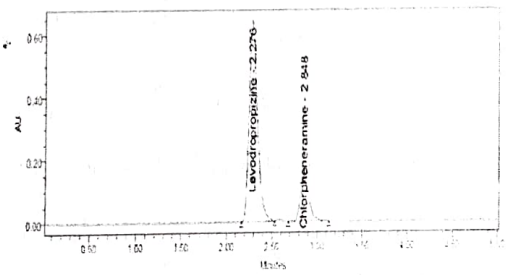
Levodropropizine and Chlorpheniramine were eluted at 2.279 min and 2.852 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table 01: System suitability parameters for Levodropropizine and Chlorpheniramine

Sr.no	Levodropropizine			Chlorpheniramine			
	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.276	2943	1.40	2.848	3796	1.34	3.2
2	2.280	3057	1.38	2.855	3765	1.35	3.2
3	2.280	2954	1.39	2.855	3824	1.34	3.2
4	2.280	2902	1.40	2.856	3819	1.36	3.2
5	2.280	3017	1.37	2.856	3731	1.34	3.2
6	2.281	2968	1.39	2.857	3835	1.31	3.2



According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

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Validation

- **Specificity**

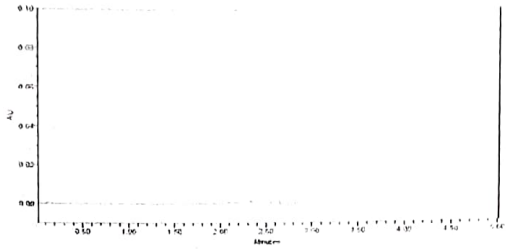

Fig 05: Chromatogram of blank

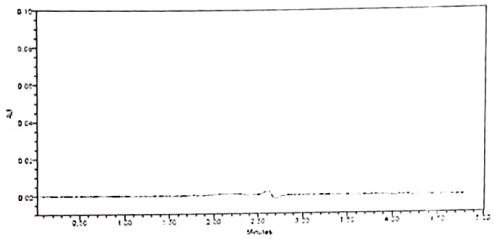

Fig 06: Chromatogram of placebo

- **Linearity**

Table 02: Linearity table forLevodropropizine and Chlorpheniramine

Levodropropizine		Chlorpheniramine	
Conc (µg/mL)	0 Peak area	Conc (µg/mL)	0 Peak area
15	1061194	1	242381
30	1994923	2	492293
45	2956215	3	685116
60	4087434	4	920941
75	5017766	5	1142880
90	6057273	6	1371032

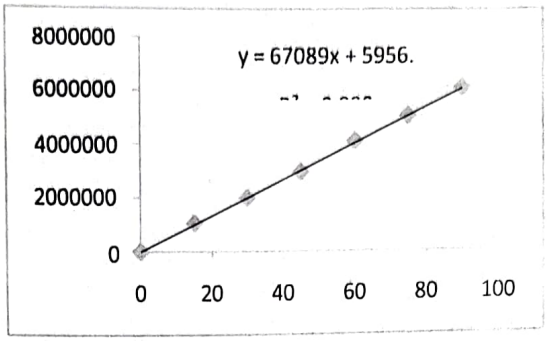

Fig 07: Calibration curve of Levodropropizine

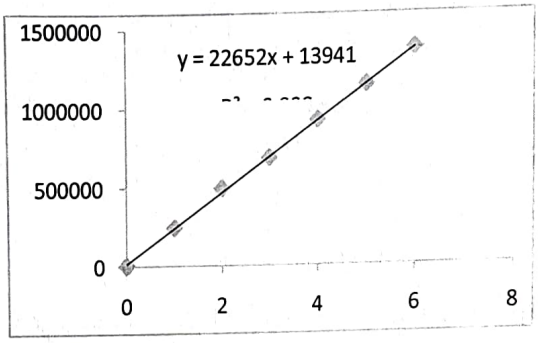

Fig 07: Calibration curve of Chlorpheniramine

Six linear concentrations of Levodropropizine (15- 90µg/ml) and Chlorpheniramine (1-6µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Levodropropizine was y = 67089x + 5956.8 and of Chlorpheniramine was y = 226526 x + 13941 Correlation coefficient obtained was 0.999 for the two drugs.

Precision System Precision

Table 03: System precision table of Levodropropizine and Chlorpheniramine

Sr. no	Area of Levodropropizine	Area of Chlorpheniramine
1.	4043221	918686
2.	4087891	918284
3.	4058297	916869
4.	4029594	924941
5.	4061933	909503
6.	4087812	919243
Mean	4061458	917921
S. D	23457.9	4974.2
%RSD	0.6	0.5

Repeatability

Table 04: Repeatability table of Levodropropizine and Chlorpheniramine

Sr. No	Area of Levodropropizine	Area of Chlorpheniramine
1.	4063209	911247
2.	4053333	908454
3.	4098738	915791
4.	4017469	921275
5.	4088506	903916
6.	4040395	917554
Mean	4060275	913040
S.D	30201.9	6375.8
%RSD	0.7	0.7

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.7% and 0.7% respectively for Levodropropizine and Chlorpheniramine. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day Precision)

Table 05: Intermediate precision table of Levodropropizine and Chlorpheniramine

S. No	Area of Levodropropizine	Area of Chlorpheniramine
1.	4043221	918686
2.	4087891	918284
3.	4058297	916869
4.	4029594	924941
5.	4061933	909503
6.	4087812	919243
Mean	4061458	917921
S. D	23457.9	4974.2
%RSD	0.6	0.5

Accuracy

Table 06: Accuracy table of Levodropropizine

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	%
50 %	30	30.562924	101.88	
	30	30.222525	100.74	
	30	29.525749	98.42	

100 %	60	59.644159	99.41	100. 73%
	60	60.428729	100.71	
	60	60.843357	101.41	
150 %	90	91.780456	101.98	
	90	90.684389	100.76	
	90	91.113059	101.24	

Table 07: Accuracy table of Chlorpheniramine

% Level	Amount Spiked (µg/mL)	Amount recovered	% Rec	Mean% Recover
50 %	2	1.972	98.59	99.03%
	2	1.994	99.72	
	2	1.969	98.44	
10 0%	4	4.005	100.1 4	
	4	3.993	99.82	
	4	3.932	98.31	
15 0%	6	5.932	98.87	
	6	5.957	99.29	
	6	5.885	98.08	

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as

100.73% and 99.03% for Levodropropizine and Chlorpheniramine respectively.

