Sharun Pharmaceuticals Private Limited
R.S. No. 195/9 & 197/4, Pangoor, Ariyar Revenue Village, Villianur, Puducherry - 605 102
TIN.No. 34740024209, C.S.T.No.: 34740024209 Dt.03.00.2016, DL.No.: 16 13 4220, 16 22 4221

Date : 10/08/2020

To

The Principal,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
TN Palayam.

Subject: Proposal for Research Collaboration – Reg.

Dear Sir,

Greetings. I am writing on behalf of **Sharun Pharmaceuticals Private Limited** to propose a collaboration that aligns with our mutual interests and scientific objectives.

We are impressed by your institution's expertise and research capabilities, particularly in the area of pharmacology and the study of natural extracts. Our organization is keen to explore the possibility of engaging JKK Munirajah Institute of Health Sciences College of Pharmacy in conducting research on the **"Develop and Validate Trioxsalen Pharmaceutical Dosage Form".**

Our interest in this project stems from our dedication to advancing pharmaceutical research and developing innovative solutions to combat hyperlipidaemia. Given the esteemed reputation of your institution, we believe that a collaboration with JKK Munirajah Institute of Health Sciences College of Pharmacy would significantly enhance our research efforts in this specific area.

In this regard, we would like to propose that your institution undertakes the research project outlined above, with funding and logistical support provided by **Sharun Pharmaceuticals Private Limited**. We are committed to ensuring the success of this project and will facilitate all necessary resources required for its completion.

Thanking you,

Sincerely,

For SHARUN PHARMACEUTICALS PVT. LTD.

Managing Director

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

SHARUN PHARMACEUTICALS PRIVATE LIMITED ★ Pangoor PUDUCHERRY 605 102

JKK Munirajah Institutes of Health Sciences College of Pharmacy ★ T.N.Palayam Erode Dt.

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY

(Approved by Tamil Nadu Govt. & Pharmacy Council of India - New Delhi, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai)
Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

13.08.2020

To

Sharun Pharmaceuticals Private Limited,
Ariyur Revenue, Villianur,
Puducherry, 605 102.

Subject: Response to Proposal for Research Collaboration – Reg.

Dear Sir,

Greetings. We greatly appreciate your interest in collaborating with JKK Munirajah Institute of Health Sciences College of Pharmacy for the research project titled **"Development and Validation of Analytical Methods for the Estimation of Trioxsalen Pharmaceutical Dosage Form by Using Uv-Spectrophometry, HPTLC and RP-HPLC"**

First and foremost, we are honoured and excited about the possibility of working with Sharun Pharmaceuticals Private Limited on this significant research endeavour. Your organization's dedication to advancing pharmaceutical research resonates with our mission to contribute to the field of pharmacology and improve healthcare outcomes.

We are enthusiastic about the potential impact of this collaboration. The research project aligns perfectly with our expertise and ongoing efforts in the area of natural extracts and their therapeutic applications. We believe that this partnership will not only enhance our research capabilities but also foster valuable contributions to the scientific community.

We would like to express our gratitude for your willingness to provide financial support and logistical assistance for this project. We are confident that this collaboration will yield substantial results and Validation of Analytical Methods for the Estimation of Trioxsalen Pharmaceutical Dosage Form.

To move forward, our team is excited to engage in this research endeavour and is committed to ensuring the successful completion of the project. Please let us know your availability, and we will coordinate a meeting that accommodates your schedule. You can reach me at **principal@jkkmihscp.org** to coordinate further.

T.N.Palayam
Erode Dt.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

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DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

We look forward to a productive partnership and the opportunity to contribute meaningfully to the advancement of pharmaceutical research.

With reference to the letter dated 10/08/2020, JKKMIHSCP is permitting the following faculty members to do collaborative research with Sharun Pharmaceuticals Private Limited and a proposal on the mentioned title "Development and Validation of Analytical Methods for the Estimation of Trioxsalen Pharmaceutical Dosage Form by Using Uv-Spectrophometry, HPTLC and RP-HPLC" is submitted along with this letter. The faculty members were assigned to do research work with Sharun Pharmaceuticals Private Limited.

