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PREFORMULATION STUDIES OF ACYCLOVIR WITH SPECIAL REFERENCE TO DEVELOPMENT OF ANALYTICAL METHODS, CRYSTAL PROPERTIES OF DRUG AND DRUG- EXCIPIENTS INTERACTION STUDIES

Ashish Kumar Gupta[1†], Minakshi Verma[2]

[1] Department of pharmacy, V B S Purvanchal University, Jaunpur, U.P. India
[2] Department of pharmacy G. S.V.M Medical College, Kanpur, U.P., India

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Corresponding Author:

†Ashish Kumar Gupta

Department of pharmacy, V B S Purvanchal University, Jaunpur, U.P. India

ABSTRACT

The main objective of this work was to develop the Preformulation studies of acyclovir. Preformulation study was mainly performed to develop simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Acyclovir, an antiHIV drug, in bulk and pharmaceutical dosage form. Various parameters like melting point, pK_a, $K_{o/w}$, flow properties of the powder drug, crystal morphology, drug excipients compatibility studies and particle size distribution were carried out. The solvent used was 0.1N HCl and the λ_{max} or the absorption maxima of the drug was found to be 255nm. The regression co-efficient obtained from the standard plots were nearing about 1.0 and which proved the linearity of the analytical methods. Calibration curves followed the linear regression. All the models followed Beer-Lambert's law and therefore can be analyzed by UV spectrophotometer. This method can be used for the determination of Acyclovir in quality control of formulation without interference of the excipients.

Keywords: Acyclovir, pKa, Ko/w, flow properties of the powder drug, crystal morphology, drug excipients compatibility studies and particle size distribution, UV-Vis Spectro photometry

INTRODUCTION

Almost all drugs are marketed as tablets, capsules or both. Prior to the development of these major dosage forms, it is essential that pertain fundamental physical and chemical properties of the drug molecule and other divided properties of the drug powder are determined. This information decides many of the subsequent events and approaches in formation development. This first learning phase is known as Preformulation [1-3]. Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing optimum drug delivery system. Before beginning the formal preformulation programs the preformulation scientist must consider the following factors: The amount of drug available, the physicochemical properties of the drug already known, therapeutic category and anticipated dose of compound and the nature of information, a formulation should have or would like to have [1-4].

There are critical differences between companies at the detailed level of knowledge and their ability to learn before doing knowledge of the underlying variables and their relationship to performance and knowledge of the future manufacturing environment and the new variables introduced by that environment [2, 4, 5].

Preformulation development studies are conducted to determine the physical and chemical characteristics of the compound of interest be it a small organic molecule, peptide or protein. These studies generate the data that are a prerequisite to dosage form development and the data required for submission of the Chemistry, Manufacturing and Controls (CMC) section of the Investigational New Drug application (IND)[4-6].

The main objective of this work was to develop the physiochemical properties of the acyclovir. The main objective of this work was to develop the Preformulation studies of acyclovir. Preformulation study was mainly performed to develop simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Acyclovir, an antiHIV drug, in bulk and pharmaceutical dosage form. Various parameters like melting point, pK_a, $K_{o/w}$, flow properties of the powder drug, crystal morphology, drug excipients compatibility studies and particle size distribution were carried out.

MATERIALS & METHODS

Chemicals and reagents used

Acyclovir was obtained from Matrix Lab, Secunderabad, India. Water was glass-double distilled and further

dids no.: 12.2015-77737978, dids Link: http://dids.info/didslink/06.2016-38182138/

purified from Milli Q water purification system. All the other chemicals were used in analytical grades.

Methods

The purpose of Preformulation study was to establish physicochemical parameters of drug, physical characteristics & compatibility with common excipients. Preformulation study was mainly performed to develop simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Acyclovir, an anti HIV drug, in bulk and pharmaceutical dosage form. Various parameters like melting point, pK_a, $K_{o/w}$, flow properties of the powder drug, crystal morphology, drug excipients compatibility studies and particle size distribution were carried out.

Physical appearance

Acyclovir was obtained from Matrix Lab, Secunderabad, India and it was inspected visually for physical appearance. It was physically characterized on the basis of organoleptic properties like color, odor, texture and taste. All these physical parameters were compared with reported in official monograph.