Sensitivity

Table 08: Sensitivity table of Levodropropizine and Chlorpheniramine

Molecule	LOD	LOQ
Levodropropizine	0.13	0.43
Chlorpheniramine	0.02	0.06

Robustness

Table 09: Robustness data for Levodropropizine and Chlorpheniramine

Sr. no	Condition	%RSD of Levodropropizine	%RSD of Chlorpheniramine
1	Flow rate (-) 0.9ml/min	0.6	0.4
2	Flow rate (+) 1.1ml/min	0.2	0.4
3	Mobile phase (-) 65B:35A	0.8	1.3
4	Mobile phase (+) 55B:45A	0.9	0.7
5	Temperature (-) 25°C	0.9	0.8
6	Temperature (+) 35°C	0.9	0.7

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. **Assay**

Resaws bearing the label claim Levodropropizine 30mg, Chlorpheniramine 2mg. Assay was performed with the above formulation. Average % Assay for Levodropropizine 99.57% and Chlorpheniramine 99.27% obtained was and respectively.

Table 10: Assay Data of Levodropropizine

Sr.no	Standard Area	Sample area	% Assay
1	4043221	4063209	99.64
2	4087891	4053333	99.40
3	4058297	4098738	100.51
4	4029594	4017469	98.52
5	4061933	4088506	100.26
6	4087812	4040395	99.08
Avg	4061458	4060275	99.57
Std dev	23457.9	30201.9	0.74
%RSD	0.6	0.7	0.7

Table 11: Assay Data of Chlorpheniramine

Sr.no	Standard Area	Sample area	% Assay
1	918686	911247	99.07
2	918284	908454	98.77
3	916869	915791	99.57
4	924941	921275	100.16
5	909503	903916	98.28
6	919243	917554	99.76
Avg	917921	913040	99.27
Std dev	4974.2	6375.8	0.7
%RSD	0.5	0.7	0.7

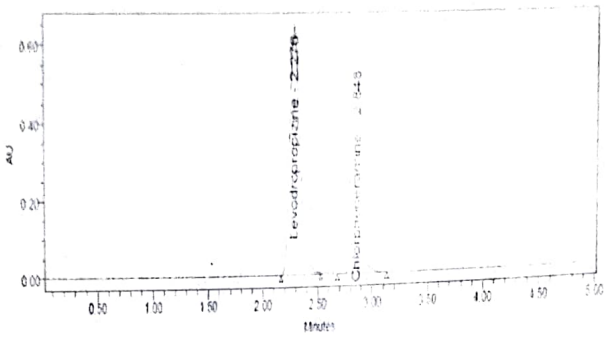

Fig 08: Chromatogram of working standard solution

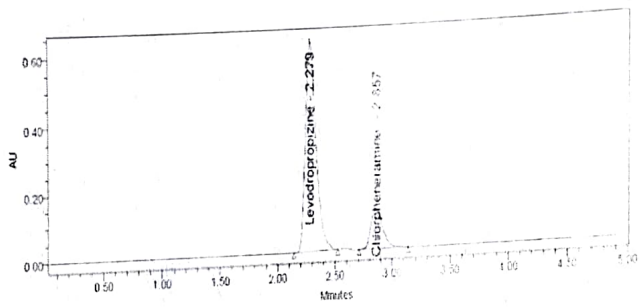

Fig 09: Chromatogram of working sample solution Degradation data

Table 12: Degradation data for Levodropropizine and Chlorpheniramine

Type of degradation	Levodropropizine			Chlorpheniramine		
	Area	% Recovered	Degraded	Area	% Recovered	Degraded
Acid	39 13 60 2	95.97	4.03	88 65 13	96.39	3.61
Base	39 41 27 5	96.65	3.35	86 55 99	94.11	5.89
Peroxide	38 22 37 5	93.74	6.26	88 48 93	96.21	3.79
Thermal	39 90 18 3	97.85	2.15	89 39 07	97.19	2.81
Uv	40 20 39 5	99.55	0.45	89 57 91	97.39	2.61
Water	40 59 29 4	98.59	1.41	91 25 03	99.21	0.79

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Kalol, Gandhinagar

6.Financial Statement

Certified that a grant of ₹5,00,000) was received from the University Grants Commission for the project titled "Development and Validation of New Analytical Method for the Simultaneous Estimation of Levodropropizine and Chlorpheniramine in Pharmaceutical Dosage Form"". The amount has been utilized as per the approved budget and guidelines

Sr.No.	Particulars	Expenditure Incurred
1	Equipment	₹ 1,50,000
2	Chemicals	₹2,25,000
3	Travel	₹75,000
4	Contingency	₹50,000
Total		**₹5,00,000**

7.Conclusion

The developed analytical method is effective for simultaneous estimation of Levodropropizine and Chlorpheniramine in pharmaceutical formulations. The method can be recommended for use in routine quality control in pharmaceutical industries. A simple, Accurate, precise method was developed for the simultaneous estimation of the Levodropropizine and Chlorpheniramine in in pharmaceutical dosage form. Retention time of Levodropropizine and Chlorpheniramine were found to be 2.276min and 2.848. %RSD of the Levodropropizine and Chlorpheniramine were and found to be 0.7 and 0.7 respectively. %Recovery was obtained as 100.73% and 99.03% for Levodropropizine and Chlorpheniramine respectively. LOD, LOQ values obtained from regression equations of Levodropropizine and Chlorpheniramine were 0.14,0.02 and 0.43, 0.06 respectively. Regression equation of Levodropropizine is y = 67089x + 5956.8 and y = 226526x+ 13941 of Chlorpheniramine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in industries.

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8. Reference

1. Patil D, Priti A, Rohan A, Sanjay K. Development and validation of stability indicating RP-HPLC method for Levodropropizine. *International Journal of Institutional Pharmacy and Life Sciences*. 2017; 7197-211.
2. Rushikesh B, Atul N, Sanjay S. Analytical method development and validation of Phenylephrine hydrochloride, Chlorpheniramine maleate, Paracetamol, and Caffeine in bulk drug and tablet dosage form by RP-HPLC. *Indo American Journal of Pharmaceutical Research*. 2013; 4330.
3. Ramya V, Vijaya Lakshmi M, Pravallika M, Buchi N. Novel HPLC-PDA method for the estimation of Chlorpheniramine maleate in bulk and dosage forms. *Indo American Journal of Pharmaceutical Research*. 2016; 6439-44.
4. Magesh AR, Dhanaraju MD. Development and validation of RP-HPLC method for simultaneous estimation of Diethylcarbamazine citrate and Chlorpheniramine maleate in pharmaceutical preparations. *Chemical Science Transactions*. 2017; 316-22.
5. Madhusudan T, Shanbhag SV. Method development and validation by RP-HPLC for simultaneous estimation of Paracetamol, Caffeine, Phenylephrine, Chlorpheniramine in tablet dosage form. *International Journal of Pharmacy and Technology*. 2017; 29960-70.
6. International Conference on Harmonisation. Validation of Analytical Procedures: Text and Methodology Q2(R1). *ICH Harmonised Tripartite Guideline*. 2005.
7. Vishnu Vardhan Reddy V, Sreenivas P, Prasanna K, Thejomoorthy K. Development and validation of new analytical method for the simultaneous estimation of Levodropropizine and Chlorpheniramine in pharmaceutical dosage form. *International Journal of Health and Clinical Biomedical Sciences*. 2021;1(1):1-7.
8. Chengalva P, et al. Analytical method development and validation for simultaneous quantification of Levodropropizine and Chlorpheniramine maleate in bulk and pharmaceutical formulation by RP-HPLC. *International Research Journal of Pharmacy*. 2019;10(4):161-165.
9. Sun YC, Shen Y, Sun GP, Wei W. Determination of phenol in the bulk drug and injection by RP-HPLC. *Chinese Journal of Pharmaceutical Analysis*. 2003;23(5):379-381.
10. Afreen F, Usha A, Satish A, Madhukar A. RP-HPLC method development and validation of Alogliptin bulk and tablet dosage form. *Asian Journal of Research in Chemistry*. 2018;11(4):643-648.

