Vertex Pharma Chemicals
60-A & 61 - A, RBF, Unit -3 Pipdic Electronic Park,
Thirubuvanai, Puducherry - 605 107.
TIN : 34880013947
CST: 34880013947 Dt : 14-07-10
DL.No. : 11 13 2992
DL.No. : 11 23 2993

Date : 27/12/2022

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 **Vertex Pharma Chemical** 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 **"Bioequivalence Evaluation of Remogliflozin Tablet Using RP-HPLC Method."**

Our interest in this project stems from our dedication to advancing pharmaceutical research and developing innovative solutions to Bioequivalence Evaluation of Remogliflozin. 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 **Vertex Pharma Chemicals.** We are committed to ensuring the success of this project and will facilitate all necessary resources required for its completion.

Thanking you,

Sincerely,

For VERTEX PHARMA CHEMICALS

Managing Director

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

VERTEX PHARMA CHEMICALS ★ Puducherry 605 107

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

06.01.2023

To

Vertex Pharma Chemical,
Electronic Park,
Puducherry.

Subject: Response to Proposal – Reg.

Dear Sir,

I hope this letter finds you well. We greatly appreciate your interest in collaborating with JKK Munirajah Institute of Health Sciences College of Pharmacy for the research project titled **"Bioanalytical Method Development and Bioequivalence Evaluation of Remogliflozin Tablets in Wister Rat Plasma Using RP-HPLC Method."**

First and foremost, we are honoured and excited about the possibility of working with **Vertex Pharma Chemical** 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 have carefully reviewed your proposal, and 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 advancements in the understanding and treatment of hyperlipidaemia.

To move forward, we propose scheduling a meeting to discuss the specific details of the collaboration, including project timelines, budget considerations, and other essential aspects. 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.

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

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

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 27/12/2022, JKKMIHSCP is permitting the following faculty members to do collaborative research with Vertex Pharma Chemical, Puducherry and a proposal on the mentioned title "Bioanalytical Method Development and Bioequivalence Evaluation of Remogliflozin Tablets in Wister Rat Plasma Using RP-HPLC Method" is submitted along with this letter. The faculty members were assigned to do research work with Vertex Pharma Chemical, Puducherry.

1. Ms. K. KANAGAPRIYA, Assistant Professor, Department of Pharmaceutical Chemistry.
2. Ms. RAJESHWARI. M. K (Reg. No: 261121516509), Student, M.Pharm.
3. Ms. KAVIYA. K (Reg. No: 261121516503), Student, M.Pharm.
4. Mr. VASUDEVAN. G (Reg. No: 261121516511), Student, M.Pharm.

Kindly permit the above faculty members to execute the above research work. We are expecting a positive reply from your end.

Thanking you,

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

Principal 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

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

BUDGET AND FACULTY DETAILS

Project Title: Bioanalytical Method Development and Bioequivalence Evaluation of Remogliflozin Tablets in Wister Rat Plasma Using RP-HPLC Method

Project Duration: 3 months

Project Budget:

S. No	Budget category	Amount in lakhs
1.	High-Performance Liquid Chromatography (HPLC) equipment	0.45
2.	RP-HPLC Column and Consumables	0.20
3.	Remogliflozin standards and reference materials	0.15
4.	Laboratory Consumables (Reagents, Solvents, etc.)	0.15
5.	Personnel Costs (Salaries, stipends for researchers)	0.15
6.	Data Analysis Software and Tools	0.10
	Total Budget	**1.20 lakhs**

Project Team:

Principal Investigator (PI):	Ms. K. KANAGAPRIYA Assistant Professor, Department of Pharmaceutical Chemistry, JKKMIHSCP.
Student-Investigators:	Ms. RAJESHWARI. M. K Ms. KAVIYA. K Mr. VASUDEVAN. G

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

Vertex Pharma Chemicals
60-A & 61 - A, RBF, Unit -3 Pipdic Electronic Park,
Thirubuvanai, Puducherry - 605 107.