Determination of Melting Point

This determination was obtained using a digital capillary melting point apparatus (Cambell Electronics, Bombay, India) by capillary fusion method. A capillary was taken and bringing it near the burner flame then sealed its one end. The open end of the capillary tube was pushed in to a small heap of drug, so that a small plug of the powder was collected in the open end and the tube was tapped gently, so that collected drug was settled down. This process was repeated several times. Then the capillary tube was placed in the melting point determination apparatus and observed the temperature at which sample changes its state from solid to liquid. The experiment was performed in triplicate. The temperature at which starts to melt was noted with the help of thermometer and it was compared with earlier reported value.

pKa Determination

Determination of the dissociation content for a drug capable of ionization within a pH range of 1 to 10 is important since solubility and consequently absorption, can be altered by orders of magnitude with changing pH. The Henderson-Hasseslebach equation provides an estimate of the ionized and unionized drug concentration at a particular pH.

For acidic compounds: pH = pKa + log ([un-ionized drug] / [ionized drug])

Partition Coefficient

Partition Coefficient (oil/ water) is a measure of a drug's lipophilicity and an indication of its ability to cross cell membranes. It is defined as the ratio of unionized drug distributed between the organic and aqueous phases at equilibrium [2-5].

$K_{o/w}$ = (C oil / C water) equilibrium.

For series of compounds, the partition coefficient can provide an empiric handle in screening for some biologic properties. For drug delivery, the lipophilic/ hydrophilic balance has been shown to be a contributing factor for the rate and extent of drug absorption. Although partition coefficient data alone does not provide understanding of *in vivo* absorption, it does provide a means of characterizing the lipophilic/ hydrophilic nature of the drug. Since biological membranes are lipoidal in nature. The rate of drug transfer for passively absorbed drugs is directly related to the lipophilicity of the molecule. The partition coefficient is commonly determined using an oil phase of octanol or chloroform and water. Drugs having values if K much greater than 1 are classified as lipophilic, whereas those with partition coefficient much less than 1 are indicative of a hydrophilic drug. Although it appears that the partition coefficient may be the best predictor of absorption rate, the effect of dissolution rate, pKa and solubility on absorption must not be neglected [3-7].

The partition coefficient [8-10] of acyclovir was determined in n-octanol and water solvent systems. Accurately weighed amount of drug (20 mg) was transferred in to a rubber stopper (wrapped with butter paper) containing 20 ml each of octanol and water and the resulting mixture was shaken onrush action shaker for 2 hours. Both the phases were separated using separating funnel and it was analyzed by spectrophotometer, to determine the amount of drug after suitable dilution.

Particle size distribution

Bulk flow, formulation homogeneity, and surface-area controlled processes such as dissolution and surface morphology of the drug particles. In general, each new drug candidate should be tested during Preformulation with the smallest particle size as is practical to facilitate preparation of homogeneous samples and maximize the drug' s surface area for interactions. Various chemical and physical properties of drug substances are affected by their particle size distribution and shapes. The effect is not only on the physical properties of solid drugs but also, in some instances, on their biopharmaceutical behavior. It is generally recognized that poorly soluble drugs showing a dissolution- rate limiting step in the absorption process will be more readily bio available when administered in a finely subdivided state rather than as a coarse material. In case of tablets, size and shape influence the flow and the mixing efficiency of powders and granules. Size can also be a factor in stability: fine materials are relatively more open to attack from atmospheric oxygen, the humidity, and interacting excipients than are coarse materials. Though microscopy is the simplest technique of estimating size ranges and shapes, it is to slow for quantitative determination the material is best observed as a suspension in non dissolving fluid. Saving is less useful technique at preformulation storage due to lack of bulk material [6, 10-13].

Particle size of the drug was measured by using Malvern instrument (Hydro 2000MU (A), Mastersizer, Malvern, UK). The drug (2gm) was dispersed with 400 ml of distilled water and it was suspended on the sample tray with an in built vacuum and compressed air system. The laser obscuration range was maintained between 8% - 12%. All the measurements were carried out in triplicate and 50^{th} percentile diameter (d 0.5) and 90^{th} percentile diameter (d 0.9) were determined. 50^{th} percentile diameter of the cumulative particle size distribution were considered as mean values and it was expressed for all formulations as mean size range.