11. Kanagala V, Prajawala K, Sandhala D, Niharika K. Method development and validation of UV and RP-HPLC method for the estimation of Capecitabine in bulk and tablet dosage form. *Asian Journal of Research in Chemistry*. 2018;11(1):123-127.
12. Rajasekhar T, Sujana K. Stability indicating RP-HPLC method development and validation for simultaneous estimation of Levodropropizine and Chlorpheniramine maleate in bulk and their pharmaceutical dosage forms. *SlideShare Presentation*. 2019.
13. Naveen Kumar A, et al. Simple, accurate, precise method was developed for the simultaneous estimation of the Levodropropizine and Chlorpheniramine Maleate in syrup dosage form. *Journal of Global Trends in Pharmaceutical Sciences*. 2018;9(2):5675-5691.
14. Reddy VV, Sreenivas P, Prasanna K, Thejomoorthy K. Development and validation of new analytical method for the simultaneous estimation of Levodropropizine and Chlorpheniramine in pharmaceutical dosage form. *International Journal of Health and Clinical Biomedical Sciences*. 2021;1(1):1-7.
15. Chen Galva P, et al. Analytical method development and validation for simultaneous quantification of Levodropropizine and Chlorpheniramine maleate in bulk and pharmaceutical formulation by RP-HPLC. *International Research Journal of Pharmacy*. 2019;10(4):161-165.
16. Sun YC, Shen Y, Sun GP, Wei W. Determination of paeonol in the bulk drug and injection by RP-HPLC. *Chinese Journal of Pharmaceutical Analysis*. 2003;23(5):379-381.
17. Afreen F, Usha A, Satish A, Madhukar A. RP-HPLC method development and validation of Alogliptin bulk and tablet dosage form. *Asian Journal of Research in Chemistry*. 2018;11(4):643-648.
18. Kanagala V, Prajawala K, Sandhala D, Niharika K. Method development and validation of UV and RP-HPLC method for the estimation of Capecitabine in bulk and tablet dosage form. *Asian Journal of Research in Chemistry*. 2018;11(1):123-127.
19. Rajasekhar T, Sujana K. Stability indicating RP-HPLC method development and validation for simultaneous estimation of Levodropropizine and Chlorpheniramine maleate in bulk and their pharmaceutical dosage forms. *SlideShare Presentation*. 2019.
20. Naveen Kumar A, et al. Simple, accurate, precise method was developed for the simultaneous estimation of the Levodropropizine and Chlorpheniramine Maleate in syrup dosage form. *Journal of Global Trends in Pharmaceutical Sciences*. 2018;9(2):5675-5691.
21. Reddy VV, Sreenivas P, Prasanna K, Thejomoorthy K. Development and validation of new analytical method for the simultaneous estimation of Levodropropizine and

SERVICE CONTRACT

This Service Contract ("Contract") is entered into by and between **SPECHROM Solutions and Swarrnim Startup and Innovation University**, Gandhinagar, Gujarat.

In consideration of the mutual promises and covenants contained herein, the receipt and sufficiency of which are hereby acknowledged, the parties agree to the following terms:

SERVICES

"The Provider agrees to develop and validate low cost and efficient analytical methods for the APIs and formulations for SPECHROM Solutions."

TERMS AND CONDITIONS

This Contract shall commence on **11th January 2021** and will remain in effect for five years. The contract may be extended for an additional period as mutually agreed by both parties. Should either party wish to terminate the contract, a written notice must be provided 90 days prior to the intended termination date.

PAYMENT

For each completed service, the Provider will submit an invoice as services are delivered. The Client agrees to make payment upon receipt of the invoice, after deducting any applicable TDS (Tax Deducted at Source).

For SPECHROM Solutions

For Swarrnim Startup and Innovation University

Swarrnim Startup & Innovation University ★ Gandhinagar

Date: 5.5.2022

To,

The Principal,

Swarrnim Science College

Swarrnim Startup and Innovation University

Gandhinagar Gujrat

Subject: Approval for Consultancy Project

Dear Sir/Madam

It is our pleasure to inform you that the project for consultancy which has been under discussion for quite some times is granted. The details are as follows:

Project Title:

"Development and Validation of Analytical Method for the Estimation of Deferasirox in Pharmaceutical Dosage Form and Its Application in Dissolution Studies"

Project Timeline:

The project is expected to be completed within the next 3 to 4 months.

Payment:

A total amount payable after successful completion of Project will be Rs. 9,00,000 plus GST will be made after raising invoice of the same after due TDS.

Should you require any further clarification regarding the project, please feel free to reach out to us. We are excited about this collaboration and look forward to a positive working experience with **Swarrnim Startup and Innovation University**.

Best Regards,

For SPECHROM Solutions

SPECHROM SOLUTIONS AHMEDABAD

Ref.No.swarrnim/RO/SCR/2022/47

Date: 05.09.2022

To,

SPECHROM SOLUTION.

SF/209, Trade Square, Sabarmati,

Ahmadabad, Gujarat.

Subject: - Submission of completion report regarding your shared problem.

Dear Sir/Madam

Please find enclosed herewith all data related to the problem shared by your prestigious company the details are as follows:

Project title: **'Development and Validation of Analytical Method for the Estimation of Deferasirox in Pharmaceutical Dosage Form and Its Application in Dissolution Studies'**

Date of assigning problem: 05.05.2022

Date of completion: 02.09.2022

Name of person: Ms. Sonal Panchal

We are thankful for providing the opportunity to support you and the profession. We will always ready to solve such problems with our best effort.

