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(Research Article)

Spectrophotometric Estimation of Acyclovir by a Novel Mixed Solvency Solubilisation Concept

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ABSTRACT

The present study involves the use of mixed cosolvency solubilisation technique to estimate the amount of Acyclovir present in tablets by spectrophotometric method in the mixture of solvents 15% urea, 8% propylene glycol and 7% PEG 6000. It was observed that solubility of Acyclovir in this solvent mixture was increased by ten folds as compared with distilled water. The method obeyed Beer's law with maximum absorbance at 251nm. The analysis of tablets indicated good correlation between estimated and label claim. The results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method. The low values of LOD and LOQ of Acyclovir in the solvent mixture indicated good sensitivity of proposed method. As urea, propylene glycol and PEG 6000 were cheaper than most of the organic solvents, these can be used as a substitute for organic solvents. The study proved that mixed cosolvency phenomenon is an effective technique in enhancement of aqueous solubility of poorly water soluble drugs. The proposed method is new, simple, accurate, non-toxic and precise method that can be successfully employed for estimation of drugs in routine analysis of tablets.

Key Words: Mixed cosolvency, Acyclovir, Urea, Solubilisation, Spectrophotometry

INTRODUCTION

The improvement of aqueous solubility of poorly water soluble drugs is of prime importance in pharmaceuticals. This is essential as most of the newly developed drugs are highly lipophilic in nature and its analysis was mainly carried out using organic solvents like methanol, chloroform, ethanol, benzene, acetone, toluene, carbon tetrachloride, diethyl ether and acetonitrile[1-3]. Most of these organic solvents are toxic, volatile and costlier. This may cause inaccuracy in analytical methods[4]. Several approaches have been used to improve the aqueous solubility. One such technique is use of hydrotropic agents like Sodium benzoate, Sodium salicylate, Niacinamide, Sodium ascorbate and Urea. Various works have been reported on use of hydrotropic solvents in estimation of various lipophilic drugs [5-10]. It was observed that hydrotropy is another type of cosolvency. Based on this approach a novel mixed solvency solubilisation phenomenon was developed to study the effect on solubility of poorly water soluble drugs. Few works based on this mixed cosolvency technique were reported to study the improvement in solubility by titrimetric estimations[11, 12]. This mixed cosolvency technique is based on the principle that instead of using one solubilizer in large concentration for a desired level of solubility, several solubilizers like hydrotropes (sodium ascorbate, urea, sodium benzoate), co-solvents (propylene glycol, PEG 200, 300, 400) and water soluble solids (PEG 4000, 6000, Cyclodextrins) in varying concentrations may be used that may show additive or synergistic enhancement in solubility. These solubilizers do not cause any toxicity and are non volatile. No work has been reported on spectrophotometric estimations of poorly water soluble drugs using

the mixed cosolvency concept. So based on the existing literature an attempt was made in this investigation to demonstrate the application of mixed solvency concept for spectrophotometric estimation of Acyclovir, a poorly water soluble drug in the bulk drug sample and tablets thereby eliminating the use of organic solvents. The total concentration of solubilizers was kept constant (30 % w/v) in all solubilizing systems. The selected solubilizers were urea (15%) as hydrotrope, propylene glycol (8%) as cosolvent and PEG 6000 (7%) as water-soluble solid. Acyclovir is an anti-viral drug, a synthetic nucleoside analogue which is active against herpes viruses. Acyclovir is activated via monophosphorylation by virus induced thymidine kinase. The molecular formula is $C_8H_{11}N_5O_3$. The chemical name is 9-[(2-Hydroxy) methyl] guanine; 2-Amino-1, 9-dihydro-9-(2-hydroxyetoxymethyl)-6H-purin-6-one. It is slightly soluble in water, whereas freely soluble in dimethylsulfoxide and dilute acids and alkali[13].

MATERIALS AND METHOD

Acyclovir bulk drug was a gift sample obtained from Remidex Pharmaceuticals, Bangalore. Urea, propylene glycol and PEG 6000 was purchased from S.D.Fine Chemicals, Mumbai. Tablets of Acyclovir were purchased from local market. Shimadzu UV/Visible recording spectrophotometer (model-UV-1601) with 1cm matched silica cells was employed. All other chemicals and solvents used were of analytical grade.

Experimental Method

Saturation solubility studies of the drug

Solubility of Acyclovir was determined by saturation aqueous solubility method in mixed cosolvents containing 15%urea, 8% propylene glycol and 7% PEG 6000 (UPEG) in distilled water. An excess amount of drug was added to 50ml beakers containing UPEG and distilled water. The beakers were shaken for 12 hours at 28 ± 1^0C. The solutions were filtered through Whatman filter paper #41, and the resulting filtrates were suitably diluted and analyzed spectrophotometrically against solvent blank.

Preparation of standard stock and calibration curve

The standard stock solution of Acyclovir was prepared by dissolving 50mg of drug in 50 ml of UPEG. From this solution 5ml of solution was taken and diluted to 100ml with distilled water to get a solution containing 50µg/ml and scanned in the entire UV range of 400-200 nm to determine the λ max of the drug. The λ max of Acyclovir was found to be 251 nm (Fig.1). Ten working standard solutions for the drug having concentration 2, 4, 6, 8,10,12,14,16,18 and 20µg/ml was prepared with distilled water from the stock solution. The absorbances of resulting solutions for the drug were measured at wavelength of 251nm and a calibration curve was plotted to get the linearity and regression equation.

