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Novel Spectrophotometric Estimation of Acyclovir Using Hydrotropic Solublizing Agent

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Abstract:

The Ultraviolet absorption spectrophotometric method for the estimation of poorly water soluble drug, acyclovir. In pharmaceutical formulation has been developed aqueous solubility of this selected model drug was in 5M Urea solution. The primary objective of the present investigation was to employ hydrotropic agent to improve solubility of poorly soluble drug, without use of costlier organic solvents. The selected wavelength for Acyclovir was 254nm.The hydrotropic solution did not interfere in method development of Acyclovir. The result analyses have been validated statistically and recovery studies. The proposed methods are new, simple, economic, accurate safe and precise.

Keywords: Acyclovir, Hydrotropic Agent, Urea, Spectrophotometric Estimation.

1 Introduction

The term hydrotropic agent was first introduced by Neuberg (1916) to designate anionic organic salts which, at high concentrations, considerably increase the aqueous solubility of poorly soluble solutes.[1] Hydrotropy is a solubilisation phenomenon whereby addition of large amount of second solute results in an increase in the aqueous solubility of another solute. Hydrotropic agents are ionic organic salts. Additives or salts that increase solubility in given solvent are said to “salt in” the solute and those salts that decrease solubility “salt out” the solute. Several salts with large anions or cations that are themselves very soluble in water result in “salting in” of non-electrolytes called “hydrotropic salts” a phenomenon known as “hydrotropism”. Hydrotropic solutions do not show colloidal properties and involve a weak interaction between the hydrotropic agent and solute.[2]

It is evident from the literature survey that more is the concentration of hydrotrope; more is the aqueous solubility of poorly water-soluble drugs. Therefore, highly concentrated solutions of hydrotropic agents were used in the present investigation. Distilled water was used in making hydrotropic solutions. sodium benzoate, niacinamide, sodium salicylate, sodium acetate, urea and sodium citrate were employed as hydrotropic agent.[3,4]

Mechanism of Hydrotrope Action:-

A hydrotrope is a compound that solubilises hydrophobic compounds in aqueous solutions. Typically, hydrotropes consist of a hydrophilic part and a hydrophobic part (like surfactants) but the hydrophobic part is generally used in small quantity to cause spontaneous self-aggregation. Hydrotropesdo not have a critical concentration above which self-aggregation 'suddenly' starts to occur. Instead, some hydrotropes aggregate in a step-wise self-aggregation process, gradually increasing aggregation size.[5]

Advantages of Hydrotropic Solubilisation:-

1) It is new, simple, cost-effective, safe, accurate, precise and environmental friendly method for the analysis (Titrimetric and Spectrophotometric) of poorly water-soluble drugs.

2) By use of hydrotropic agent we can avoids the problem of residual toxicity, error due to volatility, pollution, cost etc. [6]

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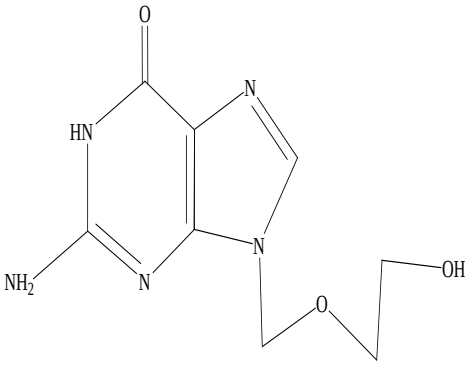
This method statistically validated for Linearity, Precision, LOD, LOQ, and Accuracy. The primary objective of this study was to employ hydrotropic solubilizing agents to enhance solubility of poor water soluble drug like acyclovir and to avoid use of organic solvents. This hydrotropic solution did not interfere in analytical method development.

Drug Profile [7]:-

Structure:-



Chemical Name : 9-[(2-Hydroxyethoxy)methyl] guanine,2-Amino-1,9-dihydro-9(2hydoxyethoxymethyl)-6H-purin-6-one.

Molecular Formula :- C8H11N5O3

Category:- Anti-Viral

Solubility:-Acyclovir is slightly soluble in water.

2 Materials and Methods

Materials

Acyclovir pure standards were received as gift samples from micro labs Pharmaceuticals GOA (India). All other reagentsused were AR grade.