For any technical support please contact person who has completed the project, the name is Ms. Sonal Panchal.

Thanking you.

Patel

Registrar

Swarrnim Startup & Innovation University
Bhoyan Rathod Gandhinagar

Development and Validation of Analytical Method for the Estimation of Deferasirox in Pharmaceutical Dosage Form And Its Application in Dissolution Studies

Research Project Report Submission to

Spechrom Solution

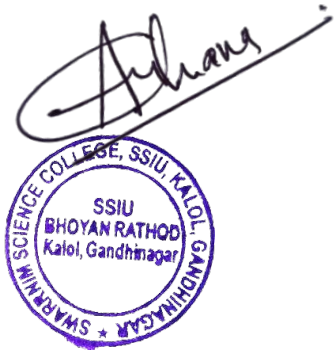
SWARNIM
STARTUP & INNOVATION
UNIVERSITY
WHERE IDEAS COME ALIVE

Submitted by:

Principal Investigator: Panchal Sonal, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat

SWARNIM SCIENCE COLLEGE, SSIU, KALOL, GANDHINAGAR
SSIU
BHOYAN RATHOD
Kalol, Gandhinagar

INDEX



- **Declaration**

I, Panchal Sonal, Principal Investigator of the project titled "Development and Validation of Analytical Method for the Estimation of Deferasirox in Pharmaceutical Dosage Form And Its Application in Dissolution Studies", certify that the project work has been carried out as per the terms and conditions of the Spechrom Solution.

Name:

Principal Investigator: Ms. Sonal Panchal

Head of Institution: Dr. Hemant Chaube

- **Acknowledgment**

I extend my sincere gratitude to the **Spechrom Solution** for funding this project. I also thank my institution, colleagues, and students who supported and contributed to the successful completion of this project. Special thanks to [mention key contributors].

- **Executive Summary**

This project focuses on the development and validation of a robust analytical method for accurately estimating Deferasirox, a widely used iron-chelating agent, in pharmaceutical dosage forms. The validated method ensures precision, accuracy, sensitivity, and reproducibility, meeting regulatory guidelines such as ICH Q2(R1). The research also extends the method's application to dissolution studies, assessing the drug's release profile from various formulations. These findings contribute to quality control and the optimization of pharmaceutical formulations, ensuring therapeutic efficacy and compliance with industry standards.

- **Detailed Report**

1. Introduction

Thalassemia (thal-uh-SEE-me-uh) is an inherited blood disorder that causes your body to have less haemoglobin than normal. Haemoglobin enables red blood cells to carry oxygen. Thalassemia can cause anaemia, leaving you fatigued. If you have mild thalassemia, you might not need treatment. But more severe forms might require regular blood transfusions.

You can take steps to cope with fatigue, such as choosing a healthy diet and exercising regularly. Thalassemia is caused by mutations in the DNA of cells that make haemoglobin - the substance in red blood cells that carries oxygen throughout your body. The mutations associated with thalassemia are passed from parents to children. Haemoglobin molecules are made of chains called alpha and beta chains that can be affected by mutations. In thalassemia, the production of either the alpha or beta chains are reduced, resulting in either alpha-thalassemia or beta-thalassemia. There are several types of thalassemia. The signs and symptoms you have depend on the type and severity of your condition.

Thalassemia signs and symptoms can include:

1. Fatigue
2. Weakness
3. Pale or yellowish skin
4. Facial bone deformities
5. Slow growth
6. Abdominal swelling

7. Dark urine

Some babies show signs and symptoms of thalassemia at birth; others develop them during the first two years of life. Some people who have only one affected haemoglobin gene don't have thalassemia symptoms.

1.4 Classification of thalassaemic disorder:[1]

Alpha-thalassemia

Four genes are involved in making the alpha haemoglobin chain. You get two from each of your parents. If you inherit:

One mutated gene, you'll have no signs or symptoms of thalassemia. But you are a carrier of the disease and can pass it on to your children.

Two mutated genes, your thalassemia signs and symptoms will be mild. This condition might be called alpha-thalassemia trait.

Three mutated genes, your signs and symptoms will be moderate to severe. Inheriting four mutated genes is rare and usually results in stillbirth. Babies born with this condition often die shortly after birth or require lifelong transfusion therapy. In rare cases, a child born with this condition can be treated with transfusions and a stem cell transplant.

Beta-thalassemia

Two genes are involved in making the beta haemoglobin chain. You get one from each of your parents. If you inherit:

One mutated gene, you'll have mild signs and symptoms. This condition is called thalassemia minor or beta-thalassemia.

Two mutated genes, your signs and symptoms will be moderate to severe. This condition is called thalassemia major, or Cooley anaemia.

Babies born with two defective beta haemoglobin genes usually are healthy at birth but develop signs and symptoms within the first two years of life. A milder form, called thalassemia intermedia, also can result from two mutated genes.

1.5 Risk factors of thalassaemic disorder:[1]

Factors that increase your risk of thalassemia include:

Family history of thalassemia. Thalassemia is passed from parents to children through mutated haemoglobin genes.

Certain ancestry. Thalassemia occurs most often in African Americans and in people of Mediterranean and Southeast Asian descent.

1.6 Chelating Agents to treat thalassaemic disorder: DEFEROXAMINE:[2]

Route: SC/V

Dose: 25-50 mg/kg/day

Schedule: over 8 to 10 hrs for 5-6 nights a week with the help of subcutaneous deferral infusion pump.

MOA: chelates loosely bound iron, iron from ferritin, hemosiderin, not from transferrin.

Excretion: urine/Faeces

Adverse effects: Local skin reaction, toxicity, infections, ophthalmic toxicity, skeletal impairment.

DEFERIPONE(KELFER):

It mobilizes iron from transferrin ferritin and hemosiderin. Dose: 75 to 100mg/kg body weight/day in 3-4 divided doses. Excretion: urine

Plasma clearance: t1/2: 53-166 min

Adverse effect: a granulocytic, GIT disturbance, transaminase elevation, arthralgias. Monitoring weekly CBC.

DEFERASIROX (EXJADE):[3]

A novel chelating agent belongs to tridentate triazole, with high affinity to iron as Fe+++ and chelates at a ratio of 2:1 (Deferasirox: Iron)

Dose: 20-30 mg/kg/day Schedule: daily, OD Excretion: Faeces

Plasma clearance: t1/2: 1-16 hours

It is available as 250/500 mg tablet for oral suspension dispersible tablets.

Factors that increase your risk of thalassemia include:

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Nanotechnology based ocular drug delivery systems such as polymeric nano- particles have revealed promising results for dose optimization, bioavailability and sustained ocular drug delivery to the posterior ocular tissue segments.