TIN : 34880013947
CST: 34880013947 Dt : 14-07-10

DL.No. : 11 13 2992
DL.No. : 11 23 2993

Date :

10.01.2023

Dear Principal,

Sub: Financial assistance for Research Project scheme of **Vertex Pharma Chemicals** - Reg.

This is to invite your attention to the reference cited and to inform that the project proposal titled **"BIOANALYTICAL METHOD DEVELOPMENT AND BIOEQUIVALENCE EVALUATION OF REMOGLIFLOZIN TABLETS IN WISTER RAT PLASMA USING RP-HPLC METHOD"** submitted by Ms. K. KANAGAPRIYA as Principal Investigator, Ms. RAJESHWARI. M. K, Ms. KAVIYA. K, Mr. VASUDEVAN. G, as Student-investigators for the project, and it has been approved. An amount of **₹ 1,20,000 /-** is sanctioned by the **Vertex Pharma Chemicals.** The budget estimate of the project is as detailed below.

SL.NO.	ITEMS	AMOUNT (₹)
1.	High-Performance Liquid Chromatography (HPLC) equipment	45000
2.	RP-HPLC Column and Consumables	20000
3.	Remogliflozin standards and reference materials	15000
4.	Laboratory Consumables (Reagents, Solvents, etc.)	15000
5.	Personnel Costs (Salaries, stipends for researchers)	15000
6.	Data Analysis Software and Tools	10000
	Total	**1,20,000**

The PI has to submit the signed Terms and Conditions (as per the guidelines) and the date of start of the project within two weeks to the undersigned. The project should be completed within six months and submit the certified soft copy of the final report, Statement of Expenditure and Utilization Certificate counter signed by the Head of the Institution for releasing the grant.

Thanking you,

Sincerely

For VERTEX PHARMA CHEMICALS

Managing Director

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

VERTEX PHARMA CHEMICALS Puducherry 605 107

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

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

PROJECT COMPLETION REPORT

Title of the project : Bioanalytical Method Development and Bioequivalence Evaluation of Remogliflozin Tablets in Wister Rat Plasma Using RP-HPLC Method

Category of the project : Research project

Date of approval of competent authority : 10/01/2023

Total cost of the project : Rs: 1,20,000/-

S.NO.	ITEMS	AMOUNT (₹)
1.	*High-Performance Liquid Chromatography (HPLC) equipment*	*45000*
2.	*RP-HPLC Column and Consumables*	*20000*
3.	*Remogliflozin standards and reference materials*	*15000*
4.	*Laboratory Consumables (Reagents, Solvents, etc.)*	*15000*
5.	*Personnel Costs (Salaries, stipends for researchers)*	*15000*
6.	*Data Analysis Software and Tools*	*10000*
	Total	***120000***

Date of start of the project : 10/01/2023

Date of completion of project : 05/04/2023

Name and Signature of Principal Investigator

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

Name and Signature of Co-investigator

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

Vertex Pharma Chemicals
60-A & 61 - A, RBF, Unit -3 Pipdic Electronic Park,
Thirubuvanai, Puducherry - 605 107.

TIN : 34880013947
CST: 34880013947 Dt : 14-07-10
DL.No. : 11 13 2992
DL.No. : 11 23 2993

LETTER OF APPRECIATION

Date :

Date: 10.04.2023

To

The Principal,

JKK Munirajah Institute of Health Sciences College of Pharmacy,

T.N. Palayam, Erode, 638506.

Subject: Completion of project – Reg.

Dear Sir,

We extend our heartfelt appreciation to you and your research team for the successful completion of the research project, "Bioanalytical MethodDevelopmentand Bioequivalence Evaluation of Remogliflozin Tablets inWisterRatPlasmaUsingRP-HPLC Method," which was supported by **Vertex Pharma Chemicals.**

Your dedication, commitment, and hard work throughout the project have resulted in valuable contributions to the field of pharmacology. The outcomes of this project have not only met but exceeded our expectations, and we are excited about the potential impact of your research findings.

We look forward to the dissemination of your research findings and anticipate that they will further enrich the academic and professional community. Your success reflects positively on our mission to support meaningful research and innovation.