Principal Investigator (PI):	DR. P. MOHANRAJ, Professor, Department of Pharmaceutical Chemistry, JKKMIHSCP.
Co-Investigators:	Mr. M. PUSHPARAJ, Assistant Professor, Mrs. K. ABHENAYA, Assistant Professor, Department of Pharmaceutical Chemistry, JKKMIHSCP.

Thanking you,

Principal Investigator

Principal
JKK Munirajah Institute of Health Science
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Principal
JKK Munirajah Institute of Health Science
College of Pharmacy, T.N.Palayam
Gobi (Tk), Erode (Dt) - 638 506

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T.N.Palayam
Erode Dt.

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DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

BUDGET AND FACULTY DETAILS

Project Title: Development and Validation of Analytical Methods for the Estimation of Trioxsalen Pharmaceutical Dosage Form by Using Uv-Spectrophometry, HPTLC and RP-HPLC.

Name of the Institution: JKK Munirajah Institute of Health Sciences College of Pharmacy, T. N. Palayam.

Project Duration: 6 months

Project Budget Estimation:

S. No	Detail of Expenditure	Amount
1.	*UV-Spectrophotometer for analytical work*	40000
2.	*High-Performance Thin-Layer Chromatography (HPTLC) equipment and supplies*	30000
3.	*Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) equipment and supplies*	45000
4.	*Trioxsalen standards and reference materials*	15000
5.	Laboratory Consumables (Glassware, Solvents, Reagents)	15000
6.	Personnel Costs	20000
7.	Data Analysis Software and Tools	15000
	Total Budget	**1.8 lakhs**

We kindly request an opportunity to discuss this funding application further. Your support will contribute significantly to the success of our project.

Thank you

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Yours Sincerely,

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

04285 - 262220 jkkmihscp@gmail.com www.jkkmihscp.org

Sharun Pharmaceuticals Private Limited
R.S. No. 195/9 & 197/4, Pangoor, Ariyar Revenue Village, Villianur, Puducherry - 605 102
TIN No.: 34740024209, C.S.T.No.: 34740024209 Dt.03.00.2016, DL.No.: 16 13 4220, 16 22 4221

Date : **Date: 26/08/2020**

To,

The Principal,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
T.N. Palayam, Erode, 638506.

Copy to: HOD/Principal Investigator/Co-investigator

Sub: Project Acceptance and Sanction Order – Reg.

Dear Sir/Madam,

We are greatly privileged to offer the grant of **Rs. 1,50,000/-** (Rupees One lakh fifty thousand only) to the project "Development and Validation of Analytical Methods for the Estimation of Trioxsalen Pharmaceutical Dosage Form by Using Uv-Spectrophometry, HPTLC and RP-HPLC". The project will be carried forward during the period of 6 months by the team members of DR. P. MOHANRAJ as a Principal Investigator, Mr. M. PUSHPARAJ, Mrs. K. ABHENAYA as a Co-investigator of JKK Munirajah Institute of Health Sciences College of Pharmacy. We would extend our continuous support throughout the implementation of the project.

Thanking You

Sincerely

For SHARUN PHARMACEUTICALS PVT. LTD.

Managing Director

Copy to:
DR. P. MOHANRAJ.
Professor, JKKMIHSCP.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

SHARUN PHARMACEUTICALS PRIVATE LIMITED ★ Pangoor PUDUCHERRY 605 102

JKK Munirajah Institutes of Health Sciences College of Pharmacy ★ T.N.Palayam Erode Dt.

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY

(Approved by Tamil Nadu Govt. & Pharmacy Council of India - New Delhi, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai)
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DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

PROJECT COMPLETION REPORT

Title of the Project: Development and Validation of Analytical Methods for the Estimation of Trioxsalen Pharmaceutical Dosage Form by Using Uv-Spectrophometry, HPTLC and RP-HPLC.