Introduction to Deferasirox:[4]

Deferasirox is an oral iron chelator. Deferasirox is a white to off white crystalline powder with a Molecular weight 373.4. Deferasirox is freely soluble in Dimethyl formamide, Dimethyl sulfoxide, slightly soluble in methanol, practically insoluble in water. Its empirical formula is C21H15N3O4 and Chemical structure is given below (Figure 1).

So, it is felt necessary to develop a liquid chromatographic (LC) procedure which would serve as a rapid and reliable method for the deferasirox.

Deferasirox iron chelating agent used in the treatment of chronic iron overload caused by blood transfusions in both adults and pediatric patients. According to the BCS and BDDCS, deferasirox is a Class II compound showing low solubility, high permeability, extensive metabolism and suspected to be substrates of efflux transporters (P- glycoprotein; Breast Cancer Resistance Protein; Multidrug Resistance Proteins). The critical parameter for deferasirox bioavailability is mainly its solubility. Solubility enhancement approaches can be implemented to increase the bioavailability of deferasirox and therefore, the dose, side effects and the overall production cost of deferasirox dosage forms can be reduced.

1.11 Clinical rational of drug:

Its main use is to reduce chronic iron overload in patients who are receiving long term blood transfusions for conditions such as beta thalassemia and other chronic anaemias. Chemically4-[(3Z,5E)-3,5bis(6-oxo-1-cyclohexa-2,4-dienylidene)-1,2,4 triazolidin-1-yl] benzoic acid.

Here is the study that shown,[50]Here is one study which involved 64 patients with known cases of β-thalassemia major or intermedia that has been treated with blood transfusion and iron chelators. Serum ferritin, serum iron, serum total iron binding, unsaturated iron-binding capacity (UIBC), and immunological parameters were assessed in deferoxamine and deferasirox-treated patients.

In deferoxamine-treated patients, serum ferritin levels were high (8160.33 ± 23[illegible]75 ng/dL) compared to deferasirox-treated patients (3000.62 ± 188.23 ng/dL; P < 0.0001), also there

were significant differences in serum iron, total iron-binding capacity and UIBC ($P < 0.0001$) in deferasirox-treated patients compared to deferoxamine-treated patients.

2. Literature Review

Deferasirox, a tridentate chelator used in the treatment of chronic iron overload conditions, has gained significant attention due to its efficacy and oral administration advantage. Analytical methods for quantifying Deferasirox in pharmaceutical dosage forms are critical for ensuring quality, efficacy, and safety. Previous studies have employed various analytical techniques, including UV-Visible spectrophotometry, High-Performance Liquid Chromatography (HPLC), and High-Performance Thin Layer Chromatography (HPTLC). Among these, HPLC is widely favoured for its precision, reproducibility, and ability to separate complex mixtures.

Validation of analytical methods is pivotal, as emphasized in guidelines such as ICH Q2(R1), which outline parameters like specificity, linearity, accuracy, and robustness. Researchers have highlighted the importance of method validation in establishing reliable protocols for quality control and regulatory compliance.

Additionally, dissolution studies play a vital role in the pharmaceutical development process by providing insights into the drug release profile, formulation behaviour, and bioavailability. Methods combining dissolution studies with validated analytical techniques have been successfully implemented to optimize dosage forms and ensure consistent therapeutic outcomes.

Despite these advancements, limited studies have addressed the integration of validated analytical methods with dissolution testing specifically for Deferasirox formulations. This gap underscores the need for research that bridges analytical development with practical applications, such as dissolution profiling, to enhance drug quality and performance in clinical settings.

3. Objectives

1. Develop a Reliable Analytical Method: To design and optimize an accurate, precise, and sensitive analytical method for the quantification of Deferasirox in pharmaceutical dosage forms.
2. Validation of the Analytical Method: To validate the developed method in compliance with ICH guidelines, assessing its accuracy, precision, specificity, linearity, robustness, and detection limits.

3. Application in Quality Control: To utilize the validated method for routine quality control analysis of Deferasirox in commercial pharmaceutical formulations.
4. Dissolution Studies Integration: To apply the analytical method in evaluating the dissolution profiles of Deferasirox formulations, ensuring compliance with regulatory standards for drug release.
5. Comparison Across Formulations: To compare the dissolution characteristics of different Deferasirox formulations, aiding in the optimization of pharmaceutical development and ensuring consistent therapeutic performance.

4. Methodology

1. Preparation of Standard and Sample Solutions:

- Standard Solution: Accurately weigh a known amount of pure Deferasirox, dissolve it in a suitable solvent (e.g., methanol or water), and dilute to obtain the desired concentration.
- Sample Solution: Weigh the pharmaceutical dosage form equivalent to the required amount of Deferasirox. Dissolve and filter to remove excipients, then dilute to achieve a concentration within the calibration range.

2. Development of Analytical Method:

- Chromatographic Conditions (HPLC):
    - Select a suitable stationary phase (e.g., C18 column) and optimize the mobile phase (e.g., acetonitrile and phosphate buffer in varying ratios).
    - Determine the flow rate, wavelength for detection (e.g., UV or PDA detection), and injection volume.
- Spectrophotometric Method:
    - Identify the maximum absorption wavelength (λmax) using a UV-Vis spectrophotometer. Optimize parameters for Beer-Lambert's law compliance.

3. Validation of Analytical Method:

- Conduct validation as per ICH Q2(R1) guidelines:
    - Specificity: Ensure the method differentiates Deferasirox from excipients and impurities.

- Linearity: Assess linearity by plotting concentration vs. peak area/absorbance over a range of concentrations.
- Accuracy: Perform recovery studies by spiking known amounts of standard into the sample matrix.
- Precision: Evaluate repeatability (intra-day) and intermediate precision (inter-day) using multiple replicates.
- Robustness: Alter chromatographic parameters (e.g., flow rate, mobile phase ratio) and assess the impact on results.
- Limits of Detection (LOD) and Quantification (LOQ): Calculate using signal-to-noise ratios.
-

4. Dissolution Studies:

- Select appropriate dissolution media (e.g., buffer solutions of specific pH).
- Use a USP dissolution apparatus (e.g., Type II paddle) to study drug release at different time intervals. Maintain standardized conditions (e.g., temperature, rotation speed).
- Collect aliquots at predetermined intervals, filter, and analyze using the validated analytical method.

5. Results and Discussion

IR Spectral Determination:

Here is the chromatogram or IR Spectra of the Deferasirox shown below which shows the different functional groups by which we identify the structure of the Deferasirox.