Power Flow Properties

When limited amounts of drugs are available Power flow properties can be evaluated by measurements of bulk density and angle of repose. Changes in particles size, and shape are generally very important an increase in crystal size or a more uniform shape will lead to a small angle of repose and a smaller Carr's index [6-9].

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Bulk Density

Bulk density was determined by measurement in graduated cylinder [8]. A quantity of 25 gm of material was weighed (M) accurately and passed through sieve (# 22) to break up any agglomerates and introduced into a 100 ml measuring cylinder without compacting. The powder was leveled carefully and the unsettled apparent volume V_0 was noted to the nearest graduated unit. The bulk density was calculated in gm/ml by the formula: $\mathbf{M/V_o}$.

Tap Density

After determination of the bulk density, the cylinder was tapped mechanically by mounting on a holder in a mechanical tapped density tester that provided a fixed drop of 14 ± 2 mm at a nominal rate of 300 drops per minute. The cylinder was tapped for 500 times initially and the tapped volume V_t was measured to the nearest graduated unit. The tapping was repeated for an additional 750 times and the tapped volume was measured. Final tapped volume was measured and tapped density was calculated by the formula: $\mathbf{M/V_t}$.

Measure of drug's compressibility

The compressibility index and Hausner ratio are measures of the propensity of a powder to be compressed. As such, they are measures of the relative importance of interparticulate interactions. In a free flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poor flowing materials there are frequently greater interparticle interactions and a greater difference between the bulks and tapped densities will be observed. These differences are reflected in the compressibility index or Carr's index (CI) and the Hausner ratio (HR) which is calculated using the following formulas [6-9]–

%Carr's Index = 100 * (TD – BD) / TD

5-15% = Good flow

15-25% = poor flow

< 40 % = very poor flow

Hausner ratio = TD / BD

<= 1.25 – good flow

Angle of repose

A glass funnel was held in place with a clamp on a ring support over a glass plate. Approximately 50 gm of material was transferred into the funnel keeping the orifice of the funnel blocked by thumb. As the thumb was removed, the powder was emptied from the funnel.

The maximum angle which is formed b/w the surface of a pile of powder and horizontal surface is called the angle of repose [7-10].

Table 1: Relationship between flow, angle of repose, Carr's index fee power flow

Flow	Angle of repose	Carr's index (%)
Excellent	<25	5-15
Good	25-30	12-16
Fair to passable	30-40	18-21
Poor	> 40	23-35
Very Poor		33-38
Extremely Poor		>40

Drug-Excipient Compatibility Studies:

Analytical Data Management Applied to Preformulation/Formulation-

Preformulation is increasingly moving toward front-loading as many number and types of studies as possible in order to reduce the risks of late stage attrition and to minimize costly problems. Despite the increase in volume, these studies cannot be allowed to delay time to market. This means that extensive characterization of greater numbers of candidate forms and evaluations based on a myriad of criteria create logistical problems in organizing, sharing, communicating, and evaluating data in a coordinated way.

Efficiency gains of 20% to 80% have been demonstrated for various steps in the workflow:

i. Reduces the time spent collecting and processing analytical data versus interpreting,

ii. Speeds up decision-making and reporting by providing a single point of access,

iii. Facilitates interdepartmental and worldwide collaboration via Web-based access [10-14].