Category of the Project: Research project

Date of approval of competent authority : 26/08/2020

Total cost of the Project : Rs: 1,50,000/-

S. NO.	ITEMS	AMOUNT (₹) IN LAKHS
1.	UV-Spectrophotometer for analytical work	0.5
2.	*High-Performance Thin-Layer Chromatography (HPTLC) equipment and supplies*	0.4
3.	Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) equipment and supplies	0.45
4.	*Trioxsalen standards and reference materials*	0.05
5.	*Laboratory Consumables (Glassware, Solvents, Reagents)*	0.10
	Total	**1.5 Lakhs**

Date of start of the project : 26/08/2020

Date of completion of project : 24/02/2021

Name and Signature of Principal Investigator

Dr. P. Perumal

Principal
JKK Munirajah Institute of Health Science
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

Name and Signature of Co-investigator

(M. PUSHPARAJ) (K. Abheraya)

Sharun Pharmaceuticals Private Limited
R.S. No. 195/9 & 197/4, Pangoor, Ariyar Revenue Village, Villianur, Puducherry - 605 102
TIN.No.:34740024209, C.S.T.No.: 34740024209 Dt.03.00.2016, DL.No.: 16 13 4220, 16 22 4221

Date :

LETTER OF APPRECIATION

Date: 26.02.2021

To,

The Principal,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
T.N. Palayam, Erode, 638506.

Subject: Completion of project – reg.

Dear Sir,

With reference to above cited subject, **Sharun Pharmaceuticals Private Limited**, extend sincere gratitude towards JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N. Palayam, for successfully completion of project "Development and Validation of Analytical Methods for the Estimation of Trioxsalen Pharmaceutical Dosage Form by Using Uv-Spectrophometry, HPTLC and RP-HPLC "

We also appreciate sincere efforts taken by DR. P. MOHANRAJ, for guiding and valuable suggestions provided for completion of this project.We look forward to the continuation of our successful partnership and to exploring new opportunities for collaboration.

Thank you

Sincerely,

For SHARUN PHARMACEUTICALS PVT.LTD.

Managing Director

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

SHARUN PHARMACEUTICALS PRIVATE LIMITED ★ Pangoor PUDUCHERRY 605 102

JKK Munirajah Institutes of Health Sciences College of Pharmacy ★ TN.Palayam Erode Dt.

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
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Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

UTILIZATION CERTIFICATE

Certified that out of Rs. 1,50000/- sanctioned by **Sharun Pharmaceuticals Private Limited** towards financial assistance for the student project titled "Development and validation of Analytical Methods for the estimating of Triexsalon Phamulical Dosage form by UV" an amount of Rs. 1,50000/- was utilized for the purpose for which it was sanctioned, leaving a balance of Rs: 0 at the close of 24/02/2021. As shown in the Statement of Expenditure annexed.

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt

Name & Signature
of the Principal Investigator
Dr. P. mohanraj

Name & Signature
of Head of Institution
Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

"DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE ESTIMATION OF TRIOXSALEN PHARMACEUTICAL DOSAGE FORM BY USING UV-SPECTROPHOMETRY, HPTLC AND RP-HPLC"

PRINCIPAL INVESTIGATOR

DR. P. MOHANRAJ, M. Pharm., Ph. D.,

Professor,

Department of Pharmaceutical Chemistry,

CO-INVESTIGATORS

Mr. M. PUSHPARAJ, M. Pharm.,

Assistant Professor,

Department of Pharmaceutical Chemistry,

Mrs. K. ABHENAYA, M. Pharm.,

Assistant Professor,

Department of Pharmaceutical Chemistry,

JKK Munirajah Institutes of Health Sciences College of Pharmacy T.N.Palayam Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

FEBRUARY-2021

JKKMUNIRAJAHINSTITUTEOFHEALTHSCIENCES

COLLEGEOFPHARMACY,

T.N-PALAYAM-638506,

GOBI (TK), ERODE (DT), TAMILNADU.