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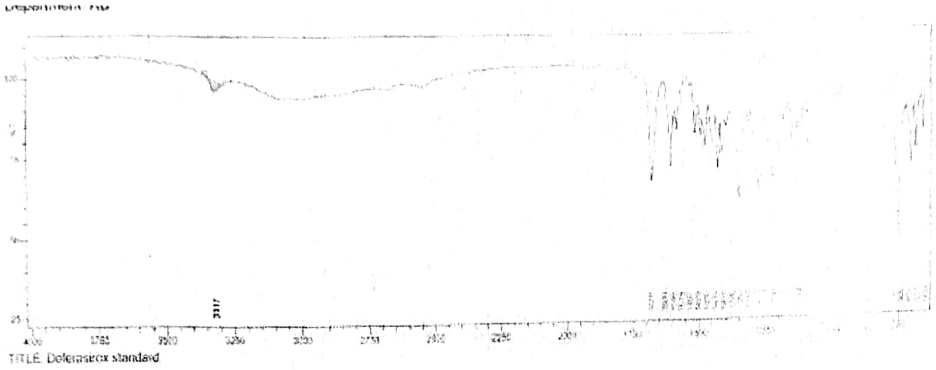
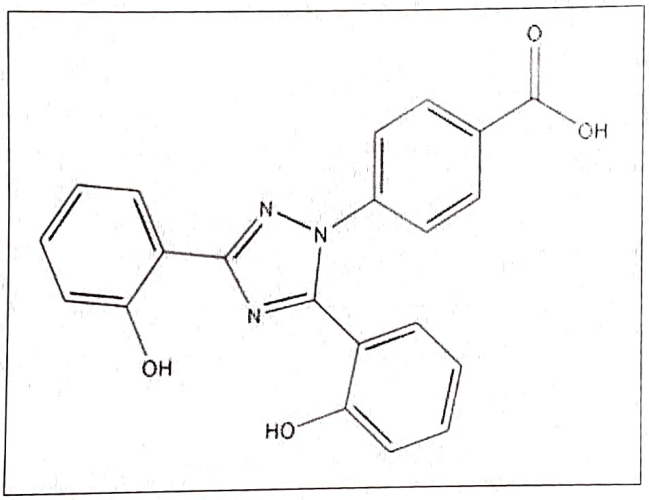


FT-IR Spectra of Deferasirox



Structure of Deferasirox

Interpretation of Standard spectra of Deferasirox from its functional group as below mentioned :

Sr. no.	Functional group	Reportedfrequency [44]	Observed frequncy (cm
1	C = O	1680 - 1630	1678

2	- OH	uj2800 - 3500	3317
3	C = C	827 - 790	852
4	C - N	1350 - 1000	1352

Melting Point Determination of Deferasirox:

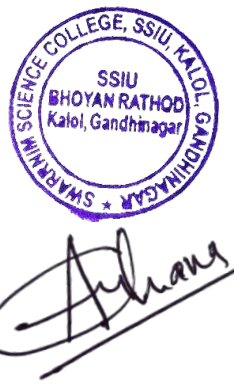
Name of Standard	Standard Melting Point[6]	Observed Melting Point
Deferasirox	260 - 262	261- 262

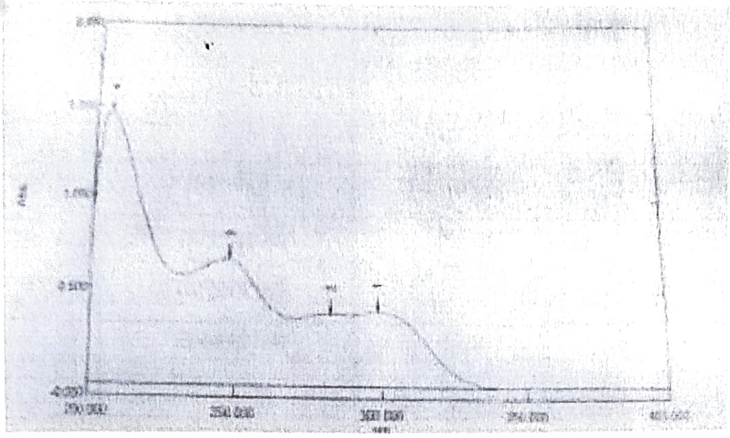
Solubility of deferasirox

Solvent	mg of Deferasirox	ml of Solvent	Solubility
Water	100	1000	Practically insoluble
Ethanol	10	10	Slightly soluble
Dimethyl Sulfoxide	1002	1	Very soluble
Heptane	100	100	Practically insoluble

Selection of Wavelength:

UV- Spectra of Deferasirox





Observation: Maximum Absorption of Deferasirox was found at 200 nm. So, 200 nm wavelength used for detection of Deferasirox.

Selection of Dissolution Media:

As per the ICH M9 guidelines, the dissolution method was performed in different pH buffers for the selection of dissolution media based on %drug release. Table 6.2.2.1 shows the results of % Drug release.

% Drug release of Deferasirox

Buffer media	%DR (15mins)	%DR (30mins)	%DR (40mins)
Water	71	71	70
0.1 N HCl	73	73	72
pH 4.5 Acetate buffer	75	75	75
pH 6.8 Phosphate buffer	77	77	77
PH 6.8 Phosphate buffer + 0.3%tween	80	80	79
PH 6.8 Phosphate buffer + 0.5%tween	92	92	92

Trials of Mobile phase

Column	Intersil ODS-3V, (150 x 4.6) mm,	Intersil ODS-3V, (150 x 4.6) mm,	Intersil ODS-3V, (150 x 4.6) mm,
Flow Rate	1.2 ml/min	1.2 ml/min	1.2 ml/min
Wavelength(UV detector)	250 nm	250 nm	250 nm
Injection Volume	5 µl	5 µl	5 µl
Column Oven Temperature	30 °C	30 °C	30 °C

System suitability Parameters data (n=5)

Sr. no.	Area of Deferasirox	T.F.	T.P.	RT
1	1201134	0.956	4257	2.90
2	1205173	0.974	4263	2.90
3	1205564	0.987	4311	2.90
4	1211825	0.993	4317	2.90
5	1213638	0.998	4303	2.90
Mean	1207467	-	-	-
SD	5149	-	-	-
% RSD	0.4	-	-	-
Tailing factor	0.95	-	-	-
Theoritical plates	4257	-	-	-