Instruments:

UV- Shimadzu 1800, Centrifuge,

Method

Preparation of 5 M Urea Solution:

30 gm of urea salts was weighed and dissolved in 100 ml water and centrifuge for 2hrs.

Selection of Solvent:-Solubility of acyclovir was determined at 28±2 0c. An excess amount of pure drug was added to screw capped 30 ml vial containing different solvents viz distilled water, methanol, ethanol, chloroform. The vials were shaken mechanically for 12 hrs at 28±20c in mechanical shaker. This solution was equilibrating for next 24 hrs and then centrifuged for 5 min at 2000rpm. Thesupernatant of each vial was diluted suitably and analyzed against spectrophotometricaly corresponding solvent blank. It was found that methanol was suitable for the study of drug. The data obtained shown in Table no.1.

Table no. 1. Solubility of Acyclovir

Solvent	Solubility
Methanol	+++
Ethanol	+
Water	+
Chloroform	++

+++ Freely Soluble,
++ Sparingly soluble,
+ Insoluble

Selection of Hydrotropic Agent and its concentration:- The Urea was selected as a hydrotropic agent. For selection of concentration of urea is carried out by checking solubility of Acyclovir in different concentrations of urea solutions. The solubility of Simvastatin in urea were determined at 28±2 ^{0}C. An excess amount of pure drug was added to screw capped 30 ml vial containing different concentrations of urea solution such as 1M, 2M, 3M, 4M, 5M, urea solution. The vials were shaken mechanically for 2 hrs. at 28±20 ^{0}C in mechanical shaker. Then centrifuged for 5 min at 2000 rpm. Then each vial analyzed spectrophotometricaly against corresponding urea solution blank. It was found that 5M urea solutions were suitable for the study of drug. The data was obtained as shown in Table no.2

Table No.2.Solubility of acyclovir in molar solution of urea

Different conc. of Urea and Drug	Observation	Remark
1M+100 mg acyclovir	Not completely soluble	-
2M`+100mg acyclovir	Not completely soluble	-
3M+100mg acyclovir	Not completely soluble	-
4M+100mg acyclovir	Not completely soluble	-
5M+100mg acyclovir	Drug completely soluble	After Centrifuge clear solution is obtain.
6M+100mg acyclovir	Drug completely soluble	After Centrifuge clear solution is obtain.

Preparation of solution:-

1.Preparation of stock solution in 5M urea:- Accurately weigh 100 mg acyclovir and transfer into 100 ml volumetric flask and dissolve with 5M urea solution and volume was make up to 100 ml with water get concentration of 1000 µg/ml (stock A).10 ml stock A was taken in 100 ml volumetric flask and dilute up to 100 ml with water to get 100 µg/ml Solution (stock B).

2.Preparation of stock solution in methanol:- Accurately weigh 100 mg acyclovir and transfer into 100 ml volumetric flask dissolve with methanol solution and volume was made up to 100 ml with Methanol get concentration of 1000 µg/ml (stock C).10 ml stock A was taken in 100 ml volumetric flask and dilute up to 100 ml with water to get 100 µg/ml Solution (stock D).

UV Spectra (graph):-

Study of spectral characteristic of acyclovir in 5M urea:- For study of spectral characteristic of acyclovir in urea, concentration of 80µg/ml solution of acyclovir in urea was selected randomly. After using 5M urea the 80µg/ml solution of acyclovir was scanned in the entire UV range 400 to 200 nm. A broad band of absorption spectrum was observed with maximum absorption at 254nm.

Study of spectral characteristic of acyclovir in methanol:- For study of spectral characteristic of acyclovir in methanol, concentration of 80µg/ml solution of acyclovir in methanol was selected randomly. After using methanol the 80µg/ml solution of acyclovir was scanned in the entire UV range 400 to 200 nm. A broad band of absorption spectrum was observed with maximum absorption at 254nm.

