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Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Acyclovir and Silymarin in Niosome Formulation

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ABSTRACT

A UV-spectrophotometric method for simultaneous estimation of Acyclovir and Silymarin in niosome formulation has been developed. Methanol is used as solvent for preparation of stock solution and further dilutions are prepared in distilled water. Acyclovir and Silymarin exhibit absorption maxima at 251nm and 287nm respectively. The system obeyed Beer's law over the concentration range of 2-10 µg/ml and 10-50 µg/ml for Acyclovir and Silymarin respectively. Method was validated for linearity, range, accuracy, precision and recovery studies as per ICH norms. Thus the proposed method was found to be rapid, specific, precise, accurate and cost effective quality control tool for the routine analysis of Acyclovir and Silymarin in niosome formulation.

Keywords: Acyclovir, Silymarin, Simultaneous Method Development, UV Spectroscopy

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INTRODUCTION

Acyclovir is a white, crystalline powder is used to treat viral disease caused by varicella, such as genital herpes, cold sores, shingles, chicken pox& herpes simples keratitis. It slows the growth and spread of the herpes virus in the body. Acyclovir will not cure herpes, but it can lessen the symptoms of the infection.[1] Mechanism of action: Acyclovir is converted by viral thymidine kinase to acyclovir monophosphate, which is then converted by host cell kinases to acyclovir triphosphate (ACV-TP). ACV-TP, in turn, competitively inhibits and inactivates HSV-specified DNA polymerases preventing further viral DNA synthesis without affecting the normal cellular processes.[2] Sylimarin is a yellow, powder, not soluble in water and is usually administered in an encapsulated form. It shows detoxifying and anti-inflammatory, Antifungal, Antiviral and ant allergic activities .which can be used to treat keratitis as there is inflammation of the cornea. Its mechanism of action includes inhibition of hepatotoxic binding to receptor sites on the hepatocyte membrane; reduction of glutathione oxidation to enhance its level in the liver and intestine; antioxidant activity; and stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration. It is orally absorbed but has very poor bioavailability due to its poor water solubility.[3]

There are various method available for the estimation of acyclovir like LC-MS-MS [4], HPTLC[5], HPLC[6], UV[7], RP-HPLC[8] LC-MS. Silymarin was estimated by TLC, UV, HPLC, Capillary electrophoresis analysis and UPLC is reported.[9]

Not a single UV method is reported so far for simultaneous analysis of Acyclovir and Silymarin in niosomal formulations. Present research work is done to develop and validate a simple UV spectroscopy based method using simultaneous equation for rapid, accurate and precise estimation of Acyclovir and Sylimarin in single formulation.

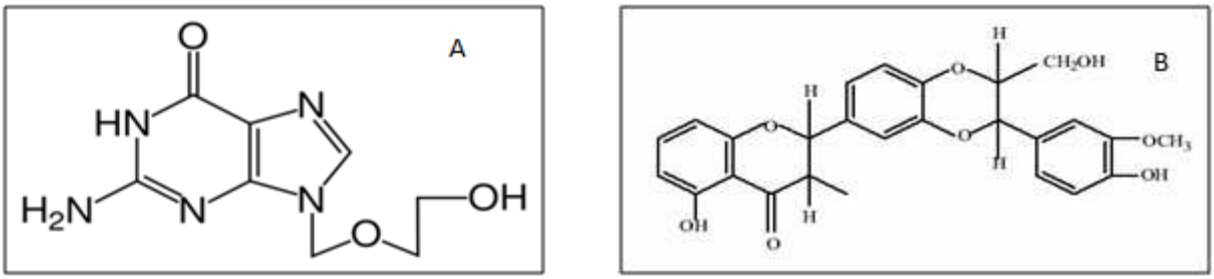

FIGURE1: STRUCTURE (A) ACYCLOVIR (B) SYLIMARIN

MATERIALS AND METHODS

Apparatus

A double beam UV-spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UVProbe2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, digital balance (Radwag.AS220/C/2 LC/GC), ultrasonicator (Steryl 40050, Mumbai, India), volumetric flasks and pipettes of borosilicate glass were used for the development and validation of proposed analytical method. [10]

Material and Reagents

Acyclovir gift sample was kindly supplied from Mahalaxmi chemicals (Mumbai), Sylimarin was purchased from Otto Chemie Pvt. Ltd (Mumbai, Maharashtra). All the reagents used in this assay were of analytical grade and the reagent solutions were prepared using pre- analyzed distilled water.