DECLARATION

This is to certify that the Research entitled **"DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE ESTIMATION OF TRIOXSALEN PHARMACEUTICAL DOSAGE FORM BY USING UV-SPECTROPHOMETRY, HPTLC AND RP-HPLC"** submitted to The **Sharun Pharmaceuticals Private Limited,** is the bonafide project work carried out in the Department of Pharmaceutical chemistry, JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode, Under the guidance of **DR. P. MOHANRAJ, M. Pharm., Ph. D, Professor, Department of Pharmaceutical Chemistry,** JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N Palayam,Gobi, Erode. During the academic year 2020-2021.

Place: T.N-Palayam

Date: 24.02.2021

DR. P. MOHANRAJ M. Pharm., Ph. D.,
Principal Investigator

Mr. M. PUSHPARAJ, M. Pharm.,
Co-Investigator

Mrs. K. ABHENAYA, M. Pharm.,
Co-Investigator

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institute of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

DECLARATION

The research work emboided in this work entitled "Development and Validation of Analytical Methods for the Estimation of Trioxsalen Pharmaceutical Dosage form by Using Uv-Spectrophometry, HPTLC and RP-HPLC" was carried out by us under the direct supervision of DR. P. Mohanraj, M. Pharm., Ph. D, Professor, Department of Pharmaceutical Chemistry, JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N Palayam, Gobi.

The Project submitted to the **Sharun Pharmaceuticals Private Limited,** during the academic year 2020-2021.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt

ACKNOWLEDGEMENT

First and for most we express our heartful sense of gratitude and faithfulnessto God 'grace and our family members, which has enabled us to finish our project work successfully.

With the blessing of our Founder chairman Dr. J.K.K Munirajah, M.Tech, (Bolton). D.Litt., and Secretary Mrs. Kasthuripriya Kirupakarmurali, M.B.A.,

J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode for providing all the facilities to carry out this work.

Our sincere gratitude to our beloved sir, Dr. P.Perumal, M.Pharm, Ph.D,FIC., Principal and Head of the Department of Pharmaceutical Chemistry, J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode for his kindly support for our project work and for his encouragement and also providing all facilities in this Institute to the fullest possible extent enabling us to complete this work.

With the immense pleasure and pride, we would to take opportunity in expressing our deep sense of gratitude to our beloved guide DR. P. MOHANRAJ, M. Pharm., Ph.D., Professor, Department of Pharmaceutical Chemistry J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode under whose active guidance, innovate ideas, constant inspiration and encouragement of the work entitled "Development and Validation of Analytical Methods for the Estimation of Trioxsalen Pharmaceutical Dosage form by Using Uv-Spectrophometry, HPTLC and RP-HPLC" has been carried out.

We also express our grateful thanks to all the teaching and non-teaching staff members of J.K.K Munirajah Institute of Health Sciences College of Pharmacy for their

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

valuable advice and cooperation.

We express our heartful gratitude to the algmighty, for giving us the right way to achieve the best of our project.

We would like to give sincere thanks to our classmates for their timely help and co-operation.

We also extend our thanks to all staff members of Department of Pharmaceutical Biotechnology, Pharmaceutical Chemistry, Pharmacognosy, Pharmaceutics and Pharmacology for their co-operation.

We would like to Thank to Sharun Pharmaceuticals Private Limited, give a Financial and moral support to completion of the project being a successful manner on the duration of 2020-2021.

Last but not least, great thanks from the heart to our beloved MOTHER and FATHER. They are our living god, as who guided us in the rightful way to achieve all our activities. They gave the incredible effort to become a successful for bright future in this world. Thanks a lot, to my parents.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
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T.N.Palayam
Erode Dt.