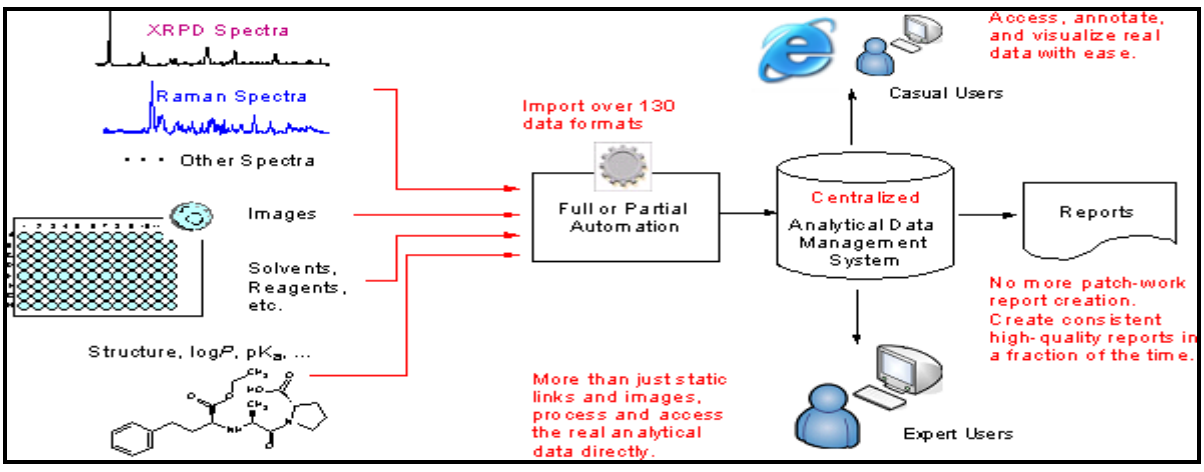

Figure 1: Preformulation Data Management Scheme

dids no.: 12.2015-77737978, dids Link: http://dids.info/didslink/06.2016-38182138/

The knowledge of drug excipients interaction is useful for the formulation to select appropriate excipients. The described preformulation screening of drug excipients interaction requires only 5mg of drug in a 50% mixture with the excipients to maximize the likelihood of obscuring an interaction. Mixtures should be examined under nitrogen to ultimate oxidation and paralytic effect at a standard heating rate on DSC, over a temperature range, which will encompass any thermal changes due to both the drug and appearance or disappearance one or more peaks in themograms of drug excipient mixtures are considered of indication of interaction [12-15].

Drug was mixed with certain proportions with all excipients to be used in our formulation and charged at 40oC / 75% relative humidity (RH) conditions for one month. The physical properties were monitored regularly. We observed any color change in the mixture and the flow of blends were not changed during the period.

Crystal Properties and Polymorphism

Many drug substances can exit in more than one crystalline from with different space lattice arrangements. This property is known as polymorphism. Polymorphs generally have different melting points, x-ray diffraction patterns and solubility even though they are chemically identical. Differences in the dissolution rates and solubilities of different polymorphic forms of a given drug are very commonly observed. When the absorption of a drug is dissolution rate limited, a more soluble and faster-dissolving from may be utilized to improve the rate and extent of bioavailability. For drugs pane to degradation in the solid state, physical form of the drug influences degradation. Selection of a polymorph that is chemically more stable is a solution in many cases. Different polymorph also leads to different morphology, tensile strength and density of power bed which all contribute of compression characteristics of materials. Some investigation of polymorphism and crystal habit of a drug substance as it relates to pharmaceutical processing is desirable during its Preformulation evaluation especially when the active ingredient is expected to constitute the bulk of the tablet mass. Although a drug substance may exist in two or more polymorphic forms, only one form is thermodynamically stable at a given temperature and pressure. The other forms would convert to the stable form with time. In general, the stable polymorph exhibits the highest melting point, the lowest solubility, and the maximum chemical stability. Various techniques are available for the investigation of the solid state. These include microscopy (including hot stage microcopy), infrared spectrophotometry, single-crystal x-ray and x-ray power diffraction, thermal analysis, and dilalometry [14-18].

The Crystal Characteristics of the pure drug was studied by using Magnus microscope. X-ray diffractometry of drug sample was investigated using Philips XRD Machine set up with generator (PW1830), Goniometry (PW 1820) and diffractometer (PW1710, Eindhoven & Almelo, Netherlands, Europe). Cu Kα radiation was used (30 kV, 50mA with a α1/α2 ratio of 0.5) to study it. The XRD patterns were recorded at diffraction angels (2θ) with 4o/min scanning speed, and 5o-45o 2θ range.