Selection of solvent

The solubility of drugs was determined in a variety of solvents as per Indian Pharmacopoeia standards. Solubility was carried out in polarto non polar solvents. The common solvent was found to be methanol and distilled water, used for the analysis of both Acyclovir and Silymarin for the proposed method.[11]

Procedure

Preparation of standard stock solution and calibration curve

10mg of Acyclovir and Silymarin were weighed individually. They were dissolved in 20ml of methanol and diluted up to 100ml by distilled water. Different aliquots of Acyclovir and Silymarin containing 10µg/ml were transferred into a series of 10 ml standard flasks using a micropipette. The maximum absorbance of Acyclovir and Silymarin was observed at 251 nm and 287 nm respectively. Correlation coefficient was 0.997 for Acyclovir and 0.998 for Silymarin .The obtained constant values are given in the **Table1**

Analytical Data

Adherence to Beer's law was studied by measuring the absorbance values of solutions varying in drug concentration. The analytical parameters such as molar absorptivity, Beer's law limits and Sand ell's sensitivity values were calculated. A linear correlation was found between absorbance and concentration ranges given in figure 3 and 4. The LOD and LOQ were calculated according to ICH guidelines as LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is standard deviation of y-intercept of regression lines (standard deviation of response) and S is slope of calibration curve .Sensitivity of the proposed methods is determined by calculating Sand ell's sensitivity (µg/cm2/0.001 Abs unit), which can be defined as smallest weight of substance that can be detected in column of unit cross-section. The calibration graphs are described by the equation Y = a + bx (Y = absorbance, a = intercept, b = slope, x = concentration in µgml−1) obtained by the method of least squares. [12]

Simultaneous Equation Method

Simultaneous estimation of drug is very important as the new combined formulation approved in market. Simultaneous estimation of drug allows the estimation of drug without prior separation of drug. So, it saves the time of analysis and also the cost of analysis because all the material and reagent require for analysis are common for the entire drug in combined formulation. Simultaneous estimation of drug is more economical for estimation of combined drug formulation, and it is widely used for the quantification of drug in combination. [13]

From the overlain spectra (Fig 2) of Acyclovir (10 µg/ml) and Silymarin (10 µg/ml), two wavelengths i.e., 251 nm as λ max of Acyclovir and 287 nm as λ max of Silymarin were selected as the working wavelength, at which both drugs showed absorbance for each other. The absorptivity of these two drugs was determined at 251 nm and 287 nm. A set of two simultaneous equations were formed using absorptivity values as given in equation (1) and (2), at

selected wavelengths. The concentrations of two drugs in Noisome Formulation were calculated using set of two simultaneous equations.

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$ ---------- (1)[13]

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$ ---------- (2)[13]

Where; Cx and Cy are concentrations of) Acyclovir and Silymarin(µg/ml) respectively in known sample solution. A1 and A 2 are absorbance of sample solutions at 251nm and 287 nm respectively. ax1and ax2 are absorptivity of Acyclovirat 251 nm and 287 nm, ay1 and ay2 are absorptivity of Silymarin at 251 nm and 287 nm. The concentration of Cx and Cy in noisome formulation can be obtained by solving equation (1) and (2). Validity of the equation was checked by using mixed standard of pure drug sample of two drugs, measuring their absorbance at respective wavelength and calculating concentration of two components.

Analysis of the Noisome Formulation

Noisome solution (1 ml) containing equivalent to 10 mg of both drugs was weighed and transferred to volumetric flask and excipient extracted with chloroform. Then drug dissolved in methanol. The sample solution was then filtered through what man filter paper. This solution was appropriately diluted to get approximate concentration of 20 µg/ml of Acyclovir and Silymarin, each, the absorbance of sample solution were measured at 251 nm and 287 nm against blank.