Once again, congratulations on the successful completion of this project, and thank you for your dedication to advancing knowledge in your field. We hope to continue our collaboration on future projects.

Thank you

Sincerely,

For VERTEX PHARMA CHEMICALS

Managing Director

Copy to:

Ms. K. KANAGAPRIYA
AssistantProfessor, JKKMIHSCP.

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

VERTEX PHARMA CHEMICALS
Puducherry
605 107

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

(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

UTILIZATION CERTIFICATE

Certified that out of Rs. ...1,20,000/-..... sanctioned by **Vertex Pharma Chemical, Puducherry.** towards financial assistance for the student project titled "..BIOANALYTICAL METHOD DEVELOPMENT AND BIOEQUIVALENCE EVALUATION OF REMOGLIFLOZIN TABLETS IN WISTEI ....RAT PLASMA USING RP-HPLC METHOD, an amount of Rs. ...1,20,000/-..... was utilized for the purpose for which it was sanctioned, leaving a balance of Rs. ...0-..... at the close of ..05/04/2023... 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

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

"BIOANALYTICAL METHOD DEVELOPMENT AND BIOEQUIVALENCE EVALUATION OF REMOGLIFLOZIN ETABONATE TABLETS IN WISTAR RAT PLASMA USING RP-HPLC METHOD"

PRINCIPAL INVESTIGATOR

Ms. K. KANAGAPRIYA, M.Pharm.,
Assistant Professor
Department of Pharmaceutical Chemistry

STUDENT-INVESTIGATORS

Ms. RAJESHWARI. M. K	**REGISTER NO: 261121516509**
Ms. KAVIYA. K	**REGISTER NO: 261121516503**
Mr. VASUDEVAN. G	**REGISTER NO: 261121516511**

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

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES COLLEGE OF PHARMACY
T.N.PALAYAM - 638 506
CARE COUNCIL CURE

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

APRIL-2023

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY,
T.N- PALAYAM-638506, GOBI (TK), ERODE (DT),
TAMILNADU.

CERTIFICATE

This is to certify that the Research entitled **"BIOANALYTICAL METHOD DEVELOPMENT AND BIOEQUIVALENCE EVALUATION OF REMOGLIFLOZIN TABLETS IN WISTER RAT PLASMA USING RP-HPLC METHOD"** submitted to The **Vertex Pharma Chemicals Pvt Ltd, Puducherry,** is the bonafide project work of **Reg.no**: **261121516509, 261121516503, 261121516511** and 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 **Ms. K. KANAGAPRIYA M. Pharm., Assistant Professor, Department of Pharmaceutical Chemistry,** JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam,Gobi, Erode. During the academic year 2022-2023.

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

Place: T.N-Palayam

Date: 05.04.2023

Dr. P. Perumal. M.Pharm,Ph.D,FIC

PRINCIPAL

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

DECLARATION

This is to certify that the Research entitled **"BIOANALYTICAL METHOD DEVELOPMENT AND BIOEQUIVALENCE EVALUATION OF REMOGLIFLOZIN TABLETS IN WISTER RAT PLASMA USING RP-HPLC METHOD"** submitted to The **Vertex Pharma Chemicals Pvt Ltd, Puducherry,** is the bonafide project work of **Reg.no**: **261121516509, 261121516503, 261121516511** was 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 **Ms. K. KANAGAPRIYA M. Pharm., Assistant Professor, Assistant Professor, Department of Pharmaceutical Chemistry,** JKK Munirajah Institute of Health Sciences College of pharmacy, T.N-Palayam, Gobi, Erode. During the academic year 2022-2023.

Place: T.N-Palayam

Date: 05.04.2023

Ms.K.KANAGAPRIYA M.Pharm.,

Principal Investigator

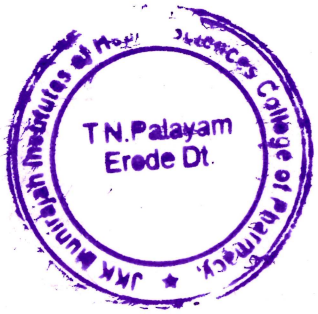
Principal
KK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

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

DECLARATION

The research work emboided in this work entitled "Bioanalytical Method Development and Bioequivalence Evaluation of Remogliflozin Tablets in Wister Rat Plasma Using RP-HPLC Method" was carried out by us under the direct supervision of Ms. K. Kanagapriya M. Pharm., Assistant Professor, Department of Pharmaceutical Chemistry, JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi.

