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Trustpuram, Kodambakkam, CHENNAI - 600 024.
Phone : +91 44 40149999, Fax : +91 44 40149989
website : http://www.medopharm.com

FACTORY:
No.34 B, Industrial Area, MALUR - 563 130.
Karnataka - India
Phone : +918151 - 232307.
Email : mmalur@gmail.com

17/07/2019

To

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

Subject: Collaboration Proposal Research – Reg.

Dear Sir,

I extend warm greetings to you on behalf of Medopharm Pvt. Ltd, chennai. It is with great enthusiasm that I write to propose a collaborative research endeavor that aligns with our shared scientific interests and objectives.

We have been deeply impressed by the remarkable expertise and research capabilities exhibited by JKK Munirajah Institute of Health Sciences College of Pharmacy, particularly in the field of pharmaceutics and the exploration of analytical methods.

We believe that your institution is ideally positioned to join forces with us in conducting a research project titled "Development and validation of Acyclovir by UV-Spectrometry method."

The rationale behind our interest in this project is rooted in our unwavering commitment to advancing pharmaceutical research to address the new development and validation of Acyclovir by UV-Spectrometry in pure and pharmaceutical dosage form.

Recognizing the esteemed reputation of your institution, we are confident that a collaboration with JKK Munirajah Institute of Health Sciences College of Pharmacy would elevate and amplify our research efforts in this specialized field.

To facilitate this collaboration, we propose that your esteemed institution leads the research project outlined above, while Medopharm Pvt. Ltd. extends comprehensive financial support and logistical resources to ensure its successful execution.

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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Karnataka - India
Phone : +918151 - 232307.
Email : mmalur@gmail.com

In order to proceed, we kindly request that you provide us with the following details:

1. **Budget Breakdown:**

   We seek a detailed breakdown of the estimated budget required for the research project. This should encompass expenses related to equipment, materials, personnel, and other pertinent costs.

2. **Faculty Credentials:**

   We would appreciate information regarding the faculty members and researchers who will be actively engaged in the project. This should include details on their qualifications, areas of expertise, and relevant experience.

We eagerly anticipate your positive response and the prospect of embarking on this significant research journey together. This collaboration holds immense potential in advancing our comprehension of Parkinson's disease and exploring innovative avenues for its treatment.

Thanking You

Sincerely,

17/7/17

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

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

22.07.2019

To

Medopharm Pvt. Ltd.,
Medo House, 25,
Puliyur 2nd Main Rd,
Trustpuram, Kodambakkam,
Chennai.

Subject: Response to Proposal for Research Collaboration – Reg.

Dear Sir/Madam,

Greetings. We wish to express our sincere appreciation for your interest in collaborating with JKK Munirajah Institute of Health Sciences College of Pharmacy on the research project titled **"Development of New Analytical Methods and Validation of Acyclovir in Pure and Pharmaceutical Dosage Form by UV-Spectrometry Method."**

First and foremost, we are honoured and excited about the opportunity to work alongside **Medopharm Pvt. Ltd**. on this vital research initiative. Your company's commitment to advancing pharmaceutical research aligns seamlessly with our institution's mission to drive innovation and excellence in the field.

We have meticulously reviewed your proposal, and we are genuinely enthusiastic about the potential benefits of this collaboration. The project's focus on the development and validation of analytical methods for Acyclovir is of great significance to the pharmaceutical industry. Your proposal resonates with our expertise in analytical chemistry and aligns with our goal of contributing valuable insights to the scientific community.

We are thankful for your willingness to provide financial support and logistical assistance for this project. We believe that this collaboration will not only enhance our research capabilities but also lead to the development of cutting-edge analytical methods that can significantly impact the pharmaceutical sector.

To advance our collaboration, we propose scheduling a meeting to delve into the project's specifics. This meeting will encompass discussions on project timelines, budget allocation, resource requirements, and other critical aspects.

Institute 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

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Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

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

We are committed to ensuring the seamless execution and success of this research endeavor. Please inform us of your availability, and we will coordinate a meeting at your convenience. For further communication and coordination, you may reach us at **principal@jkkmihscp.org**

We eagerly anticipate a productive partnership and the opportunity to contribute substantively to the advancement of analytical methods for Acyclovir. We are excited about the potential of this collaboration to enhance pharmaceutical research and development.

With reference to the letter dated 17/07/2019, JKKMIHSCP is permitting the following faculty members to do collaborative research with Medopharm Pvt. Ltd. and a proposal on the mentioned title. The faculty members were assigned to do research work with Medopharm Pvt. Ltd.

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

Thanking you,

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

Principal Investigator

Dr. P. Mohanraj

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

Principal Institutes 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: Development of New Analytical Methods and Validation of Acyclovir in Pure and Pharmaceutical Dosage Form by UV-Spectrometry Method.

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.	*Equipment and Maintenance (UV-Spectrophotometer)*	*70000*
2.	*Chemicals and Materials (Acyclovir, etc.,)*	*30000*
3.	*Laboratory Consumables (Glassware, Solvents)*	*35000*
4.	*Personnel Costs (Salaries, Stipends)*	*25000*
5.	*Miscellaneous Expenses*	*15000*
	Total Budget	**1.75 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

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

Yours Sincerely,

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

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Email : mmalur@gmail.com

02.08.2019

To

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

Subject: Sanction Order for Research Project Funding – Reg.