Calibration Curve in hydrotropic agent (5M urea):- Accurately weigh 100 mg acyclovir and transfer into 100 ml volumetric flask dissolve with 5 M urea solution and volume was made up to 100 ml with water get concentration of 1000 µg/ml (stock A).10 ml stock A was taken in 100 ml volumetric flask and dilute up to 100 ml with water to get 100 µg/ml Solution (stock B).Finally from stock B different concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100µg/ml were selected and scanned at selected wavelength 254 nm. The data was obtained as shown in Table no.3

Table no.3. Calibration of acyclovir in 5M urea

Concentration in µg/ml	Absorbance		
	Sample 1	Sample 2	Sample3
10	0.1495	0.1415	0.1453
20	0.2813	0.2861	0.28
30	0.4315	0.4296	0.4356
40	0.5640	0.5654	0.5718
50	0.7136	0.7104	0.7156
60	0.8459	0.8495	0.8432
70	0.9381	0.9766	0.9817
80	1.1213	1.1254	1.1193
90	1.2647	1.2716	1.2684
100	1.4051	1.4136	1.4157

Calibration Curve in Reference Solution (Methanol):-

10 ml stock C was taken in 100 ml volumetric flask and dilute up to 100 ml with methanol to get 100 µg/ml Solution (stock D).Finally from stock D different concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100µg/ml were selected and scanned at selected wavelength 254 nm. The data was obtained as shown in Table no. 4.

Table no. 4 Calibration of acyclovir in Methanol

Concentration in µg/ml	Absorbance		
	Sample 1	Sample2	Sample 3
10	0.1200	0.1256	0.1246
20	0.2981	0.2837	0.3100
30	0.4567	0.4583	0.4513
40	0.5837	0.5883	0.5856
50	0.7940	0.8003	0.7991
60	0.8489	0.8392	0.8453
70	1.0051	1.0324	1.0037
80	1.1345	1.1296	1.1385
90	1.2946	1.3001	1.2979
100	1.4337	1.4367	1.4358

Validation

1.Linearity:-

Linearity range:-Calibration curve was prepared for acyclovir in 5M urea at 254 nm and entire data at the selected wavelength as summarized in graph. The readings were plotted by taking absorbance in Y-axis and concentration in X-axis. Whereas calibration curve was prepared for acyclovir in methanol at 254 nm and entire data at the selected wavelength as summarized in graph. The readings were plotted by taking absorbance in Y-axis and concentration in X-axis.

2.Precision:-

Interday Precision in Hydrotropic Agent (Urea):- For intraday precision study 3 Concentration of 40, 50, 60µg/ml was selected.From the stock solutionB 4, 5, 6ml were taken in the 10 ml volumetric flask and dilute it up to 10 ml with distilled water and scanned these replicates at selected wavelength (λ_{max}) 254 nm. Three readings were taken in a day of each dilution as shown in Table no. 7

Table no.7.Interday precision study of acyclovir in 5M urea

Concentration in µg/ml	Absorbance		
	Sample 1	Sample 2	Sample 3
40	0.5637	0.5687	0.5591
50	0.7204	0.7139	0.7095
60	0.8394	0.8431	0.8417

Interday Precision in Methanol :-For intraday precision study 3 replicates of 40, 50, 60µg/ml was selected. From the stock solution D, 4, 5, 6ml were taken in the 10 ml of volumetric flask and dilute it up to 10 ml with methanol and scanned these replicates at selected wavelength (λ_{max}) 254 nm. Three readings are taken of each dilution as shown in Table no. 8

Table no.8.Interday precision study of acyclovir in methanol

Concentration in µg/ml	Absorbance		
	Sample 1	Sample 2	Sample 3
40	0.5681	0.5594	0.5631
50	0.7931	0.7859	0.7881
60	0.8634	0.8691	0.8582

Intraday Precision in Hydrotropic Agent 5M Urea:- For intraday precision study 3 replicates of 40, 50, 60µg/ml was selected. From the stock solution B 4, 5, 6ml were taken in the 10 ml volumetric flask and dilute it up to 10 ml with distilled water and scanned these replicates at selected wavelength (λ_{max}) 254 nm. Three readings were taken in three days of each dilution as shown in Table no.9

Table no. 9. Intraday precision of acyclovir in 5 M urea

Concentration in µg/ml	Absorbance		
	Day 1	Day 2	Day 3
40	0.5627	0.5693	0.5631
50	0.7139	0.7212	0.7184
60	0.8456	0.8329	0.8387

Intraday Precision in Methanol:-

Day to day variation study: - For day to day variation study 3 Replicates of 40, 50, 60µg/ml were selected. From the stock solution D 4, 5, 6ml were taken in the 10 ml volumetric flask and dilute it up to 10 ml with methanol and scanned these replicates at selected wavelength (λ_{max}) 254 nm. Three readings are taken in three days of each dilution as shown in Table no. 10.