The Project submitted to the **Vertex Pharma Chemicals Pvt. Ltd., Puducherry,** during the academic year 2022-2023.



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

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.,

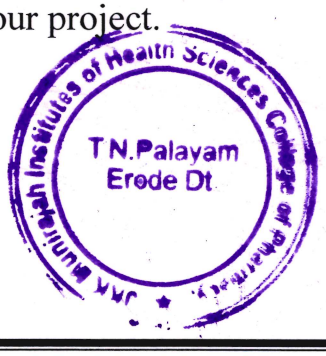
J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode for providing all the facilities to carry out this work.

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 alsoproviding 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 Ms.K.Kanagapriya M.Pharm., Assistant Professor, Department of Pharmacology 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 "Bioanalytical Method Development and Bioequivalence Evaluation of Remogliflozin Tablets in Wister Rat Plasma Using RP-HPLC Method" 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 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.



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

We would like to give sincere thanks to our classmates for their timelyhelp 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 Vertex Pharma Chemicals Pvt. Ltd., Puducherry, to give a Financial and moral support to completion of the project being a successful manner on the duration of 2022-2023.

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,
Gobi (Tk), Erode (Dt) - 638 506

1. INTRODUCTION

The pharmaceutical analysis may be a branch of chemistry, which involves the series of process for the identification, determination, quantitation, and purification.

Analytical instrumentation plays a crucial role within the production and evaluation of recent products and within the protection of consumers and therefore the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air.There are mainly two forms of analytical methods. They're as follows:

1. **Qualitative analysis**: This method is employed for the identification of the chemical compounds.

2. **Quantitative analysis:** This method is employed for the determination of the amount of the sample.

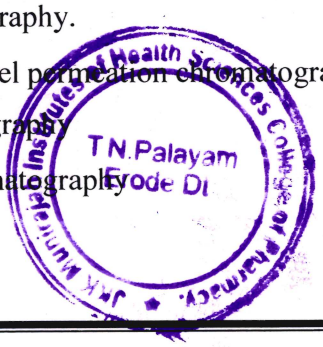
High-performance liquid chromatography (or high liquid chromatography, HPLC) could be a specific style of chromatography generally employed in biochemistry and analysis to separate, identify, and quantify the active compounds.[1]

CLASSIFICATION OF CHROMATOGRAPHIC TECHNIQUES

1. According to the character of stationary and mobile phase1 Gas Solid Chromatography.
2. Gas Liquid Chromatography.
3. Solid Liquid Chromatography.
4. Liquid Liquid Chromatography.

I. According to mechanisms of separation, chromatographic methods are divided into

- Adsorption chromatography.
- Partition chromatography.
- Ion exchange chromatography.
- Ion pair chromatography.
- Size exclusion or gel permeation chromatography
- Affinity chromatography
- Chiral phase chromatography



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

TYPES OF HPLC [2, 3]

Types of HPLC generally depend upon phase system employed in the method.

A. BASED ON MODE OF SEPERATION:

There are two modes, normal phase mode and reverse phase mode.

1. Normal phase chromatography: This method separates analytes supported polarity. NP- HPLC uses a polar stationary phase and a non-polar mobile phase. The polar analyte interacted with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity, and therefore the interaction between the polar analyte and also the polar stationary phase increases the elution time.

2. Reversed phase chromatography: Reversed phase HPLC (RP-HPLC or RPC)incorporates a non-polar stationary phase and an aqueous, moderately polar mobile phase. The binding of the analyte to the stationary phase is proportional to the contact area round thenon-polar segment of the analyte molecule upon association with the ligand within theaqueous eluent.