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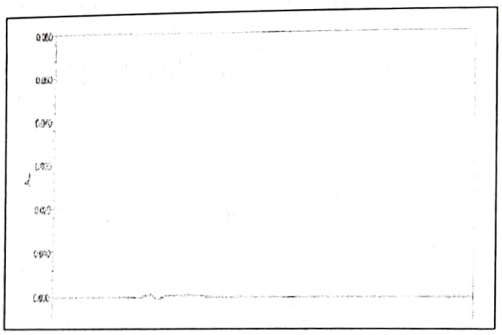
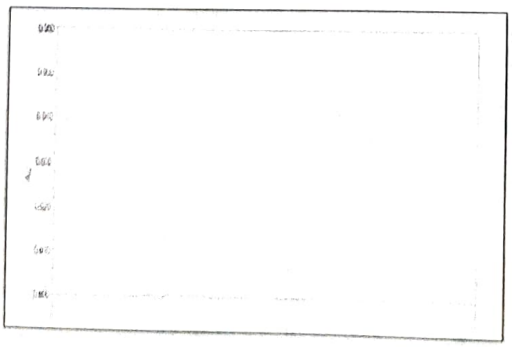
Specificity data:

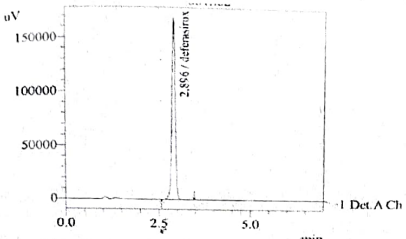
Sr. no.	Samples	Retention time of Deferasirox (minutes)	Area	Purity angle	Purity threshold	Peak Purity
1	Blank solution (6.8 phosphate buffer + tween 20)	No peak observed at the retention time of Deferasirox peak	NA	NA	NA	NA
2	Excipient blend (placebo)solution	No peak observed at the retention time of Deferasirox peak	NA	NA	NA	NA
3	Standard solution (For 500 mg)	2.90	1201134	0.055	0.281	Pass
4	Sample solution (For 500 mg)	2.90	1062606	0.072	0.286	Pass



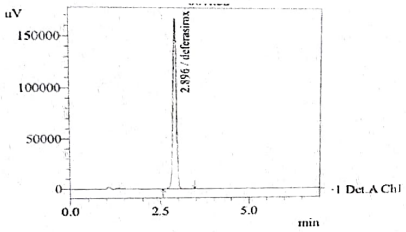
Chromatogram of blank solution at 250 nm



Chromatogram Excipient blend (placebo) solution at 250 nm



Chromatogram of standard solution (for 500mg strength) at 250 nm



Analytical method validation was performed

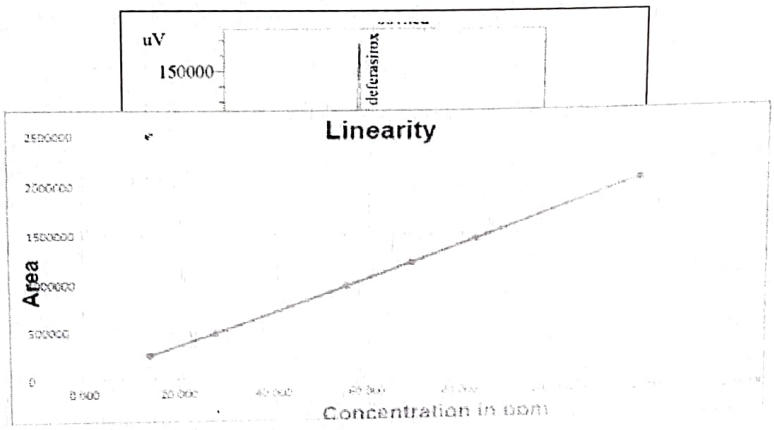
Chromatogram of sample solution (for 500mg strength) at 250 nm

Linearity and working range (n= 6):

The peak area of Deferasirox at each concentration level was determined and the linearity graph was plotted against concentration.

Level	Concentration of Deferasirox (ppm)	%RSD
L1- 20%	14	0.5
L2- 40%	28	0.5
L3- 80%	56	0.2
L4- 100%	70	0.4
L5- 120%	84	0.9
L6- 170%	119	0.01

Correlation coefficient(R^2)	**1.00**	-
Slope of regression line	**17297.91**	-
Y- Intercept	**4577.83**	-



Calibration curve of Deferasirox

Accuracy (n= 4):

It was evaluated by recovery study. Known amount of API and excipient blend spiked in 900 ml of dissolution media at level (50 %, 100 % and 150 %). The % recovery and % mean recovery at each level for Deferasirox is shown in below Table respectively.

Accuracy data of Deferasirox

Replicates	% Recovery	%Mean	SD	% RSD
1	100.97	100.48	1.34	1.34
2	98.95			
3	101.51			

1	100.15	99.74	0.35	0.36
2	99.62			
3	99.45			
1	100.41	100.26	0.20	0.20
2	100.34			
3	100.03			

Precision:

Reproducibility result of Deferasirox

Sr. no.	Area of Deferasirox	T.F.	T.P.	RT
1.	1201134	0.956	4257	2.90
2.	1205173	0.974	4263	2.90
3.	1205564	0.987	4311	2.90
4.	1211825	0.993	4317	2.90
5.	1213638	0.998	4303	2.90
Mean	**1207467**	-	-	-
SD	**1859.4**	-	-	-
% RSD	**0.154**	-	-	-
Tailing factor	**0.95**	-	-	-
Theoretical plates	**4257**	-	-	-

Repeatability (n=6):

Repeatability result of Deferasirox

Sample	Area of Deferasirox	% Drug release of Deferasirox
1	1062606	93
2	1002334	87
3	990443	86

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4	962080	84
5	1019366	89
6	996902	87
Mean	**1005621.83**	**88**
SD	**33613.18**	**3.5**
% RSD	**3.3**	**4.0**

Comparison of Method precision and Intermediate precision:

Precision	**Sample no.**	**% Deferasirox**
Method precision	Sample 1	93
	Sample 2	87
	Sample 3	86
	Sample 4	84
	Sample 5	89
	Sample 6	87
Intermediate precision	Sample 7	94
	Sample 8	87
	Sample 9	86
	Sample 10	84
	Sample 11	89
	Sample 12	86
Overall (n= 12)	**Mean**	**87**
	SD	**3.5**
	% RSD	**4.0**

Summary of validation parameters:

Parameters	**Deferasirox**
Filter validation	Complies

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Filter compatibility	Complies
Filter saturation	Complies
Filter precision	Complies
System suitability test (%RSD)	0.4
Specificity	Complies
Linearity (R^2 Value)	1.00
Accuracy (% RSD)	For 50 % - 1.34
	For 100 % - 0.36
	For 150 % - 0.20
Reproducibility	0.154
Repeatability	3.5
Intermediate precision	4.0
Flow rate	Complies
Wavelength	
Column oven temperature	
Buffer composition	
Stability of solution	Complies
Mobile phase stability	

6. Financial Statement

Certified that a grant of ₹ 9,00,000 was received from the Spechrom Solutions for the project titled Development and Validation of Analytical Method For The Estimation of Deferasirox in Pharmaceutical Dosage Form And Its Application in Dissolution Studies . The amount has been utilized as per the approved budget and guidelines.