ABSTRACT:

A new simple, accurate, rapid and precise Gradient High performance liquid chromatographic (HPLC) method was developed and

Validated for the determination of Trioxsalen C18 Column (4.6 x 150 mm and 3.5 µm) and flow rate of 1.2 ml/min with a load of

20µl. phosphate buffer (PH-3.9) was used as mobile phase A Methanol was used as Mobile Phase B and Diluent as Water: acetonitrile

in the composition of 50:50. The Detection was carried out at 248nm.This newly developed method was successfully utilized for the

Quantitative estimation of Trioxsalen in pharmaceutical dosage forms. This method was validated as per ICH guidelines.

Keywords:

UV -Ultraviolet visible, µg -Micro gram, ml- Milliliter, nm- Nano meter, RP-HPLC-Reverse phase chromatography

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T.N.Palayam
Erode Dt.

INTRODUCTION

The goal of every chemical analysis is to obtain the required information within a period of time acceptable to the customer. This means that the analyst must know what information is needed to solve the current problem. Once the goals and limits of the analysis are defined and the literature search is completed, a more detailed plan of action must be developed.

The analyst selects the method or combination of methods most likely to provide the desired information. A good analyst is always alert to the chemistry involved in the analysis as well as instrumental techniques. Thus, a combination of chemical and instrumental techniques may be used. Major components to be considered in planning an analysis are shown in fig each component is important in obtaining reliable information from the analysis.

Field sampling and laboratory sub-sampling procedures must be designed to ensure integrity of results. Proper procedures must be used to store both sample and standard. All samples must be properly labeled and recorded. Laboratory operations are often performed on samples before measurement. Analytical chemistry is a branch of chemistry that deals with the separation, identification and determination of components in a sample. It is the science of making quantitative measurements, which requires background knowledge of chemical and physical concepts of chemistry.

Analytical method development

Documentation starts at the very beginning of the development process. A system for full documentation of development studies must be established. All data relating to these studies must be recorded in laboratory notebook or an electronic database. The number of drugs introduced into the market is increasing every year.

These Drugs may be either new entities or partial structural modification of the existing one. Very often, there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopeias. This happens the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (Resulting in their withdrawal from the market).development of Patient resistance and introduction of better drugs by competitors, under these conditions, standards and analytical

procedures for these drugs may not be available in the Pharmacopeia, it becomes necessary, Therefore to develop newer analytical methods for such drugs.

Analytical method validation

Analytical monitoring of a pharmaceutical productor of specific ingredients within the product is necessary to ensure its safety efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specification selaborated during product development. Analytical validation is the corner stone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do. All new methods developed are validated

OBJECTIVE

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH, typical analytical performance characteristics that should be considered in the validation of the types of methods are:

- Accuracy
- Precision
- Specificity
- Detection limit
- Quantitation limit
- Linearity
- Range
- Ruggedness
- Robustness
- System suitability

Principal
JKK Munirajah Institute of Health Sciences
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T.N.Palayam
Erode Dt.

MATERIAL AND METHODS

High Performance Liquid Chromatography (HPLC)

Reagents and Chemicals

• Trioxsalen working standard powder was gifted by Sankalp healthcare and Allied products Pvt.ltd. Mumbai and was used without purification.

• Trioxsalen tablets USP containing 25mg were purchased from local pharmacy (TROID-25, Manufactured by Resilient Cosmeceuticals Pvt.ltd)

• HPLC grade water, methanol, acetonitrile and monobasic potassium phosphate for the preparation of the buffer were purchased from Merck specialties pvt.ltd. (Mumbai, India)

• All solutions were filtered through 0.45 micron membrane filters purchased from Merck specialties Pvt.ltd, (Mumbai, India)

• All chemicals were of analytical grade unless stated otherwise and used as received. HPLC grade water was used to prepare all solutions.

Preparation of standard drug solution in methanol

Weighed accurately 10 mg of Trioxsalen RS and transferred into a 100ml standard flask. About 25ml of methanol was added and the solution was sonicated for 10 minutes, finally the volume was made up to 100ml with methanol.

The resultant solution had a concentration of 100 mcg/ml (stock solution).