Dear Sir,

We are pleased to inform you that your proposal titled "Development of New Analytical Methods and Validation of Acyclovir in Pure and Pharmaceutical Dosage Form by UV-Spectrometry Method" submitted by DR. P. MOHANRAJ, M. Pharm., Ph. D., as the Principal Investigator, Mrs. J. PRIYA, Assistant Professor, Mr. M. PUSHPARAJ, Assistant Professor and Mrs. K. ABHENAYA, Assistant Professor as the Co-Investigators has been approved for funding.

The funding for this project, in the amount of **₹ 1.75 lakhs**, has been sanctioned by Medopharm Pvt. Ltd. The approved budget for the project is as follows:

S.No	Detail of Expenditure	Amount in lakhs
1.	Equipment and Maintenance (UV-Spectrophotometer)	₹0.40
2.	Acyclovir Standards and Reference Materials	₹0.25
3.	Laboratory Consumables (Glassware, Solvents)	₹0.25
4.	Personnel Costs (Salaries, Stipends)	₹0.40
5.	Data Analysis Software and Tools	₹0.20
6.	Miscellaneous Expenses (Travel, Publication)	₹0.25
	Total Budget	**₹1.75 lakhs**

In accordance with the terms and conditions, we request that you submit the signed Terms and Conditions (as per the provided guidelines) and the project's start date within two weeks from the date of this letter.

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

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Phone : +918151 - 232307.
Email : mmalur@gmail.com

Furthermore, the project is expected to be completed within 6 months, and upon completion, you are required to submit the certified soft copy of the final project report, Statement of Expenditure, and Utilization Certificate. The Utilization Certificate must be counter-signed by the Head of the Institution for the release of the grant.

We believe that this research project has the potential to contribute significantly to our academic and research goals. We wish you and your team the very best in successfully executing the project. Please feel free to reach out to us for any clarifications or assistance required during the course of the project.

Thank you

Sincerely,

02/8/19

Copy to:

1. Mrs. J. PRIYA, Associate Professor, Department of Pharmaceutical Chemistry.
2. Mr. M. PUSHPARAJ, Assistant Professor, Department of Pharmaceutical Chemistry.
3. Mrs. K. ABHENAYA, Assistant Professor, Department of Pharmaceutical Chemistry.

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

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

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COLLEGE OF PHARMACY

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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 : Development of New Analytical Methods and Validation of Acyclovir in Pure and Pharmaceutical Dosage Form by UV-Spectrometry Method.

Category of the Project : Research project

Date of approval of competent authority : 02/08/2019

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

S. No	Detail of Expenditure	Amount in lakhs
1.	Equipment and Maintenance (UV-Spectrophotometer)	₹0.40
2.	Acyclovir Standards and Reference Materials	₹0.25
3.	Laboratory Consumables (Glassware, Solvents)	₹0.25
4.	Personnel Costs (Salaries, Stipends)	₹0.40
5.	Data Analysis Software and Tools	₹0.20
6.	Miscellaneous Expenses (Travel, Publication)	₹0.25
	Total Budget	**₹1.75 lakhs**

Date of start of the Project : 02/08/2019

Date of completion of Project : 03/02/2020

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

Name and Signature of Principal Investigator

Dr. P. mohanaraj

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

Name and Signature of Co-Investigators

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Karnataka - India
Phone : +918151 - 232307
Email : mmalur@gmail.com

Date:12.02.2020

To

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

LETTER OF APPRECIATION

Dear Sir,

We would like to take opportunity to thank you for your time spending and valuable Research work to our organization. Once again, we extend our sincere gratitude to you and your team for your efforts in making the "Development of new analytical methods and its validation of Acyclovir in pure and Pharmaceutical dosage form by UV-Spectrometry method" a resounding success.

We look forward to the continuation of our successful partnership and to exploring new opportunities for collaboration.

Thank you

Sincerely,

12/2/20

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.

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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 the accounts of the JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N.Palayam, Erode. in respect of **"Development of new analytical methods and its validation of Acyclovir in Pure and Pharmaceutical Dosage form by UV-Spectrometry Method"**, Research Project of DR. P. MOHANRAJ M. Pharm, Ph.D., Principal Investigator (PI) have been audited by me with reference to the Vouchers. Books of accounts, norms of expenditure and relevant guidelines there to. The Statement of expenditure of Research Project duly signed by me is enclosed, for the year 2019-2020.

1. It is hereby certified that the total grants of Rs. 1,75,000/- (Rupees One Lakh Seventy-Five thousand only) has been sanctioned to the Principal Investigator (P.I.)

2. The P.I. has received Rs. 50,000/- (Rupees Fifty thousand only) towards the 1 Installment.

3. The P.I. has incurred the total expenditure of Rs. 1,75,000/- (Rupees One Lakh Seventy-Five thousand only) for the Research Project against 1 Installment.

The Original Vouchers and stamped receipts for the above-mentioned statement of Accounts is retained in College Institute office and will be made available when required.

Date: 3/2/2020
Place: T. N. Palayam

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

Name and Signature of Principal Investigator

Dr. P. Mohanraj

Name and Signature of Co-Investigators

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 OF NEW ANALYTICAL METHODS AND ITS VALIDATION OF ACYCLOVIR IN PURE AND PHARMACEUTICAL DOSAGE FORM BY UV-SPECTOMETRY METHOD"

PRINCIPAL INVESTIGATOR

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

Professor,

Department of Pharmaceutical Chemistry,

CO-INVESTIGATORS

Mrs. J. PRIYA, M. Pharm.,

Associate Professor,

Department of Pharmaceutical Chemistry,

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-2020

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 **"DEVELOPMENT OF NEW ANALYTICAL METHODS AND ITS VALIDATION OF ACYCLOVIR IN PURE AND PHARMACEUTICAL DOSAGE FORM BY UV-SPECTOMETRY METHOD"** submitted to The **Medopharm Pvt. Ltd., Bangalore,** 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.