Sr.No.	Particulars	Expenditure Incurred
1	Equipment	₹2,99,000
2	Chemicals	₹4,01,000
3	Personnel	₹1,45,000
4	Miscellaneous	₹55,000
Total		₹ 9,00,000

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7.Conclusion

In the present study, dissolution testing of Deferasirox and its method validation was carried out. The specificity of the method was determined by evaluating the interference from blank and placebo. The peak purity of drug in dissolution samples was demonstrated by using a Photo Diode Array (PDA) detector. Data suggests that % RSD was found to be within the limit of acceptance criteria and no interference was observed from blank and placebo at the retention time of Deferasirox peaks. Hence, the method can be termed as specific. For linearity, according to acceptance criteria correlation coefficient value should be not less than 0.999. Correlation coefficient value for linearity of Deferasirox was found to be 1.000. Hence, the method is linear within its range. Accuracy was determined from lowest concentration of sample to highest concentration of sample (i.e. at 50 %, 100 % and 150 %). According to acceptance criteria, individual % recovery should be in the range of 95.0 % to 105.0 %. The % recovery for Deferasirox was found to be 101.1% which is within acceptance criteria. Hence, the method can be termed as accurate. The above developed dissolution method was statistically validated in terms of specificity, linearity, accuracy, precision and robustness. The method was found to be specific, linear, accurate, precise and robust. Thus, above developed dissolution method can be applied for routine quantitative analysis of Deferasirox in tablet dosage form.

Recommendations:

Optimization of Dissolution Media: Investigate the use of various dissolution media with differing pH levels to simulate in vivo conditions more effectively and ensure comprehensive drug release profiling.

Stability Studies: Extend the analytical method's application to stability studies of Deferasirox under different environmental conditions (e.g., temperature, humidity) to assess the formulation's shelf life.

Comparison Across Dosage Forms: Evaluate the dissolution behaviour of Deferasirox in different dosage forms (e.g., tablets, dispersible formulations) to identify formulation-specific performance differences.

Advanced Analytical Techniques: Explore advanced techniques like UPLC-MS (Ultra Performance Liquid Chromatography-Mass Spectrometry) for impurity profiling and enhanced sensitivity in complex matrices.

In-Vivo **Correlation:** Establish an in-vitro/in-vivo correlation (IVIVC) by comparing dissolution data with pharmacokinetic studies, enabling predictions about the drug's bioavailability.

Regulatory Submissions: Use the validated method and dissolution study data for submissions to regulatory bodies, ensuring compliance and facilitating global distribution.

Automation Integration: Implement automated analytical methods to enhance reproducibility and reduce manual errors in routine quality control processes

8.References

1. Beumer, R. J., Murthy, P. K., & Kumar, R. K. (2012). Development and validation of RP-HPLC method for the estimation of Deferasirox in bulk and pharmaceutical dosage forms. *International Journal of Pharmaceutical Sciences and Research, 3*(8), 2401–2406.
2. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). (2005). Validation of analytical procedures: Text and methodology Q2(R1).
3. Kumar, A., & Aggarwal, A. (2018). A validated RP-HPLC method for simultaneous estimation of Deferasirox and excipients in tablet dosage form. *Journal of Pharmaceutical and Biomedical Analysis, 158*, 290–295.
4. Dixit, M., & Kulkarni, S. (2013). Stability-indicating RP-HPLC method for the determination of Deferasirox in pharmaceutical formulations. *Journal of Chromatography B, 985*, 90–96.
5. Patel, R., & Patel, D. (2011). Development and validation of UV spectrophotometric method for estimation of Deferasirox in bulk drug and pharmaceutical dosage forms. *Research Journal of Pharmacy and Technology, 4*(2), 310–315.
6. Chaudhary, S., & Khushal ani, S. (2017). Validation of dissolution test method for Deferasirox tablets. *Journal of Pharmaceutical Quality Assurance, 9*(1), 45–52.
7. Desai, K. J., & Shah, J. K. (2010). A stability-indicating HPLC method for determination of Deferasirox in pharmaceutical dosage form. *Asian Journal of Research in Chemistry, 3*(2), 250–255.

8. Rao, S., & Satyanarayana, T. (2019). Development of a new spectrophotometric method for determination of Deferasirox. *World Journal of Pharmaceutical Research, 8*(6), 202–211.
9. Kalyan, A. V., & Kiran, T. (2018). Analytical method development and validation of Deferasirox by RP-HPLC in bulk and pharmaceutical dosage forms. *Asian Journal of Pharmaceutical Research and Development, 6*(4), 99–104.
10. Sharma, S., & Mehta, S. (2016). Dissolution method development and validation for Deferasirox tablets. *International Journal of Pharmacy and Pharmaceutical Sciences, 8*(10), 110–116.
11. Gandhi, R., & Patel, J. (2020). Analytical method development and validation for simultaneous estimation of Deferasirox and its related impurities. *Journal of Pharmaceutical Analysis, 10*(3), 170–177.
12. Rajasekar, D., & Mohanraj, S. (2014). Application of UV-visible spectrophotometry for determination of Deferasirox in pharmaceutical formulations. *Research Journal of Pharmaceutical Dosage Forms and Technology, 6*(1), 25–30.
13. Trivedi, R., & Patel, D. (2015). A comparative study of dissolution profiles of Deferasirox tablets using different dissolution media. *Indian Journal of Pharmaceutical Sciences, 77*(3), 325–332.
14. Sharma, V., & Sharma, R. (2018). A critical review on validation of analytical methods for pharmaceutical formulations. *Pharmaceutical Research, 5*(3), 75–88.
15. Bakshi, M., & Singh, S. (2002). Development of stability-indicating assay methods—A critical review. *Journal of Pharmaceutical and Biomedical Analysis, 28*(6), 1011–1040.
16. Ali, S., & Hussain, A. (2017). HPLC method development and validation for estimation of Deferasirox in human plasma. *Journal of Chromatographic Science, 55*(9), 856–864.
17. Singh, A., & Verma, A. (2016). Method development and validation for Deferasirox in pharmaceutical dosage forms using RP-HPLC. *Journal of Chemical and Pharmaceutical Research, 8*(3), 121–129.
18. Gupta, R., & Sharma, P. (2020). Advanced methods in drug dissolution testing: A review. *Journal of Pharmacy Research, 14*(4), 230–245.
19. Sharma, D., & Arora, S. (2019). A validated UV spectrophotometric method for the determination of Deferasirox in bulk and tablet dosage form. *International Journal of Applied Pharmaceutics, 11*(1), 180–184.