VALIDATION OF THE DEVELOPED METHOD

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lambert's concentration range was found to be 2-10 µg/ml for Acyclovir and 10-50 µg/ml for Silymarin. The linearity data for method is presented in **Table1.**

Accuracy

Accuracy means test output match with true value. To study the accuracy of proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%).Here to a pre-analyzed sample solution, standard drug solutions were added and then percentage of drug content was calculated.[14] The % recovery of the added pure drug was calculated as % recovery = [(Ct–Cs)/Ca] x 100, where Ct is the total drug concentration measured after standard addition; Cs, drug concentration in the formulation sample; Ca, drug concentration added to formulation.[15] The result of recovery studies are reported in **Table 3.**

Precision

Inter-day and Intra-day precision

The repeatability of the method was confirmed by the formulation analysis, repeated for six times with the same concentration. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intra-day and inter-day analysis i.e. the analysis of formulation was repeated three times in the same day at an interval of one hour and on three successive days, respectively. The amount of drugs was determined and % RSD was also calculated.[11] Study expresses within laboratory variation in different days. The result of same are presented in **Table 2**

Ruggedness Study

It expresses the precision within laboratories variations like different analyst.[10] Ruggedness of the method was assessed by for the standard 3 times with different analyst by using same equipment. The results of the same are presented in **Table 5.**

Limit of Detection (LOD) and Limit of Quantization (LOQ)

The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines were used to calculate the LOD and LOQ. [16] The LOD and LOQ were calculated by using the average of slope and standard deviation of response (Intercept). The LOD and LOQ of Acyclovir and Silymarin by proposed methods were determined using calibration standards.

LOD=3.3 δ/S
LOQ=10 δ/S

Where, S is the slope of the calibration curve and δ is the standard deviation of response (intercept). [17]The results of the same are shown in **Table 1**

Sensitivity

Sensitivity of drug viz Acyclovir and Silymarin was separately evaluated by estimating sand ell's sensitivity (µg/cm2/0.001Absunit) to determine the minimum amount of substance that can be quantified in column of unit cross section.[18] **Table 1**