UV SPECTROPHOTOMETRIC ANALYSIS

The first requirement of any preformulation study is the development of a simple analytical method for quantitative estimation in subsequent steps. Most of drugs have aromatic rings and/or double bonds as part of their structure and absorb light in UV range, UV spectroscopy being a fairly accurate and simple method is a performed estimation technique at early preformulation stages. The absorption Co-efficient of the drug can be determined by the formula:-

E = AF / X

Where , A = Absorbance

F= dilution factor

X = weight of drug (mg)

It is now possible to determine connectration of drug in any solution by measuring absorbance.

C = AF / E (mg/ ml)

UV-Spectroscopic Scanning of Drug in the Selected Media

For this step, the media that was found to exhibit adequate solubility was used as a media for further analysis. 100 mg of the drug was weighed accurately and transferred to a 100 ml clean, dry precalibrated volumetric flask. The volume was made up to the mark with the media being used. 1 ml of this solution was pippeted out into a 100 ml clean dry pre calibrated volumetric flask and the volume made up to the mark with the same media to obtain working strength of 10 mcg/ml. This solution was scanned in the UV region (200-400 nm) and the λmax (if any) was noted together with the corresponding absorbance value.

Construction of the Calibration Curve

100 mg of drug was weighed into a clean, dry pre-calibrated 100 ml volumetric flask and treated with 0.1 N HCl. After ensuring that the drug has dissolved following adequate shaking, the volume was up with the media. This resulted in the formation of sop called 'stock solution'. From this stock solution 1,2,3,4 and 5 ml portions were withdrawn independently into separate, clean, dry, precalibrated 200 ml volumetric flasks The volume of each of the system was made up with the same 0.1N HCl to obtain required solutions with concentrations ranging from 5 to 20 mcg/ml. The absorbance values of each of these solutions were recorded at the absorption maximum of 255 nm against

dids no.: 12.2015-77737978, dids Link: http://dids.info/didslink/06.2016-38182138/

the same 0.1 N HCl that has earlier been used in the preparation of a series of drug solution as blank.

RESULTS & DISCUSSION

Physical appearance:

The drug was characterized for the physical appearance and Organoleptic properties, it was like that-

Appearance - White to off white crystalline powder

Odor – Odorless or slight odor

Taste – Bitter taste

Texture - Fluffy

Melting point:

The melting point of acyclovir was 255-257°C, matched with reported melting point which was matched with that.

pKa Determination: pKa of acyclovir was 2.19 & 9.28.

Partition Coefficient: $K_{o/w}$ of acyclovir was 0.024 and drug was found hydrophilic in nature.