Table no.10. Intraday precision of acyclovir in methanol

Concentration in µg/ml	Absorbance		
	Day 1	Day 2	Day 3
40	0.5734	0.5693	0.5832
50	0.8103	0.7949	0.7939
60	0.8564	0.8437	0.8459

Analyst to Analyst variation study in Hydrotropic Agent (5M Urea):-

Analyst to analyst study: - For analyst to analyst study 3 Replicates of 40, 50, 60µg/ml were selected. From the stock solution D 4, 5, 6ml were taken in the 10 ml volumetric flask and dilute it up to 10 ml with distilled water and scanned these replicates at selected wavelength (λ_{max}) 254 nm. Three readings are taken by three different analyst as shown in Table no. 11

Table no. 11. Analyst to analyst study of acyclovir in 5M urea

Concentration in µg/ml	Absorbance		
	Analyst 1	Analyst 2	Analyst 3
40	0.5624	0.5717	0.5629
50	0.7193	0.7094	0.7120
60	0.8487	0.8399	0.8413

Analyst to Analyst variation study in Methanol:-

Analyst to analyst study: - For analyst to analyst

study 3 Replicates of 40, 50, 60µg/ml were selected. From the stock solution D 4, 5, 6ml were taken in the 10 ml volumetric flask and dilute it up to 10 ml with methanol and scanned these replicates at selected wavelength (λ_{max}) 254 nm. Three readings are taken by three different analyst as shown in Table no. 12.

Table no.12 Analyst to analyst study of acyclovir in methanol

Concentration in µg/ml	Absorbance		
	Analyst 1	Analyst 2	Analyst 3
40	0.5613	0.5893	0.5713
50	0.7841	0.7935	0.7879
60	0.8694	0.8549	0.8617

3.Limit of Detection and Limit of Quantitation:-
The limit of detection (LOD) and limit of quantitation (LOQ) was based on the standard deviation of the response and the slope of the constructed calibration curve.
The LOD is calculated by following formula:-
LOD = $\frac{3.3\,\sigma}{S}$
The LOQ is calculated by following formula:-
LOQ = $\frac{10\,\sigma}{S}$
Where σ = Standard deviation of the response
S = Slope of calibration curve

4. Accuracy:-
Accuracy study in 5M Urea:-
Accuracy of proposed method was determined by performing recovery studies. A fixed concentration (20µg/ml) of drug solution from dosage form was taken and pure standard drug as 3 different concentrations (10, 20, 30µg/ml) within beers range was added. The absorbance of solution were taken at 254 nm. The determination with each concentration was repeated 3 times and results are shown in Table no. 13.

Table no.13.Determination of accuracy in 5 M urea using acyclovir tablet

Mixture	Concentration of Acyclovir Tablet (µg/ml) [A]	Standard Added [B]	Absorbance [A+B]
1. 50%	20	10	0.4512
	20	10	0.4693
	20	10	0.4498
2. 100%	20	20	0.5773
	20	20	0.5731
	20	20	0.5819
3. 150%	20	30	0.7381
	20	30	0.7292
	20	30	0.7239

Accuracy study in Methanol
Accuracy of proposed method was determined by performing recovery studies. A fixed concentration (20µg/ml) of drug solute on from dosage form was taken and pure standard drug as 3 different concentrations (10, 20, 30µg/ml) within beers range was added.The absorbance of solution were taken at 254 nm. The determination with each concentration was repeated 3 times and results are shown in Table no. 14.