Optimization of HPLC conditions

Optimization was performed by injecting 20microliter standard drug solution (diluted to the UV linearity range) into the column, keeping a run time of 30 minutes.

Mobile phase selection

Selection of mobile phase was performed by trial and error method.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

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T.N.Palayam
Erode Dt.

Different combinations of Acetonitrile, methanol and phosphate buffer were tested and the data's obtained are given below;

Table 1 : Solvent system selection trial and error data.

Methanol acetonitrile	90:10	--
Methanol : acetonitrile	80:20	--
Methanol :acetonitrile	70:30	--
Methanol acetonitrile	60:40	--
Methanol: acetonitrile	55:45	--
Water: acetonitrile	50:50	--

Phosphate buffer (5. 8): acetonitrile 50:50 Peak obtained but with least resolution, tR more

pH of the buffer selected is a critical factor in sample retention as it keeps the sample substance in its fully ionized or neutral state.

• A rule of thumb is to choose a buffer with a PKa value ±2 unit of with that of the drug substance.

The drug Trioxsalen has got a Pka value of 2.7 and considerably the pH of the mobile phase has been selected.

Preparation of buffer

Phosphate buffer was used

- It was prepared as USP; A 5.04g concentration of the phosphate buffer was used. It was prepare by taking
- 0.84g of monobasic potassium phosphate in a 250ml standard flask and making up to the volume to get a

- Phosphate solution. Further this solution was simply used as a part of organic solvent after adjusting the Desired pH using Orthophosphoric acid and/ NaOH.

Optimization of mobile phase

- Phosphate buffer (pH 5.4): Acetonitrile (ACN) system was optimized by changing the ratio of the solvents.
- Table shows the different ratio that has been tried.

Table 2 : optimization of mobile phase

Phosphate buffer pH (5.4):CAN 50:50
Phosphate buffer pH (5.4):CAN 60:40
Phosphate buffer pH (5.4):CAN 70:30
Phosphate buffer pH (5,4):CAN 80:20
Phosphate buffer pH (5,4):CAN 55:45

Mobile phase: Phosphate buffer pH (5.4): ACN (55:45) was chosen as themobile phase, which gives a chromatogram with good resolution for Trioxsalen. Preparation of Mobile phase:

500ml of the mobile phase was prepared instantly and utilized for the analytical study. It constituted 275ml of phosphate buffer (pH (5.4) and 225ml of ACN.

Optimization of other chromatographic parameters

The tR (Retention time) was found to be 6.18 min. and the pump pressure and the stop time was set as 400 Bar and 15 minutes respectively.

- Isocratic mode of elution was performed
- Column was used in an ambient temperature condition

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• The detection wavelength was selected as 248nm, from the UV spectroscopic data of Trioxsalen.

• Peaks were analyzed at different flow rates (0.8-1.2ml/min.) and the optimum resolution with least retention time and peak tailing was obtained at a flow rate of 1.2ml/min.

• The tailing factor (<1.5) and theoretical plate count (>3000) was found to be optimum.

Preparation of calibration curve

Accurately pipette out 0.1, 0.2, 0.3, 0.4 and 0.5ml, respectively from the standard stock solution (100mcg/ml) into 5 and 10ml' volumetric flasks which were labeled appropriately and the volume was made up to 10 ml using HPLC grade methanol. Corresponding solutions had a concentration of 10, 20, 30, 40, and 50µg/ml respectively.

Development of chromatogram

1. Preparation of mobile phase

• Prepared by mixing 275ml of phosphate buffer with 225ml of acetonitrile.

• Then the mixture was filtered through 0.45 urn membrane filter under vacuum.

• The obtained mobile phase was further sonicated for 10 minutes for the removal of entrapped air.

2. Initial instrumental setup

- Fill the mobile phase in the mobile phase reservoir. Purging was carried out for the removal of any dissolved air bubbles. Then the mobile phase was allowed to run through the column at a flow rate of 1.2ml/min. Sample was injected only after the stabilization of the base line.
- The detection wavelength was adjusted at 248nm. Stop time was set as 15 minute. Pump pressure was set as 400 bar.