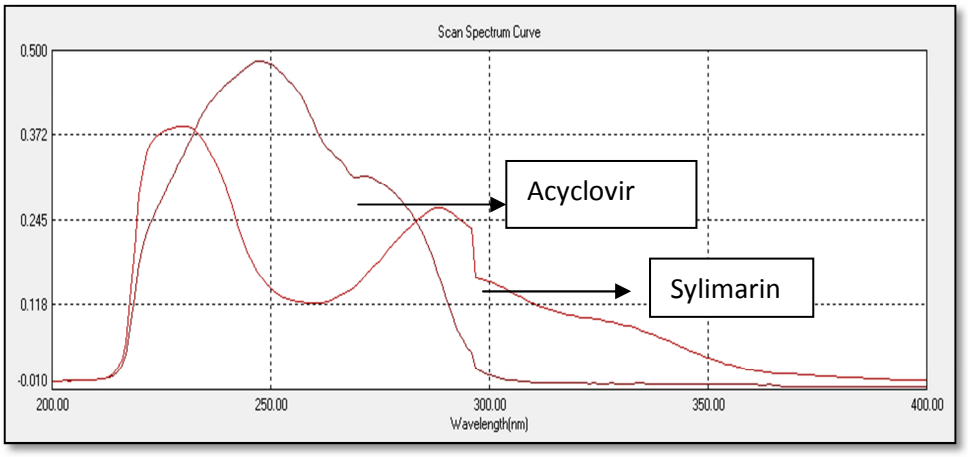

Figure 2: Overlay of maximum absorption of Acyclovir and Silymarin

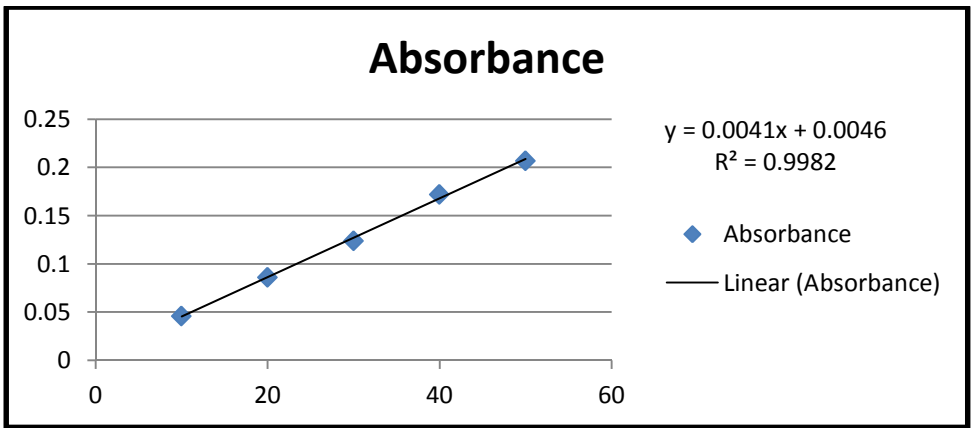

Figure 3: Calibration of Silymarin

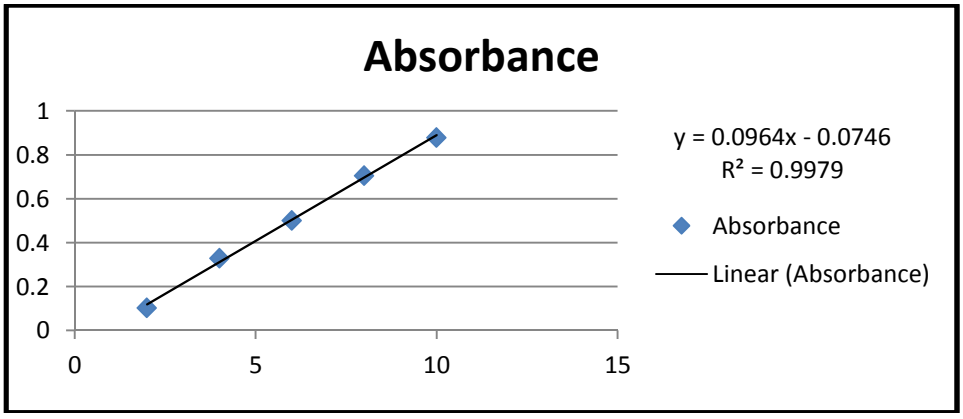

Figure 4: Calibration of Acyclovir

Table 1 Result of validation parameter

Parameter	Acyclovir	Silymarin
λmax (nm)	251 nm	287 nm
Linearity range(µgmL−1)	2-10µg/ml	10-50µg/ml
Linearity equation	Y=0.0964x-0.0746	Y=0.0041x-0.0046
Correlation coefficient	0.9979	0.9982
Slope (b)	0.0964	0.0041
intercept (a)	0.0746	0.0046
Molar absorptivity (Lmol−1 cm−1)	21619.2	1929.76
Sand ell's sensitivity (µg cm−2)	0.0104	0.25
LOD (µgmL−1)	2.54375	3.3
LOQ (µgmL−1)	7.708333	10

Table 2 Interday and Intraday precision

Drug	Interday precision		Intraday precision	
	%Amount found±SD*	% RSD	%Amount found± SD*	% RSD
Acyclovir	98.38±0.7259	0.7378	97.29±0.8	0.907
Sylimarin	101.96±0.7114	0.0069	102.17±1.008	0.986

Table 3 Recovery study

Concentration of the drug added to the formulation	Acyclovir % Recovery ±SD*	%RSD	Sylimarin % Recovery ± SD*	%RSD
80%	100.31±0.682	0.680	100.30±0.797	0.795
100%	99.86±0.3060	0.306	99.58±0.838	0.841
120%	99.88±0.1266	0.126	100.42±0.770	0.766

Table 4 Ruggedness study

Tablet Formulation	Drug	%Amount found ± S.D*
Analyst 1	Acyclovir	99.98±0.26
	Sylimarin	95.54±0.054
Analyst 2	Acyclovir	99.16±0.26
	Sylimarin	95.63±0.054

RESULTS AND DISCUSSION

The UV scan of standard solution between 200 – 400 nm showed the absorption maxima at 251 nm for Acyclovir and for Silymarin287nm. The Beer- Lambert's concentration range is for Acyclovir2-10µg/ml and for Silymarin 10-50 µg/ml at respective selected wavelength. The regression coefficient for Acyclovir and Silymarin was found to be 0.989 at 251nm and 0.997 at 287 nm respectively which indicated good correlation between concentration and absorbance within the concentration range tested. Percentage estimation of Acyclovir 249.373± 0.296 and Silymarin in niosomal formulation was found by method is 149.685±0.144. The intra- and inter-day precision was found to be less than ± 2%.

CONCLUSION

As of the result UV spectroscopic method portray in this paper for the estimation of Acyclovir and Silymarin from niosomes formulation is simple, accurate, sensitive, reproducible and economical. As this proposed method utilizes inexpensive solvents and could be applied for routine analysis in quality control laboratories.

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