Table no.14 Determination of accuracy in methanol using acyclovir tablet

Mixture	Concentration of Acyclovir Tablet (µg/ml) [A]	Standard Added [B]	Absorbance [A+B]
1. 50%	20	10	0.4392
	20	10	0.4251
	20	10	0.4486
2. 100%	20	20	0.5933
	20	20	0.5821
	20	20	0.5957
3. 150%	20	30	0.7988
	20	30	0.8032
	20	30	0.8069

3 Results and Discussions

Selection of Hydrotropic Agent Concentration:- The Urea was selected as a hydrotropic agent. For selection of concentration of urea is carried out by checking solubility of Acyclovir in different concentrations of urea solutions. The solubility of Acyclovir in urea were determined at 28±2 ^{0}c. An100mg amount of pure drug was added to screw capped 30 ml vial containing different concentrations of urea solution such as 1M, 2M, 3M, 4M, 5M, urea solution. The vials were shaken mechanically for 2 hrs. at 28±2^{0}c with mechanical shaker. Then centrifuged for 5 min at 2000rpm. Solubility observes by visual inspection. The data obtained was shown in table no.15

Table no.15.Solubility of acyclovir in molar solution of urea

Concentration of Urea Solution	**Observation**	**Remark**
1M+100 mg acyclovir	Not completely soluble	-
2M`+100mg acyclovir	Not completely soluble	-
3M+100mg acyclovir	Not completely soluble	-
4M+100mg acyclovir	Not completely soluble	-
5M+100mg acyclovir	Drug completely soluble	After Centrifuge clear solution is obtain.
6M+100mg acyclovir	Drug completely soluble	After Centrifuge clear solution is obtain.

Study of spectral characteristic of Atenolol in 5M urea:- For study of spectral characteristic of acyclovir in urea, concentration of 80µg/ml Solution of acyclovir in urea was selected randomly and was scanned in the entire UV range 400 to 200 nm. A broad band of absorption spectrum was observed with maximum absorption at 254nm as shown in fig.1.

Urea Solution having 238 nm λ max and acyclovir having 254 nm λ max. So that urea solution was not interfere in method development of Acyclovir.

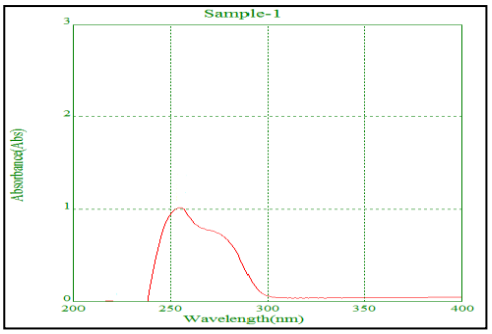


Fig. no.1 UV in spectra of acyclovir 5M urea.

Study of spectral characteristic of acyclovir in Methanol:-For study of spectral characteristic of acyclovir in methanol, concentration of 80µg/ml Solution of acyclovir in methanol was selected randomly and was scanned in the entire UV range 400 to 200 nm. A broad band of absorption spectrum was observed with maximum absorption at 254nm as shown in fig no.2. Methanol having 240 nm λ max and acyclovir having 254 nm λ max. So that methanol solution was not interfere in method development of Acyclovir.

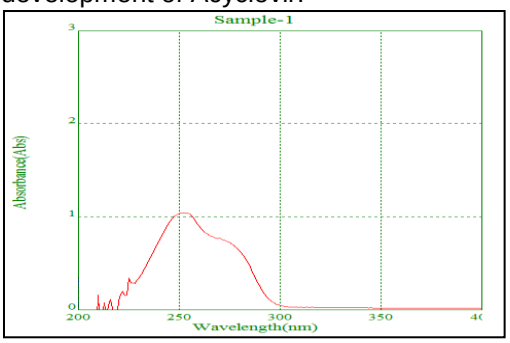
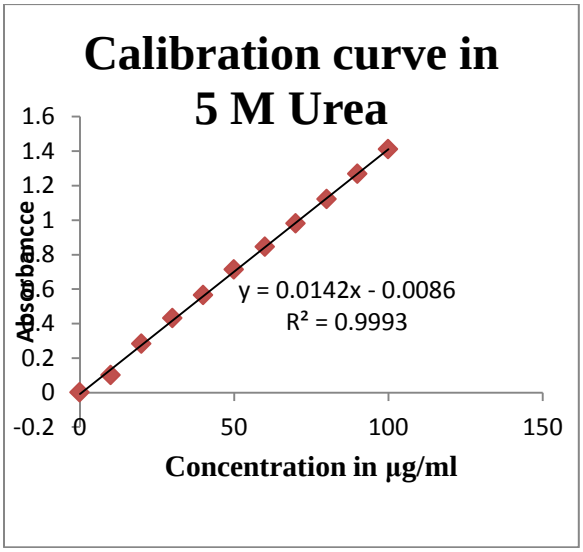