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

Place: T.N-Palayam

Date: 3/2/2020

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

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

DECLARATION

This is to certify that the Research entitled **"DEVELOPMENT OF NEW ANALYTICAL METHODS AND ITS VALIDATION OF ACYCLOVIR IN PURE AND PHARMACEUTICAL DOSAGE FORM BY UV-SPECTOMETRY METHOD"** submitted to The **Medopharm Pvt. Ltd., Bangalore,** 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 2019-2020.

Place: T.N-Palayam

Date: 3/2/2020

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

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

Mrs. J. PRIYA, M. Pharm.,
Co-Investigator

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

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

DECLARATION

The research work emboided in this work entitled "Development of New Analytical Methods and its Validation of Acyclovir in Pure and Pharmaceutical Dosage form by Uv-Spectometry Method" 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 **Medopharm Pvt. Ltd., Bangalore,** during the academic year 2019-2020.

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

ACKNOWLEDGEMENT

First and for most we express our heartful sense of gratitude and faithfulness to 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 of New Analytical Methods and its Validation of Acyclovir in Pure and Pharmaceutical Dosage form by Uv-Spectometry 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.

T.N.Palayam
Erode Dt

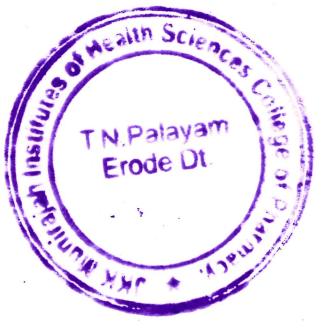
Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

We would like to give sincere thanks to our classmates for their 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 Medopharm Pvt. Ltd., Bangalore give a Financial and moral support to completion of the project being a successful manner on the duration of 2019-2020.

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

INTRODUCTION

1. Ultraviolet spectroscopy 1.1 Introduction [1-2]:

The absorption of electromagnetic radiation of wavelengths between 200 and 800nm by molecules which have 7 electrons of atoms possessing unshared electron pairs can be employed for both qualitative and quantitative analysis; ay such, it fs known as spectrophotometry, As a wide variety of pharmaceutical substances absorb radiation in the near-ultraviolet (200 380nm) and visible (380 800nm) regions of the electromagnetic spectrum, the technique is widely employed in pharmaceutical analysis.

The relationship between the concentration of analytes and the intensity of light absorbed is the basis of quantitative applications of spectrophotometry. In addition, features of absorption spectra such as the molar absorptivity, spectral position, and shape and breadth of the absorption band are related to molecular structure and environment and therefore can be used for qualitative analysis.

The absorption of near ultraviolet or visible light by molecules occurs because of the interaction of the electric field ons. The intensity, position in the spectrum, and appearance by this interaction depends on the energies of the with molecular electrons the spectral band produce molecular electrons and their dynamic characteristics with respect to the rest of the molecule.

Laws of UV Spectroscopy

Beer's law

The intensity with the number of absorbing am of monochromatic radiation decreases exponentially molecules, in which absorbance is proportional to the concentration.

Formula: [= 1.e*°

Ambert's Law

The intensity of a beam of parallel radiation decreases exponentially as it passes through a medium of homogenous thickness that is proportional to the thickness of the solution. Formula: = Io.e*

1.3 Instruments used in UV [3] Experimental Procedure:

The following simulation illustrates the procedures for making Spectrophotometric measurements.

First, the intensity of light passing through a blank is measured. The intensity is the number of photons per second. The blank is a solution that is identical to the sample solution except that the blank does not contain the solute that absorbs light. This measurement is necessary because the cell itself scatters some of the light.

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

Second, the intensity of light passing through the sample solution is measured. Cn practice instruments measure the power rather than the intensity of light. The power is the energy per second, which is the product of intensity (photons per second) and the energy per photon). Third, the experimental data is used to calculate two quantities: the transmittance (T) and the absorbance (A).

$$T=Io$$

$$A= - \log io\ T$$

The transmittance is simply the fraction of light in the original beam that passes through the sample and reaches the detector. The remainder of the light, 1-T is the fraction of the light absorbed by the sample. (Do not confuse the transmittance with the temperature, which often is given the symbol T.)

In most applications, one wishes to relate the amount of light absorbed to the Concentration of the absorbing molecule. It turns out that the absorbance rather than the transmittance is most useful for the purpose. If no light is absorbed, the absorbance is zero (100% transmittance), each unit in absorbance corresponds with an order of magnitude in the fraction of light transmitted, For A=1, 10% of the light is transmitted (T=0.10) and 90% is absorbed by the sample, For A=2.1% of the light is transmitted and 99% is absorbed.

Using the simulation below, perform the following steps:

- Measure the intensity of light passing through the blank.
- Measure the intensity of light passing through the sample. Calculate the transmittance,
- Calculate the absorbance.

There are two major classes of devices:

- Single beam Spectrophotometer.
- Double beam spectrophotometer.

Single beam spectrophotometer

In a single instrument all the light pass through the sample cell. It must be measured by removing the sample. This was the earliest design, but still in common use in both teaching and industrial labs.