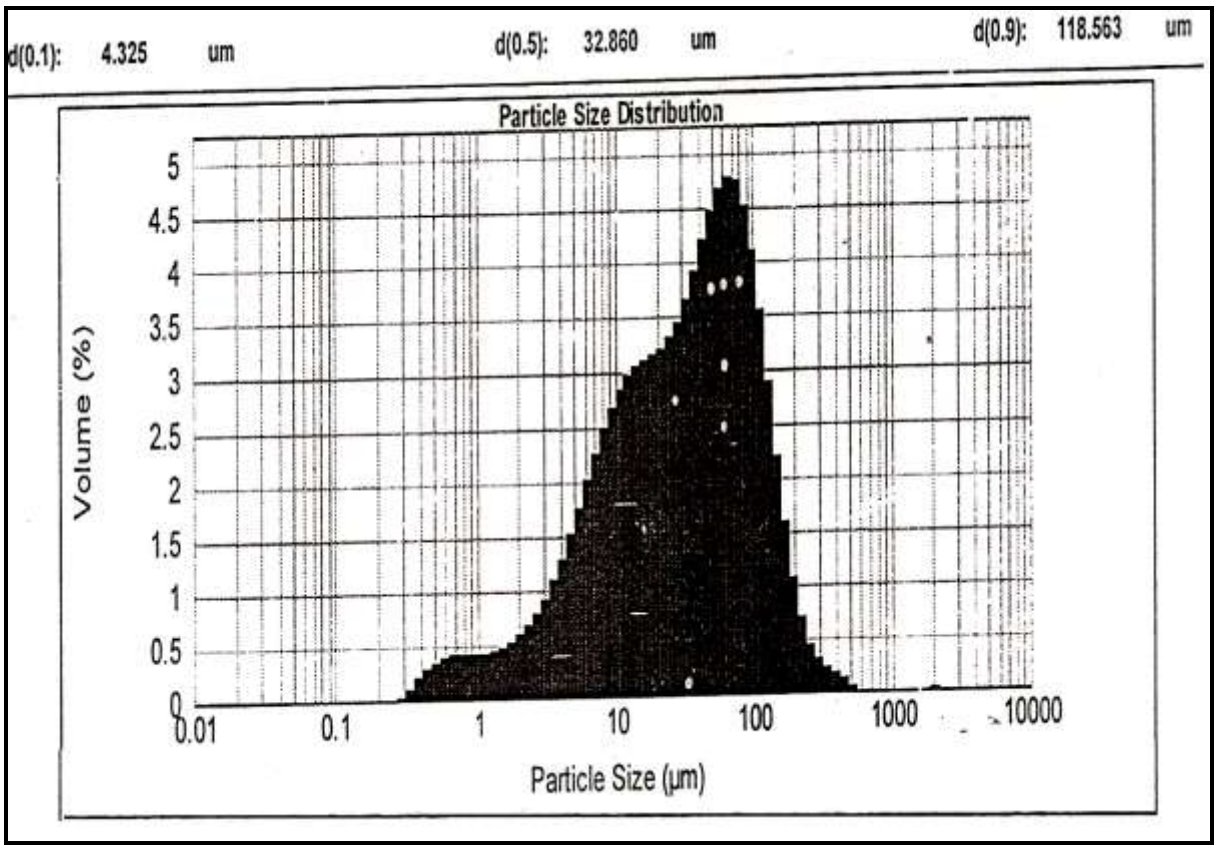

Figure 2: Particle size distribution graph

Particle size of powder drug was d (0.1) – 4.325 µm, d (0.5) – 32.860 µm and d (0.9) – 118.563 µm.

Table 2: Powder flow properties of drug

S.N0	(W)(gm)	(V_0) (ml)	(V) (ml)	Bulk density (gms/ml)	Tapped density (gms/ml)	Compressibility index [1-(V/V0)]*100	Hausner's ratio TD/BD
1	25	70	43	0.357	0.581	38.554	1.627
2	25	69.5	42	0.36	0.595	39.496	1.653
3	25	70	42.5	0.357	0.588	39.286	1.647
Average (± S.D)	-	-	-	0.358 (±0.0014)	0.588 (±0.0157)	39.112 (±0.404)	1.642± (0.01111)

The Carr's index, angle of repose values revealed that the API was having poor flow ability which was confirmed by referring to standard literature. Bulk density and Tapped density values confirming to API specification given in standard official books. All the flow parameters like angle of repose, compressibility index and Hausner's ratio are suggestive of poor flow of the model drug. Therefore it was concluded that to formulate the drug into microspheres dosage form can able to increases flowability.

dids no.: 12.2015-77737978, dids Link: http://dids.info/didslink/06.2016-38182138/

Table 3: Physical observation for drug-excipient compatibility studies

Mixtures	Ratio used	Initial observation	Observation							
			1st week		2nd week		3rd week		4th week	
			(4°c)	40°c/ 75%RH	(4°c)	40°c/ 75%RH	(4°c)	40°c/ 75%RH	(4°c)	40°c/ 75%RH
D		White	NC	NC	NC	NC	NC	NC	NC	NC
D+S	1:2	White	NC	NC	NC	NC	NC	NC	NC	NC
D+P	1:5	White	NC	NC	NC	NC	NC	*	NC	*
D+H 15cps	1:5	White	NC	NC	NC	NC	NC	NC	NC	NC
D+H 50cps	1:5	White	NC	NC	NC	NC	NC	NC	NC	NC
D+C	1:10	White	NC	NC	NC	NC	NC	NC	NC	NC
D+M	1:10	White	NC	NC	NC	NC	NC	NC	NC	NC

Where, D-Drug, S-Starch 1500, P-Povidone, H15cps-HPMC 15cps, H50cps-HPMC 50cps, C-Crospovidone, M-Magnesium stearate, *Few moist lumps, NC- no change.