3. Size exclusion chromatography: Size exclusion chromatography (SEC), also called as gel permeation chromatography or gel filtration chromatography mainly separates particleson the idea of size. It's also useful for determining the tertiary structure and quaternary structure of proteins and amino acids. This method is widely used for the mass determination of polysaccharides.

4. Ion exchange chromatography: In Ion-exchange chromatography, retention relies on the attraction between solute ions and charged sites absolute to the stationary phase. Ions of the identical charge are excluded. This way of chromatography is widely utilized in purifying water, Ligand-exchange chromatography, Ion-exchange chromatography of proteins, High- pH anion-exchange chromatography of carbohydrates and oligosaccharides, etc.

5. Bio-affinity chromatography: Separation supported specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands.

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

B.BASED ON ELUTION TECHNIQUE

1. Isocratic elution: A separation that employs one solvent or solvent mixture of constant composition.

2. Gradient elution: Here two or more solvent systems that differ significantly in polarity are employed. After elution is begun; the ratio of the solvents is varied in an exceedingly programmed way, sometimes continuously and sometimes in a series of steps. Separation efficiency is greatly enhanced by gradient elution.

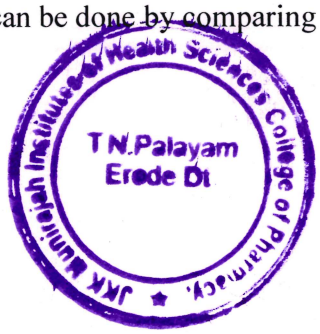
C.BASED ON SCALE OF OPERATION [4]

1. Analytical HPLC No recovery of individual components of substance.
2. Preparative HPLC Individual components of substance are often recovered.

D. BASED ON VARIETY OF ANALYSIS

1. Qualitative analysis: It's done to spot the compound, detect the presence of impurity, to seek out out the amount of components etc... This can be done by using retention time values.

6. Quantitative analysis: It's done to see the amount of the individual or several components in an exceedingly mixture. This can be done by comparing the height area of thequality and sample.



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

INSTRUMENTATION

The HPLC instrumentation involves:

1. Solvent reservoir
2. Pump
3. Injector
4. Column
5. Detector
6. Integrator and
7. Display system.

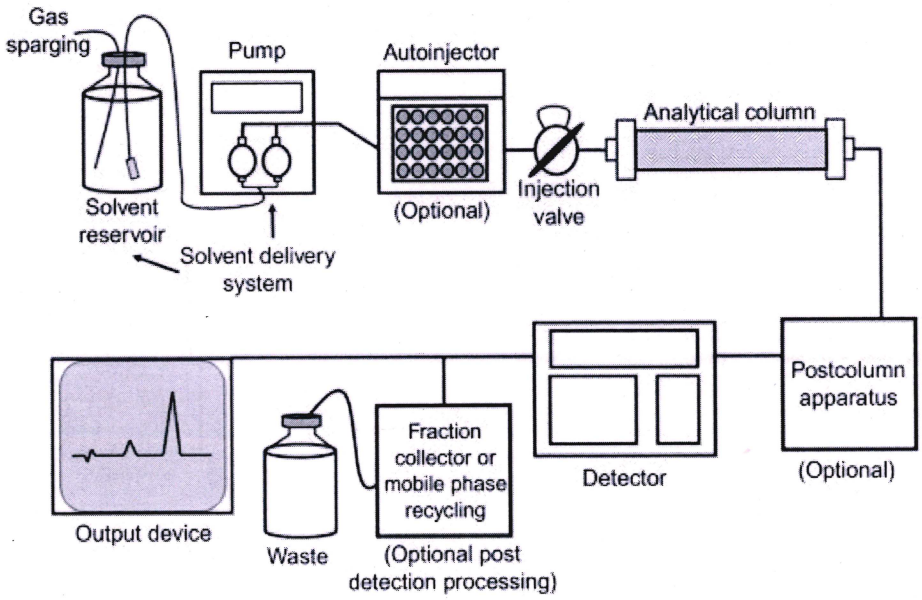


Fig.1: Instrumentation of HPLC.