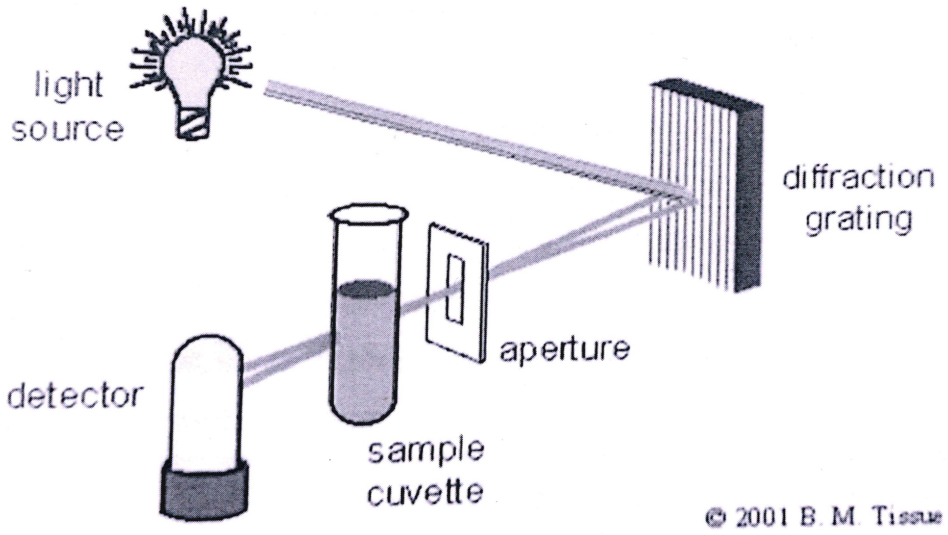


Fig 1: Single beam spectrophotometer

The light source

It is important that the power of the radiation source does not change abruptly t is imp, its length range. The electrical excitation of deuterium or hydrogen at low over its wave's pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and ultraviolet photon.

Both deuterium and hydrogen lamps emit radiation in the range 160 - 375nm. Quartz windows must be used in these lamps and quartz cuvettes must be used, Quartz windows must be used because glass absorbs radiation of wavelengths less than350nm.

The diffraction grating and the slit

The prism splits light into its component colors. A diffraction grating does the same work, but more efficiently.

The arrows show the way the various wavelengths of the light are sent off in different directions. The slit only allows light of a very narrow range of wavelengths through into the rest of the spectrometer.

By gradually rotating the diffraction grating, allow light from the whole spectrum (a tiny part of the range at a time) through into the rest of the instrument.

The rotating discs

Each disc is made up of a number of different segments. The machine has three different sections other designs may have a different number.

The light coming from the diffraction grating and slit will hit the rotating disc and one of three things can happen.

If it hits the transparent section, it will go straight through and pass through the cell containing the sample. A mirror onto a second rotating disc then bounces it. This disc is rotating such that when the light arrives from the first disc, it meets the mirrored section of the second disc. That bounces it onto the detector.

If the original beam of light from the slit hits the mirrored sections of the first rotating disc, it is bounced down along the path. After the mirror, it passes through a reference cell (more about that layer).

Finally, the light gets to the second disc, which is rotating, in such a way that it meets the transparent section. It goes straight through to the detector.

If the light meets the first disc at the black section, it is blocked — and for avery short while no light passes through the spectrometer. This just allows the computer to make allowance for any current generated by the detector in the absence of any light.

The sample and reference cells

These are small rectangular glass or quartz containers. They are often designed so that the light beam travels a distance of Icm through the contents. The sample cell contains a solution of the substance if testing means-usually very dilute. The solvent is chosen so that it does not absorb any significant amount of light in the wavelength range. The reference cell contains pure solvent.

The detector and the computer

For each wavelength of light passing through the spectrometer, the intensity of the light passing through the reference cell is measured. Detects transmittance intensity of light. The photo resistor is a very sensitive electrical device that detects the intensity of light that is being transmitted through the sample.

The photo resistor measures in Ohms, the units of resistance. However, the spectrophotometer contains a processing system that converts resistance values to the absorbance of light.

Computer displays absorbance values for the user. A computer screen that has a separating operating system from the spectrophotometer usually functions as the output device. Both the spectrophotometer and the computer internally connect, and the information output from the spectrophotometer saves directly on the computer. However, some spectrophotometers have an imbedded screen on which the output displays.

Double beam spectrophotometer

In a double beam instrument, the light is split into two beams before it reaches the sample. One beam is used as a reference the other beam passes through the sample. Some double beam instruments have two detectors and the sample reference beams are measured at the same time. In other instruments, the two beams pass through a beam chopper, which blocks one beam at one time. The detector alternates between measuring the sample beam and the reference beam.