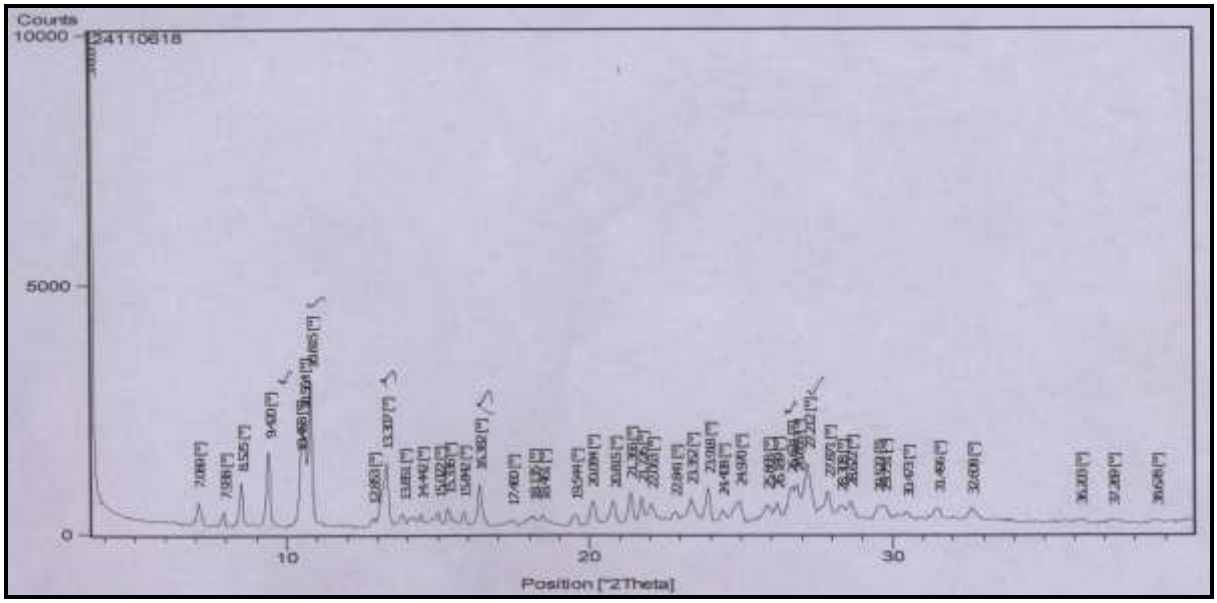

Figure 3: XRD Pattern of Drug

The powder X ray Diffraction pattern of selected samples was recorded and there was no significant change in XRD patterns of drug with starch, HPMC, Crospovidone, Magnesium stearate indicating absence of interaction between drug and these excipients. The XRD pattern of the drug and the excipients are as follows and were found to be compatible. From the XRD it is clearly proved that drug was crystalline in nature.

VALIDATION OF ANALYTICAL METHODS

Accuracy (Recovery studies)

In order to establish the reliability and suitability of the proposed method, recovery methods were carried out by the standard addition method. For the recovery studies, the drug was incorporated into the placebo. For this 1000mg of drug was added into the placebo. This was then dissolved in 900ml of media. This was then sonicated with media. This solution was filtered using 0.45µm membrane filter and 3ml of above solution was transferred into 200ml clean precalibrated flask. The absorbance of these solution was taken at 255nm.The % recovery of drug was calculated using the standard which was prepared as follows. 56.4 mg of standard was weighed into clean 50ml precalibrated flask which was sonicated with media and made upto the mark. From this 3ml of solution was transferred into 200ml flask and made up with media and its absorbance was noted.

Table 4: Recovery Analysis data

	% Recovery							
S.No	Amount of drug added	Conc. (µgm/ml)	I	II	III	Average	SD	RSD
1	1000	0.07	104.6	104.9	104.8	104.8	0.153	0.146

Filter paper interference:
The standard was prepared as described above and it was filtered using whatman filter paper 0.45µm and 0.41µm filter paper and its absorbance was noted as follows.

Table 5: Filter paper interference analysis data

SLNO	Circle No	Absorbance	% Interference
1	No filtration	0.6308	NA
2	whatman-0.45 micron	0.639	-1.30
3	whatman-41	0.6603	-4.68

System precision:
Precision is the measure of either the degree of reproducibility or of repeatability of analytical method under normal operating condition. Precision was carried out by doing the assay in inter–day basis (in two consecutive days). 10 tablets were assayed on two different days. The standard and test were prepared as already described.