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Table 3: Fixed instrumental parameters

Mobile phase flow rate 1.2ml/min.

Pump pressure 400 Bar

Stop time 15 min.

Detection wavelength 248 nm

3. Sample pretreatment

Each dilution of standard Trioxsalen solutions was passed through 0.22mm PVDF syringe filter for the removal of any minute particles.

4. Recording of chromatogram

First, the mobile phase (Phosphate buffer: ACN (55:45) was filled in the mobile phase reservoir.

The mobile phase was purged to remove the entrapped air then, the mobile phase was allowed to pass through the column at a flow rate of 1.2ml/min. for a certain period of time until the base line becomes stable.

After the stabilization of base line, 20ml of each concentration of the standard analyte solutions were injected into the column and the chromatogram was recorded at 248nm for 15 min. The retention time and area under the curve were noted and given under the table, the HPLC chromatograms of standard dilutions as well as the calibration plot was also given below.

Statistical evaluation of the calibration plot The calibration curve was plotted with peak area in the Y-axis and concentration (mcg/ml) in the X-axis. A linear plot was obtained within this concentration range. The correlation coefficient was obtained as 0.9999 and the regression equation was found to be Y=2,046,185.10x + 378,114.00.

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5. Preparation of sample solution

Details of analyzed dosage Form:

Trioxsalen tablets USP in the brand name TRO1D-25

Label claim: Trioxsalen tablets USP

Batch No: STH1505

Mfg.Date:JAN.2016

Exp.Date:DEC.2018

Mfd. by: Resilient Cosmeceuticals PvLtd.

Accurately weighed 10 no's of TROID-25, Trioxsalen tablets and peeled off the outer film coating. Then, it was finely powdered and a weight equivalent to l0mg (0.1235) was taken.

The weighed amount of drug was transferred into a stoppered flask, about 50rnl of methanol was added and sonicated for 10 minutes, and then the aggregate solution obtained was transferred into a 100ml standard flask by aiding filtration through whatmann filter paper (No.1).

The stoppered flasks as well as the residue obtained were washed through the filter paper, using methanol and the volume was made up to 100ml with the same. The final solutions constituted a concentration of l00mcg/ml. Suitable dilutions for the drug analysis were prepared from the final 100 mcg/ml solution. After the preparation, sample solutions were allowed to run through the column as mentioned above (in preparation of calibration curve) to obtain the respective chromatograms.

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Table 5: chromatographic data-sample solution

SI. No.	Conc, of Trioxsalen Sample solution (mcg/ml)	Peak area	Retention time (R)
1.	20	4101192	6.18
2.	30	6118546	6.18
3.	40	8230211	6.18

Estimation of the amount of Trioxsalen in tablet dosage form Quantitative estimation of drug was performed by comparing areas of the chromatograms obtained for the sample drug solution taken in three different concentrations with that of the standard.

Amount of drug present per table Peak area for sample x . = Peak area of standard dilution factor of sample dilution factor of standard x weight of standard x average weight -r weight of sample

Each tablet contains {label claim) 25mg

Average weight of 10 tablets = 0.3089g

Weight of sample taken, equivalent to 10 mg = 0.1235g

Weight of standard taken = 0.0100g

Table 6: Assay results

Concentration (mcg/ml)	Peak area (Sample)	Peak area (Standard)	Amount Present per tablet (gm)	Label Claim (%)
20	4101192	4130181	0.0248	99.2
30	6118546	6195272	0.0247	98.8
40	8230211	8260363	0.0249	99.6

Table 7: Assay results

Average content per tablet (in gm)	Average percentage label claim
0.0248	99.2%

6. Validation of the proposed method

a. Accuracy

Accuracy of the proposed method was determined by recovery study. The recovery studies were performed by standard addition method at three concentrations (80%, 100%, and 120%) and percentage recovery was calculated. 10 tablets of Troid-25 (containing 25mg of Trioxsalen) were taken, peeled off the outer film coat and weighed them accurately and finely powdered in a glass mortar, A weight equivalent to l0mg of Trioxsalen was weighed and transferred into a stoppered flask.