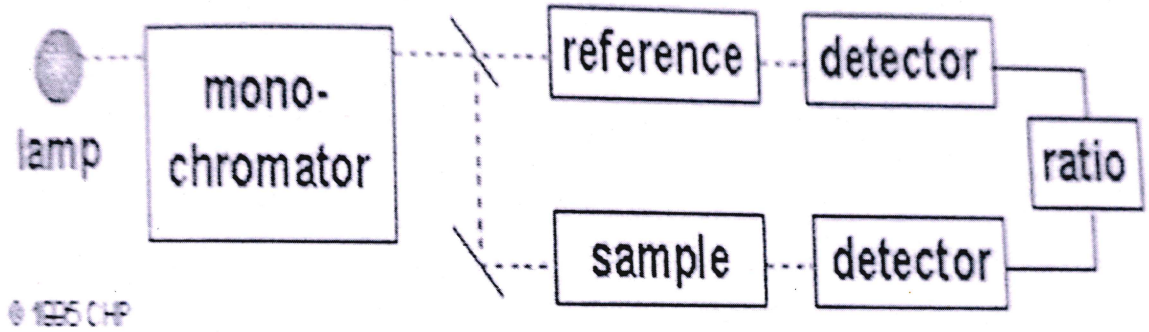


Fig 2 : Double beam spectrophotometer

Advantages of instrumental method

- A small amount of sample is needed for analysis.
- Determination by instrument method is considerably fast.
- Complex mixture can be analyses either with or without their separation.

Lamination of instrumental method

- In general instrumental methods are costly because of cost, maintenance and trained personnel required for handiling
- The sensitivity and accuracy depends on the type of instrument
- Specialized training for handiling instrument is required
- There is frequent need of checking result with other methods.

Instrumental methods are not only important in pharmaceutical analysis for analysing basic drugs or chemicals or its formulation but plays a significant rose in other fields also, various instruments are used as diagnostic tools in medicinal profession without which diagnosis of disease and its treatment would not have been possible. For eg. X-rays, ultrasound, NMR, scanning instruments.

Method of analysis of UV

- Direct comparison method
- Single component method
- Simultaneous method
- Calibration method
- Geometric method
- Geometric correction method
- Orthogonal polynomial method
- Absorption ratio method

Application of UV spectroscopy

- Determination of food colours in mixtures
- Thermal analysis of DNA by UV visible spectrometry
- Kinetics rate law investigation by UV visible spectrometry
- Multi components analysis of a vitamin B mixture by UV visible
- Automated measurements of the colour of red wines
- Fully automated water nitrate analysis
- Determination of drug dissolution studies by UV spectroscopy

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Analytical method development

Analytical method development and validation plays an important role in the discovery, development and manufacture of pharmaceuticals. The official test methods that results from these process are used by quality control laboratories to ensure the identity, purity, potency and performance of drug products.

Recent progress in methods development has been usually a result of improvements in analytical instrumentation. This is especially true for chromatographic detectors. Isocratic and gradient reverse phase high performance liquid chromatography has evolved as the primary techniques for the analysis on non-volatile active pharmaceutical ingredients and impurities. The HPLC detectors of choice for many types of methods development is the photo-diode array detectors because it can used for both qualitative and quantitative analysis.

The new (CSPs) allow trace level of enantiomeric impurities to be measured. Gas chromatography remains the method of choice for the analysis of volatile compounds. Gas chromatography with mass spectrometry detection (GC MS) is increasingly being used to identify impurities and to determine active ingredient peak purity is stressed sample. Advance in laboratory robotics and automation are beginning to be applied to methods development and validation. Development teams are using laboratory robotics to develop automated methods for high volume test.

Analytical method requirement is required for

- Herbal products
- New process and reactions
- New molecules
- Active ingredients (macro analysis)
- Residues (microanalysis)
- Impurity profiling
- Components of interest in different matrices

Analytical method validation: Analytical method validation is "the collection and evolution of data, from the process design stage throughout production, which establishes scientific evidence that a process is capable of consistency delivering quality products".

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Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and give the required accuracy, precision, sensitivity, ruggedness, etc. The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics, which need to be evaluated. Typical validation characteristics, which should be considered, are listed below:

- Specificity
- Linearity and Range
- Accuracy
- Precision
- Robustness
- System suitability

Selectivity and Specificity

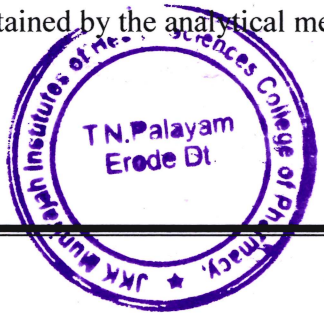
The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. On the other hand, if the method determines or measures quantitatively the component of interest in the sample matrix without separation, it is said to be specific.

Hence one basic difference in the selectivity and specificity is that, while the former is restricted to qualitative detection of the components of a sample, the latter means quantitative measurement of one or more analyte.

Linearity and range

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results (e.g., percentage, parts per million) obtained by the analytical method.



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For assay tests, the ICH requires the minimum specified range to be 80 to 120 percent of the test concentration, and for the determination of an impurity, the range to extend from the limit of quantitation, or from 50 percent of the specification of each impurity, whichever is greater, to 120 percent of the specification.