Table 6: precision data

SL. No	% Amount determined										SD	RSD
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**		
1.	101.62	99.30	99.64	102.46	104.25	101.01	100.72	101.24	100.66	100.87	1.405	**1.389**
2.	100.69	102.67	100.54	99.82	104.78	103.90	99.30	100.39	101.44	101.71	1.776	**1.749**

Linearity:
Linearity of drug was detected at 0 % to 150 % of the working limit and graph of concentration Vs absorbance was plotted. A Stock of 1100 µg was prepared accurately and from this 1.5, 3, 4.5 ml of solution was pippeted and made up to the volume with media. The absorbance was noted and linearity curve was and correlation coefficient was found.

Table 7: Linearity curve data

S.No	Concentration (µg/ml)	Absorbance	Response ratio
1.	0	0	**0**
2.	7.7	0.2625	**0.0341**
3.	15.4	0.5213	**0.0339**
4.	23.1	0.7877	**0.0341**
Mean	-	-	**0.0340**
SD	-	-	**1.155**
RSD	-	-	**0.3396**

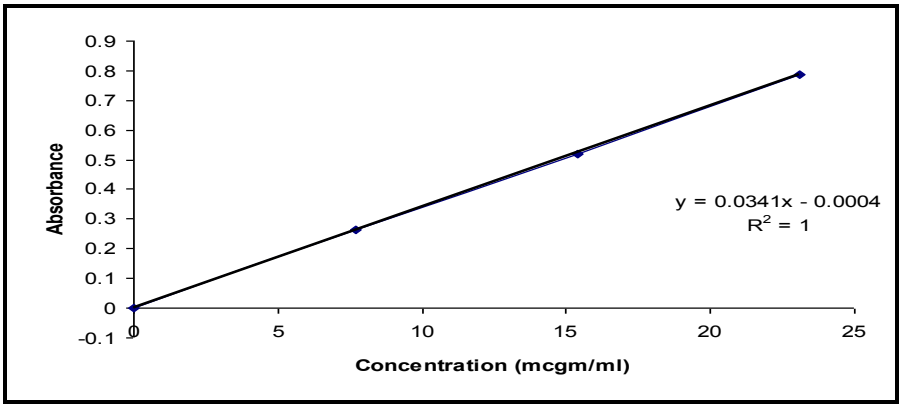

Figure 4: Linearity curve

The regression co-efficient obtained from the standard plots were nearing about 1.0 and which proved the linearity of the analytical methods. All the calibration curves followed the linear regression. All the models followed Beer-Lambert's law and therefore can be analyzed by UV spectrophotometer.

CONCLUSION

Preformulation studies have a significant part to play in anticipating formulation problems and identifying logical path in both liquid and solid dosage form technology. The need for adequate drug solubility cannot be overemphasized. Stability studies in solution will indicate the feasibility of parental or other liquid dosage form and can identify methods of stabilization. In parallel solid-state stability by DSC, TLC and HPLC in the presence of tablet and capsule excipient will indicate the most acceptable vehicles for solid dosage form.

By comparing the physicochemical properties of each drug candidate with in a therapeutic group, the preformulation scientist can assist the synthetic chemist to identify the optimum molecule, provide the biologist with suitable vehicles to elicit pharmacological response and advise the bulk chemist about the selection and production of the best salt with appropriate particle size and morphology for subsequent processing. Various parameters like melting point, pK_a, $K_{o/w}$, flow properties of the powder drug, crystal morphology, drug excipients compatibility studies and particle size distribution were carried out. The solvent used was 0.1N HCl and the λ_{max} or the absorption maxima of the drug was found to be 255nm. The regression co-efficient obtained from the standard plots were nearing about 1.0 and which proved the linearity of the analytical methods. Calibration curves followed the linear regression. All the models followed Beer-Lambert's law and therefore can be analyzed by UV spectrophotometer. This method can be used for the determination of Acyclovir in quality control of formulation without interference of the excipients.

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