In the column the separation occurs. The parts include:

Solvent Reservoir: The contents of mobile phase are present in glass container. In HPLC the mobile phase or solvent may be a mixture of polar and non-polar liquid components. Counting on the composition of sample, the polar and non-polar solvents are varied.

RESULTS AND DISCUSSION

The new RP- HPLC method was established for RGE and then applied on pharmaceutical dosage forms.

Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase comprising of buffer0.2%TEA(pH adjusted to 3 with Orthophosphoric acid) and methanol in the ratio 22:78 % v/v gave a better resolution and sensitivity.

The detection was carried out by using UV detector at 227 nm using Phenomenex C18 column (250 x 4.6 mm, 5µm). Among the several flow rates tested, the flow rate of 0.9 ml/min was found to be the best for RGE with respect to retention timesand theoretical plates.

The retention time was found to be 8.4min for RGE. The asymmetry factor or the tailing factor was found to be 1.1 for RGE, which shows symmetrical nature of the peak.

System suitability parameters such as retention time, tailing factor, capacity factorand number of theoretical plates were calculated. The number of theoretical plates was found to be around 2364 for RGE, which indicates efficient performance ofthe column. These parameters represent the specificity of the method.

Linearity range was calculated by the visual inspection of plot of peak area as a function of analyte concentration and the corresponding calibration curve was shown in table 3 and calibration curve was shown in fig.47

The withdrawal efficiency of RGE from rat plasma at concentration of 50, 100, 150 % and their accuracy was shown in table 4.

The validation of the proposed method was verified by system precision. The

%RSD for system precision of RGEwas shown in table 5.

The robustness of the method was studied by changing the experimental conditions deliberately. No significant changes in the chromatographic parameters were observed when changing the experimental conditions (pH, mobile phase ratio and flow rate) shown in table 6.

Pharmacokinetic study

Pharmacokinetics parameters such as Cmax,Tmax, AUC 0-t, AUC 0-∞,t1/2, of testformulation was compared with that of reference formulation.

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TABLES AND FIGURES

Table.3: Calibration curve study

S.NO	Conc(ng/ml)	Peak area
1.	100	7601
2.	200	10640
3.	300	14463
4.	400	19201
5.	500	24550
6.	600	29957

This method proved to be linear between 100 to 600 ng/ml of RGE in rat plasma with a distinctive calibration curve of correlation equation y =47.26x+1023, correlation coefficient>0.9906 shown in Fig.47.

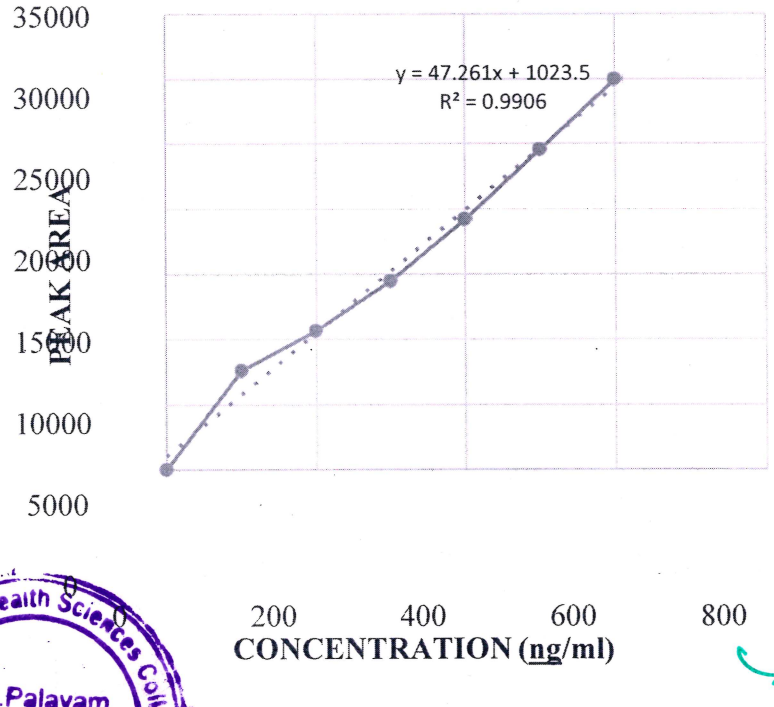

Fig.47: Calibrartion curve of RGE

DISCUSSION

Two drugs are considered to be bioequivalent if they are pharmaceutically equivalent and their bio availabilities are so similar that they are unlikely to produce clinically significant difference in regard to safety and efficacy.