Fig.no:2 UV in spectra of acyclovir in methanol

Calibration Curve in 5M Urea:-The calibration curve was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in graph no.1. The absorbance of concentration from 10-100µg/ml were taken and repeated thriceand mean of absorbance was calculated show in table no. 16. The calibration graph plotted by taking means absorbance and drug conc. solution.The drug obeyed beer's law in the concentration range of 10-100µg/ml.the SD and % RSD were calculated in table no. 16 and it was found that the low standard deviation and less than 2 % RSD obtain.

Table no. 16. Calibration of acyclovir in5M urea

Concentration in µg/ml	Absorbance			Mean	SD	%RSD
	Sample 1	Sample 2	Sample3			
10	0.1495	0.1415	0.1453	0.1457	0.004	2.7586
20	0.2813	0.2861	0.28	0.2824	0.003215	1.14
30	0.4315	0.4296	0.4356	0.4317	0.003055	0.7088
40	0.5640	0.5654	0.5718	0.5658	0.005508	0.9783
50	0.7136	0.7104	0.7156	0.7127	0.002517	0.3535
60	0.8459	0.8495	0.8432	0.8453	0.003055	0.3615
70	0.9381	0.9766	0.9817	0.98	0.003606	0.3679
80	1.1213	1.1254	1.1193	1.1217	0.003055	0.2720
90	1.2647	1.2716	1.2684	1.2673	0.003512	0.2770
100	1.4051	1.4136	1.4157	1.4117	0.005292	0.4190



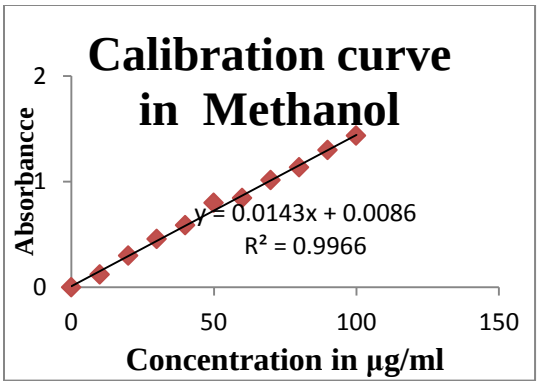
Graph no.1 Calibration curve in 5 M urea

Calibration Curve in Methanol:-The calibration curve was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in graph no.2. The absorbance of concentration from 10-100µg/ml were taken and repeated thrice and mean of absorbance was calculated show in table no. 17. The calibration graph plotted by taking means absorbance and drug conc. solution.The drug obeyed beer's law in the concentration range of 10-100µg/ml. the SD and % RSD were calculated in table no. 17 and it was found that the low standard deviation and less than 2 % RSD obtain.

So drug obeyed beer's law in the concentration range of 10-100µg/ml in 5M urea solution as well as in methanol solution.

Table no.17. Calibration of acyclovir in methanol

Concentration in µg/ml	Absorbance			Mean	SD	%RSD
	Sample 1	Sample2	Sample 3			
10	0.1200	0.1256	0.1246	0.1239	0.002646	2.1512
20	0.2981	0.2837	0.3100	0.2976	0.01352	4.5521
30	0.4567	0.4583	0.4513	0.4559	0.003606	0.7925
40	0.5837	0.5883	0.5856	0.5857	0.002517	0.4302
50	0.7940	0.8003	0.7991	0.7973	0.003215	0.4033
60	0.8489	0.8392	0.8453	0.8448	0.004583	0.5430
70	1.0051	1.0324	1.0037	1.0133	0.01619	1.5982
80	1.1345	1.1296	1.1385	1.1336	0.004509	0.3970
90	1.2946	1.3001	1.2979	1.2974	0.003	0.2313
100	1.4337	1.4367	1.4358	1.4347	0.001528	0.1065



Graph.no.2. Calibration curve in methanol

Linearity:-

The linear fit of the system was illustrated graphical. Least square regression analysis was carried out for the slope, intercept and co-relation coefficient. The equation of calibration curve for Acyclovir in urea obtained as Y=0.014x-0.008 and Y=0.014x+0.008 in Methanol. The co-relation coefficient r^2 of determination was found to be 0.999 in urea and 0.996 in methanol. The Calibration curve was found to be linear.