To this, accurately weighed 8mg of Trioxsalen RS was addedand extracted with 25ml of methanol initially by sonication for a period of 10 minutes. The solution was transferred to 10ml standard flask, through a whatmann No: 1 filter paper. The residue was further extracted twice with 10ml each of methanol and passed through the same filter paper and the volume was finally made up with methanol (10mcg/ml).

About 1.5 ml of this solution was transferred into a 10ml standard flask and the volume was made up to 10ml using methanol (to obtain a concentration within the linearity range).20ul of this solution was injected into the column to obtain the corresponding chromatogram.

A standard solution of the same concentration (10mcg/ml) was prepared and chromatogram was developed in order to determine the peak area. In a similar way recovery studies for 100% and 120% were conducted and peak heights and peak areas for each were measured in triplicates for each level. The results are statistically evaluated and the data obtained are given below.

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RESULTS AND DISCUSSION

This research work was done to develop simple, accurate, and economic methods for the estimation of Trioxsalen in commercial tablet dosage forms. By fulfilling this objective,four individual methods were developed. Proper selection of the method depends on the nature of the sample, molecular weight and olubility. The selected drug Trioxsalen for present study was polar. Polar compounds can be separated by Reverse phase chromatography and hence RP-HPLC method was selected for separation of the drug in tablet dosage form.

C18 column was chosen as stationary phase and a mixture of buffer and acetonitrile was used as mobile phase. Then it was proceeded with different ratios for the achievement of suitable resolution. During selection and optimization of mobile phase, it was observed that the retention time of Trioxsalen is decreased with increase in the proportion of organic modifiers like acetonitrile in the mobile phase. The sharpness of the peak is achieved by increasing the proportion of acetonitrile whereas an increase in the proportion of aqueous phase resulted in broadening of the peak.

The pH of the mobile phase was chosen to be at least two units away from the pKa of the drug ubstance. The drug has got a pKa of 2.7 and consequently the pH of the buffer for the composition of mobile phase was selected as 5.4. The selection of wavelength was based on the Xmax obtained by the scanning of standard drug solution within a range of 200-400nm in a UV spectrometer and it was taken as 248nm. Phosphate buffer (pH 5.4): Acetonitrile in the ratio 55:45, v/v, was chosen as the mobile phase. The flow rate was set as 1.2ml/min., pump pressure 400 Barr and the stop time 15 minutes.

The established system gave good resolution and optimum retention time with appropriate tailing factor (<2) number of theoretical plates (>2000). After setting up the method in the laboratory drug solutions were prepared and analyzed. The Rt was found to be 6.18.

Calibration curve for the drug was plotted by using the peak area in the ordinate and concentration in the abscissa. A linearity range of 10-50mcg/ml was obtained with a good correlation of 0.9999. TROID-25, the marketed product was analyzed by the developed method and gave good results. Evaluating the peak area, percentage label claim of Trioxsalen was found to be 99.2% and the amount present per tablet, 0.0248gm.

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• Accuracy was determined by recovery study employing the standard addition method at three levels (80%, 100%, 120%). The percentage recovery was found to be more than 99%,

• The precision of the method was studied by two methods; repeatability and intermediate precision. The percentage RSD was found to be<2. The LOD and LOQ of the method were obtained respectively as 0.28mcg/ml

• The developed HPLC method ensued good, accurate, reproducible and reliable analytical results and therefore can be extended for the estimation of Trioxsalen in its tablet dosage formulation.

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CONCLUSION

More rapid, precise, specific, sensitive, economic, and reproducible, gradient reverse phase HPLC method was developed and validated for simultaneous determination of Trioxsalen tablets. The method was validated for specificity, linearity, and precision as per ICH guidelines.

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