The bioanalytical method was developed, validated and the pharmacokinetic parameters were studied. From the acquired data of the two brands of RGE for bioequivalence study were compared. Remo 100mg tablets was taken as a reference formulation to evaluate the pharmokinetic profile of Zucator 100mg tablets was takenas a test formulation. Cmax ,Tmax,AUC 0-∞,t½ of test formulation was found to be similar of the reference formulation. The pharmacokinetic parameters calculated for two formulations shows that there is no significant difference by statistical analysis and hence two products are measured to be bioequivalent.

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SUMMARY AND CONCLUSION

SUMMARY

The bio-analytical method was developed for bioequivalence study of two Remogliflozin formulation in wistar rat plasma by using RP-HPLC. The method was validated for its transferability to other user or other laboratory. The HPLC method developed uses 0.2% TEA (pH adjusted to 3 with Orthophosphoric acid) buffer: methanol (22:78v/v). The peaks obtained for the drug of interest and internal standard by the present method are well resolved from each other without any interference from the plasma. The peaks are symmertrical with acceptable tailing factor. The retention time of RGE and that of internal standard was shorter and proves the methodis rapid.

The results of linearity, intraday and interday precision study and capability of theextraction method were within the limits of bioanalytical method development. The method was linear with a correlation coefficient of acceptable agreement, which is suitable for the evaluation of RGE in plasma and other biological fluids.

The method demonstrated relative recoveries with acceptable RSD. The lower limit of quantification (LLOQ) for RGE was found to be nanograms lesser then unity. Hence the developed method is sensitive for evaluation of RGE for trace amounts.

The ruggedness and robustness of the method was studied by changing the experimental conditions deliberately.

Peak purity studies, with peak purity index values closer to unity reveals that the method developed was specific for the evaluation of RGE in plasma and otherbiological fluids.

Pharmacokinetics parameters such as C_{max},T_{max},AUC $_{0\text{-}t}$, AUC $_{0\text{-}\infty}$,$t_{1/2}$, of testformulation was compared with that of reference formulation.

The C_{max} for the test and reference formulation was found to be 51.99and 57.04 ng/ml respectively. The t_{max} for the test and reference formulation was found to be 6.3and 8.4min respectively. $AUC_{0\text{-}t}$ for the test and reference formulation was found to be 310.17 and 343.23 ng/ml. $AUC_{0\text{-}\infty}$ for the test and reference formulation was foundto be 501.42 and 465.36 ng/ml respectively.

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The $t_{1/2}$ for test product and reference formulations was found to be 1 respectively.

Pharmacokinetics parameters such as C_{max},T_{max}, AUC, AUC $_{0-t}$, AUC $_{0-\infty}$,$t_{1/2}$ are compared for the bioequivalence study by statistical analysis.

Analysis of RGE concentration in 9 time intervals for collected at different time intervals showed no statistically significant and therefore zucator 100 mg (test formulation) was found to be bioequivalent to remo 100mg (reference formulation).

CONCLUSION

The simple bioanalytical method for bioequivalence study of two RGE formulations in wistar rat plasma using RP-HPLC was developed with satisfactory accuracy and precision. The method consists of sample preparation by protein precipitation, followed by chromatographic separation and UV detection. No interfering peaks were observed at the elution time of RGE (standard) and Rosuvastatin (internal standard).Suitable accuracy and precision of the proposed method was demonstrated over the concentration range of 100-600ng/ml. The methodis accurate, precise, and specific and can be successfully applicable to bioanalysis involving the pharmacokinetic study of RGE in wistar rats. The statistical analysis shows no significant difference between two products and hence zucator (test formulation) was found to be bioequivalent to remo (reference formulation).

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