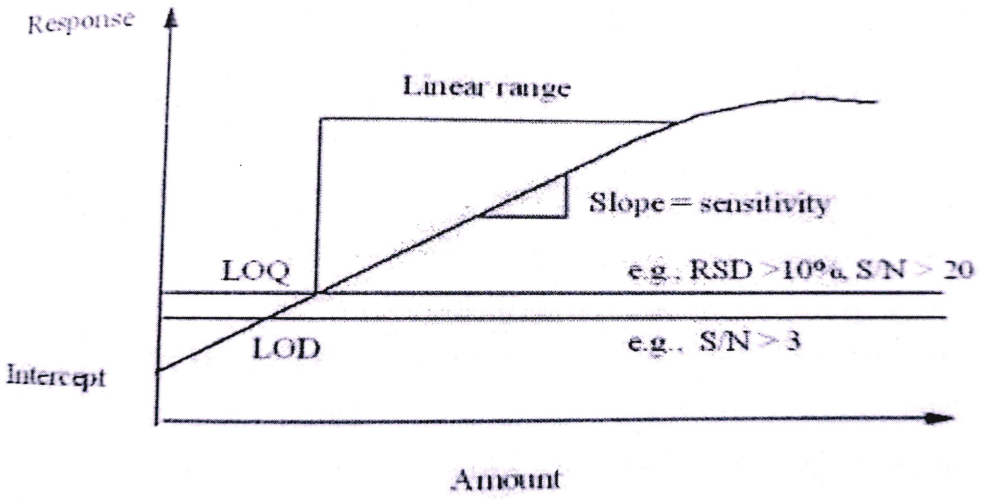

Fig 3: Linearity and range

Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added. This is sometimes termed trueness.

Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay. Dosage form assays commonly provide accuracy within 3-5% of the true value

The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e. three concentrations and three replicated of each concentration).

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances

Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Determination of Repeatability:

Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time.

It is normally expected that at least six replicates be carried out and a table showing each individual result provided from which the mean, standard deviation and co-efficient of variation should be calculated for set of n value. The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several time in a standard situation. (RSD below 2% for built drugs, RSD below 2% for assays in finished product).

Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at

- Different Occasions
- Different Laboratories
- Different Batch of Reagents
- Different Analysts
- Different Equipments

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied.

System suitability

The term system stability has been defined as the stability of the samples being analyzed in a sample solution. It is a measure of the bias in assay results generated during a preselected time interval, for example, every hour up to 46 hours, using a single solution. System stability should be determined by replicate analysis of the sample solution. System stability is considered appropriate when the RSD, calculated on the assay results obtained at different time intervals, does not exceed more than 20 percent of the corresponding value of the system precision.

Parameters used for analysis

The quantitative results obtained were subjected to the following statistical analysis:

- ❖ Sample mean
- ❖ Standard deviation
- ❖ Relative standard deviation (%RSD) or coefficient of variance (%C.V)
- ❖ Standard error of mean (S.E)

R.S.D-S.D/MEAN S.D=$\sqrt{(x-x)^2/n-1}$ S.E-S.D/Vn

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i. Ultra-Violet (UV) detector

It is divided in to three types they are fixed wavelength, variable wavelength, and diode array detectors.

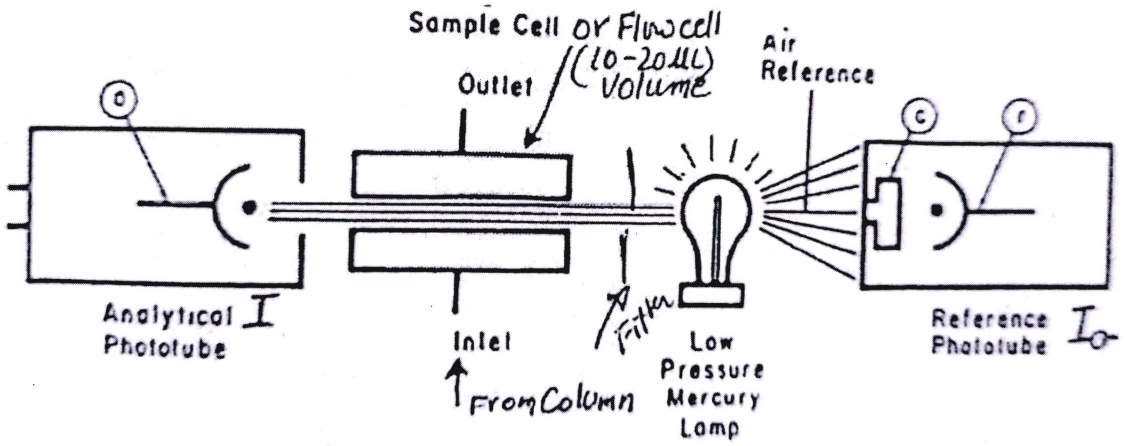

Fig 4: Ultra-violet (UV) detector

It measures the ability of a sample to absorb light. This can be accomplished at one or several wavelengths

Fixed wavelength detectors

It is the most common and inexpensive detector. The use of suitable & is determined by the nature of the light source used. Deuterium lamp can be used over a range of wavelength (covers a continuum of wavelengths), hence covering most of the UV spectral region.

Variable wavelength detectors

It is less sensitive than fixed wavelength but the detection wavelength can be varied.

Deuterium source is mostly used because it provides continuum source. This can be combined with a suitable monochromator in dual beam mode.

Diode Array detectors (DAD)

There are two major advantages of diode array detection

In the first, it allows for the best wavelength to be selected for actual analysis. This is particularly important when no information is available on molar absorptivities at different wavelengths.

The second major advantage is related to the problem of peak purity. Often, the peak shape in itself does not reveal that it actually corresponds to two (or more) components. [9-10]

Advantages:

UV wavelength-For the greatest sensitivity max should be used, which detects all sample components that contain chromophores. UV wavelengths below 200nm should be avoided because detector noise increases in this region. Higher wavelengths give greater selectivity.