Precision:-Precision of method was assessed by interday, intraday study and analyst to analyst study by analysing concentration of 40 , 50 , 60 µg/ml of acyclovir, the results are reported in table no. 17(interday), 19 (intraday), 21 (analyst to analyst) in urea solution and table no..18 (interday), 20 (intraday), 22(analyst to analyst) in Methanol solution

Precision of method were assessed by interday, intraday study and analyst to analyst study for 5 M urea solution and methanol solution. In 5M urea solution the % RSD for interday (0.4662%), intraday (0.7174%), and analyst to analyst (0.7334%). And in methanol solution % RSD were found to be for interday (0.6506%), intraday (1.0910%), analyst to analyst (1.3081%). So % RSD in 5 M Urea solution and in methanol was less than 2.

Table no. 17 Interday precision study of acyclovir in 5M urea

Concentration in µg/ml	Absorbance			Mean	SD	%RSD
	Sample 1	Sample 2	Sample 3			
40	0.5637	0.5687	0.5591	0.5627	0.004933	0.8777
50	0.7204	0.7139	0.7095	0.7114	0.005568	0.2831
60	0.8394	0.8431	0.8417	0.8415	0.00200	0.2378

Table no. 18. Interday precision study of acyclovir in methanol

Concentration in µg/ml	Absorbance			Mean	SD	%RSD
	Sample 1	Sample 2	Sample 3			
40	0.5681	0.5594	0.5631	0.5637	0.004509	0.8008
50	0.7931	0.7859	0.7881	0.7886	0.004041	0.5128
60	0.8634	0.8691	0.8582	0.8639	0.005508	0.6382

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Table no.19Intraday precision of acyclovir in 5M urea

Concentration in µg/ml	Absorbance			Mean	SD	%RSD
	Day 1	Day 2	Day 3			
40	0.5627	0.5693	0.5631	0.56411	0.004583	0.8125
50	0.7139	0.7212	0.7184	0.7175	0.004041	0.5635
60	0.8456	0.8329	0.8387	0.8389	0.006506	0.7763

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Table no.20Intraday precision of acyclovir in methanol

Concentration in µg/ml	Absorbance			Mean	SD	%RSD
	Day 1	Day 2	Day 3			
40	0.5734	0.5693	0.5832	0.5753	0.007211	1.2540
50	0.8103	0.7949	0.7939	0.7994	0.009539	1.1938
60	0.8564	0.8437	0.8459	0.8483	0.007	0.8254

Table no.21 Analyst to analyst study of acyclovir in5M urea

Concentration in µg/ml	Absorbance			Mean	SD	%RSD
	Analyst 1	Analyst 2	Analyst 3			
40	0.5624	0.5717	0.5629	0.5659	0.005196	0.9196
50	0.7193	0.7094	0.7120	0.7133	0.005131	0.7194
60	0.8487	0.8399	0.8413	0.8427	0.004726	0.5612

Table no. 22. Analyst to analyst study of acyclovir in methanol

Concentration in µg/ml	Absorbance			Mean	SD	%RSD
	Analyst 1	Analyst 2	Analyst 3			
40	0.5613	0.5893	0.5713	0.5734	0.01418	2.4712
50	0.7841	0.7935	0.7879	0.7884	0.004583	0.5815
60	0.8694	0.8549	0.8617	0.8616	0.007506	0.8717

Limit of Detection:-Based on the standard deviation of the response and slope .The detection limit may express as:

$$LOD = \frac{3.3\,\sigma}{S}$$

Where,

σ= standard deviation of response

S= slope of calibration curve.

For LOD in hydrotropic agent (urea) was found to be 0.8554µg/ml and in methanol it was found to be 1.2746µg/ml. LOD in urea solution is lower than LOD in methanol so method with urea solution is more sensitivity than method with methanol.

Limit of Quantitation:-Based on the standard deviation of the response and slope. The quantitation limit may express as:

$$LOQ = \frac{10\,\sigma}{S}$$

Where,

σ= standard deviation of response

S= slope of calibration curve.