2. AIM AND OBJECT IVES OF THE WORK

Method: Ultra violet spectrophotometry

❖ **The aim of the present work is to**

Develop UV spectrophotometric method for the estimation of Acyclovir in it's pharmaceutical dosage form

❖ **Plan of work**

The plan of work involves the following

- Literature review
- Collection of drug.
- Study of physicochemical properties of drug
- Find out the solubility of drug
- Selection of solvent

Steps involved in UV spectrometry method

- To standardize the developed method
- Analyse the marketed formulation for their reliability and accuracy
- To validate the developed method for their repeatability and recovery

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SCOPE AND PLAN OF WORK

METHOD [7-8]

- **Instruments Used**

Balance:

Single pan electronic balance sartorious GE412

UV visible spectrophotometer:

Systronics 2203(smart)

Matched quartz cells corresponding to 1 cm path Jength

- **Reagents**

Ethanol

Distilled water

Standard Acyclovir

Tablet brand used - Acivir

Standardisation of method

- **Wavelength selection**

Preparation of stock solution

Standard stock solution of Acyclovir was prepared by dissolving 800mg of drug is dissolved Ethanol and make up with same solvent and further dilution with Distilled water. Then the solution was further diluted to get the concentration of 30, 40, 50, 60, 70, 80pg/ml. The solutions were scanned inthe UV region between 200-400 nm.

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Beer's law concentration range

Standard stock solution of Acyclovir was prepared by dissolving 800mg of drug is dissolved Ethanol and make up with same solvent and further dilution with distilled water to get concentration range from 1 to 1000 pg/ml .The solutions were scanned in the UV region between 200-400nm and their absorbance were measured at 269.7 Using the absorbance values against concentrations calibration curve was plotted and shown in Fig.7.From the graph it was found that Acyclovir obeys Beer's law between 60-100pg/ml. The data was given in Table-3.

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RESULTS AND DISCUSSION

UV SPECTOMETRY METHOD:

Wave length selection:

The concentration of 30 µg/ml standard stock solution was prepared and observes the absorbance at different wavelength and the wavelength with maximum absorbance was selected as wavelength.

Table 2(a)

S.NO	CONCENTRATION	WAVELENGTH(nm)	ABSORBANCE
1	30	230.0	0.062
2	30	240.0	0.094
3	30	250.0	0.113
4	30	260.0	0.184
5	30	270.0	0.219
6	30	2880.0	0.211
7	30	290.0	0.198
8	30	300.0	0.166
9	30	310.0	0.133
10	30	320.0	0.101

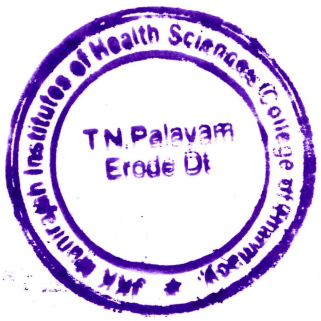

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Table 2(b)

S.NO	CONCENTTRATON	WAVELENGTH(nm)	ABSORBANCE
1	30	261.0	0.190
2	30	262.0	0.194
3	30	263.0	0.197
4	30	264.0	0.201
5	30	265.0	0.203
6	30	266.0	0.204
7	30	267.0	0.207
8	30	268.0	0.211
9	30	269.0	0.215
10	30	270.0	0.219

Table 2(c)

S.NO	CONCENTRATION	WAVELENGTH(nm)	ABSORBANCE
1	30	269.1	**0.215**
2	30	269.2	**0.217**
3	30	269.3	**0.219**
4	30	269.4	**0.220**
5	30	269.5	**0.221**

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6	30	269.6	**0.222**
7	30	269.7	**0.223**
8	30	269.8	**0.222**
9	30	269.9	**0.220**
10	30	270.0	**0.219**

Table 2(d)

S.NO	CONCENTRATION	WAVELENGTH(nm)	ABSORBANCE
1	30	269.7	0.223
2	40	269.7	0.284
3	50	269.7	0.325
4	60	269.7	0.365
5	70	269.7	0.386
6	80	269.7	0.400

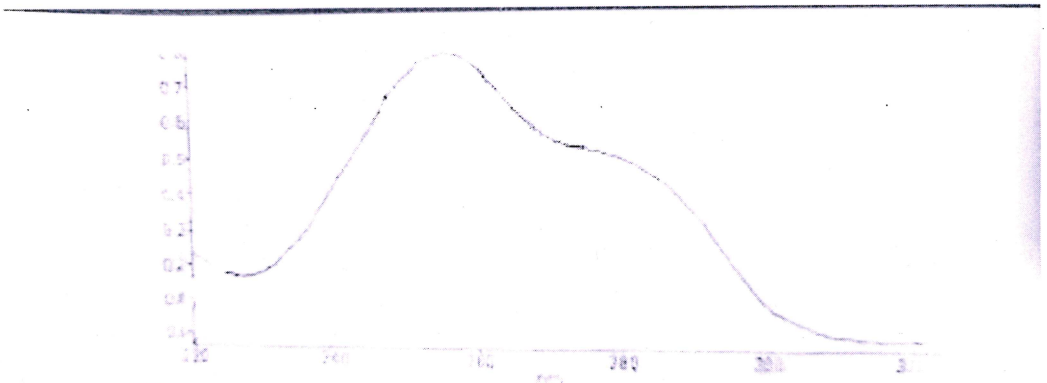

Fig.6 Spectrum of Acyclovir

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❖ **Beer's law range**

The solutions were scanned in the UV region between 200-400nm and their absorbance were measured at 269.7 .Using the absorbance values against concentrations calibration curve was plotted and shown in Fig.7. From the graph it was found that Acyclovir obeys Beer's law between 60-100yg/ml. The data was given in Table-3.