For LOQ in hydrotropic agent (urea) was found to be 2.5922µg/ml and in methanol it was found to be 3.8624µg/ml. The LOD &LOQ in hydrotropic agent (urea) was found to be less than methanol. Method with using urea as hydrotropic agent was more sensitivity than method using methanol.

Accuracy (Recovery test):-To assess the accuracy of proposed method, recovery experiment was performed at three different level that are 50%, 100% and 150%.To pre-analysed sample solution, a known amount of standard drug solution was added at three different levels and absorbance's were recorded.

The percent recovery was then calculated by using the following formula:

$$\%\ \text{Recovery} = \frac{(A-B)}{C} \times 100$$

Where, A= Total amount drug estimated.

B= Amount of drug found on pre-analysed bases.

C= Amount of pure drug added.

The recovery value for acyclovir tablet in urea was found to be 103.59% and in methanol it was found to be 99.14% as shown in table no. 23 and table no.24

The percent recovery of acyclovir in 5 M urea was higher than the methanol solution. But there was no more difference between these two values.

So that hydrotropic agent (urea) suitable solvent for increasing the solubility of acyclovir without use of organic solvent (Methanol).This method is simple, precise and accurate for the analysis of Acyclovir and hence this method was validated as per ICH guideline.

4 CONCLUSION

By this study we can concluded that solubility is most important characteristic of a drug for qualitative and quantitative analysis. Solubility can be enhanced by hydrotropic techniques. This study presents a spectrophotometric evaluation of Acyclovir exposing the advantages of using hydrotropy agent as regards to simplicity, lower cost, and better sensitivity and precluding the use of organic solvents. The method is simple, precise and accurate for the determination of Acyclovir in bulk and tablet dosage form. The results summary of method in urea and methanol was given in table no. 25 and 26

Table no.23 Determination of accuracy in 5M urea using acyclovir tablet

Mixture	Concentration of Tablet (µg/ml) [A]	Standard Added [B]	Absorbance [A+B]	Mean (Absorbance)	%RSD	%Recovery
1. 50%	20	10	0.4512			103.59%
	20	10	0.4693	0.4567	2.3861	
	20	10	0.4498			
2. 100%	20	20	0.5773			
	20	20	0.5731	0.5774	0.7623	
	20	20	0.5819			
3. 150%	20	30	0.7381			
	20	30	0.7292	0.7304	0.9824	
	20	30	0.7239			

Table no.24 Determination of accuracy in methanol using acyclovir tablet

Mixture	Concentration of Tablet (µg/ml) [A]	Standard Added [B]	Absorbance [A+B]	Mean (Absorbance)	%RSD	%Recovery
1. 50%	20	10	0.4392			99.14%
	20	10	0.4251	0.4376	2.7010	
	20	10	0.4486			
2. 100%	20	20	0.5933			
	20	20	0.5821	0.5903	1.2297	
	20	20	0.5957			
3. 150%	20	30	0.7988			
	20	30	0.8032	0.8029	0.5050	
	20	30	0.8069			

Table no. 25.Results of Validation Parameters for Acyclovir in Urea solution

Sr. No.	Parameters	Results
1	λ_{max}	254nm
2	Beer's range	10-100µg/ml
3	Correlation coefficient (r^2)	0.999
4	Regression equation	Y=0.014x-0.008
5	Intercept	-0.00891
6	Slope	0.0142
7	Precision RSD	
	Interday precision	0.6328%
	Intraday precision	0.7174%
	Analyst to Analyst study	0.7334%
8	LOD	0.8554 µg/ml
9	LOQ	2.5922 µg/ml
10	% Recovery	103.59%

Table no. 28. Validation Parameters for Acyclovir in Methanol

Sr. No.	Parameters	Results
1	λ_{max}	254nm
2	Beer's range	10-100µg/ml
3	Correlation coefficient (r^2)	0.996
4	Regression equation	Y=0.014x+0.008
5	Intercept	0.008591
6	Slope	0.01432
7	Precision RSD	
	Interday precision	0.6506%
	Intraday precision	1.0910%
	Analyst to Analyst study	1.3081%
8	LOD	1.2746µg/ml
9	LOQ	3.8624µg/ml
10	% Recovery	99.14 %

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DECLERATION OF CONFLICT INTEREST
Author declares no conflict of interest.

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