Table-3

Beer's law concentration range

S.NO	CONCENTRATION	ABSORBANCE
1	1	0.049
2	2	0.051
3	3	0.068
4	4	0.077
5	5	0.079
6	6	0.087
7	7	0.089
8	8	0.103
9	9	0.111
10	10	0.115
11	20	0.159
12	30	0.217

13	40	0.279
14	50	0.300
15	60	0.380
16	70	0.440
17	80	0.508
18	90	0.552
19	100	0.600
20	200	1.075
21	300	1.600
22	400	2.099
23	500	2.569

calibration graph of Acyclovir:

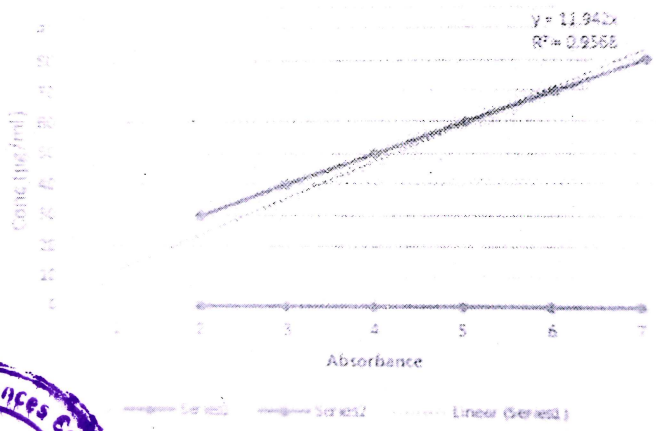

Fig.7 Beer's law range

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Quantitative estimation of Acivir tablet

The quantitative estimation was carried out in tablet formulations by taking concentrations of 60-100µg/ml. The brand of formulations shows the percentage purity values ranges from 100.8-102.0%W/W. the percentage deviations values were found to be between ± 0.8-±2.0. the data was given in table-4.

Table-4

Quantitative estimation of acivir tablet

S no	Concentration (µg/ml)	Labelled claim (mg/cap)	Amount present (mg/cap)	Percentage label claim (%w/w)	Percentage deviation
1	60	300	306.08	102.0	±2.0
2	70	300	305.99	101.9	±1.9
3	80	300	303.54	100.8	±0.8

❖ **Stastical data estimation of acivir tablets**

The quantitative results obtained were subjected to Stastical analysis to find out standard deviation and standard error values. The relative standard deviation values are below, indicating the precision of the methodology and low standard error values show the accuracy of the method. The data was given in table 5

Table 5

Stastical data of acivir tablets

S no	Concentration (µg/ml)	Percentage label claim (%w/w)	Standard deviation (SD)	Relative standard deviation (RSD)	Standard error of mean (SE)
1	60	102.0	0.011	0.021	0.0049
2	70	101.9	0.012	0.023	0.0053

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3	80	101.8	0.012	0.023	0.0053
4	90	101.2	0.013	0.025	0.0058
5	100	100.8	0.012	0.023	0.0053

Repeatability studies

The repeatability of the method was confirmed by the assay procedures with three different concentration of three replicates each. The repeatability values vary from 100.3 to 102.0 %w/w. the results obtained in repeatability test express the precision of given method. The data was given in table 6

Table 6

Repeatability studies

S no	Concentration (µg/ml)	Labelled claim (mg/cap)	Amount present (mg/cap)	Percentage label claim (%w/w)	Percentage deviation
1	70	300	307.00	102.0	±2.0
2	70	300	303.73	101.2	±1.2
3	70	300	301.06	100.3	±0.3
4	80	300	302.93	100.7	±0.7
5	80	300	304.12	101.3	±1.3
6	80	300	305.28	101.7	±1.7

❖ **Recovery studies:** The validation of the proposed method was further conformed by recover studies. The recovery values vary from 100.10 to 100.30 %w/w. these serves as a good index of accuracy and reproducibility of the study. The data was given in table 7

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Table 7

Recovery studies

S no	Concentration (µg/ml)	Amount added (mg)	Amount recovered (mg)	Percentage recovery	Percentage deviation
1	70	10	10.7	110.1	±0.1
2	70	20	24.4	122.1	±2.1
3	70	30	39.1	130.4	±0.4
4	80	10	10.7	109.9	±0.1
5	80	20	24.9	121.4	±1.4
6	80	30	39.3	131.0	±1.0

The stability of the drug was analysed for 120 minutes the absorbents was recorded and the drug is stable up to 120 minutes and sample is analysed within that time.

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SUMMARY AND CONCLUSION:

UV SPEETOMETRY METHOD:

It reports a UV spectrophotometric method for the estimation of acyclovir in pure and pharmaceutical dosage form

In standardization of method , initially the solvent and the wavelength were selected. After the different concentration solution was prepared and scanned in the UV region between 200-400 nm and the absorbance maxima was found at 269.7nm. by using the absorbance values against concentrations calibration curve was plotted. From the graph it was found that Acyclovir obeys beer's law between 60-100µg/ml.

The brand of formulation shows the percentage purity values ranges from 100.8 to 102.0 %w/w. the percentage deviation values were found to be between ±0.8 to **±2.0.**

the repeatability values vary from 100.7 to 102.0 %w/w. the results obtained is repeatability test expresses the precision of the given method.

The stability of the drug was analysed for 120 minutes. The absorbance were recorded and the drug is stable up to 120 minutes ans the sample is analysed within that time.

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