Analysis of Acyclovir in tablets using UPEG

Twenty tablets were weighed and powdered. Powder equivalent to 200mg Acyclovir was transferred to 50ml volumetric flasks containing 40ml of UPEG. The flasks were shaken for about 10min to solubilize the drug. Then volume was made up to the mark with distilled water. From this 5ml of solution was pipette into 50ml volumetric flask containing distilled water to get a concentration of 200µg/ml. From this 5ml was pipette into 100ml volumetric flask containing distilled water to get of 10µg/ml and absorbance was measured at 251nm against solvent blank and drug content was calculated.

Validation of the Proposed Method

The proposed method was validated for the following parameters[14].

Recovery studies

In order to check the accuracy and reproducibility of the proposed method, recovery studies were conducted. Tablet powder equivalent to 200 mg of Acyclovir was transferred to a 100ml volumetric flask containing UPEG. Pure Acyclovir drug sample containing 10 mg was added to the same volumetric flask. The flask was shaken for 10 mins to solubilize the drug. Then solution was filtered through Whatman filter paper #41. The filtrate was diluted with distilled water appropriately and absorbance was measured at 251nm against corresponding solvent blank. Drug content was calculated and % recovery was calculated. Similar procedure was repeated using 20 mg and 30 mg of pure Acyclovir as spiked concentration. The drug contents were determined and % recoveries were estimated.

Precision
Precision was determined by studying the repeatability and intermediate precision. The standard deviation, coefficient of variance and standard error were calculated for the drug.

Inter- day and Intra- day precision
The intra-day concentration of the drug was calculated on the same day at an interval of one hour. Whereas the inter day concentration of drug was calculated on three different days within the laboratory conditions.

Linearity
The absorbances of appropriate dilutions of standard stock solutions were measured as per the developed method to confirm the linearity.

Limit of detection (LOD) and Limit of Quantitation (LOQ)
The LOD and LOQ of Acyclovir by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3σ /S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response.

RESULTS AND DISCUSSION
The results of saturation solubility studies of Acyclovir in the mixed cosolvents revealed that solubility of Acyclovir in mixed cosolvents containing 15%urea, 8% propylene glycol and 7% PEG 6000 was found to be 5mg/ml whereas the solubility in distilled water was found to be 0.525mg/ml. These results indicated that aqueous solubility of Acyclovir was increased to ten folds in mixed cosolvents as compared to distilled water. The Beer- Lambert's concentration range for Acyclovir in mixed co-solvents was between 2-20 µg/ml. To check drug stability and precipitation of drug in solvent, a part of solution were kept in room temperature for 48 hours. The results revealed that estimation of Acyclovir can be done without substantial effect on drug stability as no precipitation was observed. From this study it is obvious that there was no interference of urea or PEG 6000 or propylene glycol in estimation of Acyclovir at the wavelength of 251nm. Based on this a large number of poorly water soluble drugs having λ max above 250 nm may be tried for estimation by the proposed method provided that their preliminary solubility studies confirmed the enhancement of solubility in the mixed cosolvents. Urea, propylene glycol and PEG 6000 are cheaper than most of the organic solvents and can thus may be better substitutes for expensive organic solvents that are used in routine analysis of pharmaceuticals.
The estimated label claim in 4M urea and 4M sodium acetate was 99.41±0.7252 indicating good correlation between estimated and those claimed by the manufacturers. Percent label claims were very close to 100 with low values of standard deviation and standard error. The results of percent label claim were shown in Table 1. The recovery studies showed proposed method is accurate and reproducible. The results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method. Accuracy, reproducibility and precision of the proposed methods were further confirmed by percent recovery values, which were close to 100 with low values of standard deviation and standard error as shown in Table 2. Repeatability results indicated the precision under the same operating conditions over a short interval time and inter-assay precision. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for the method co-efficient of variation were not more than 1.0% indicates good intermediate precision. The low values of LOD and LOQ, 0.2720 and 0.8180 for Acyclovir in the mixed UPEG indicated good sensitivity of proposed method. (Table 3).

CONCLUSION
It was thus concluded that mixed solvency solubilisation phenomenon is an effective technique in the enhancement of solubility of a poorly water soluble drug. Hence the proposed method is new, simple, accurate, non-toxic and precise method. This method can be successfully employed for estimation of drugs in routine analysis of tablets.

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Table 1: Analysis of tablet formulations of Acyclovir

Tablet Formulation	Label Claim (mg)	% Label claim Estimated* (Mean ± S.D.)	Standard error
Commercial Tablet+15%urea, 8% propylene glycol and 7% PEG 6000 (UPEG)	200mg	99.41±0.7252	0.2961

* Average of six determinations

Table 2: Result of recovery studies

Formulation	Amount of Acyclovir tablet powder(mg)	Amount of standard drug added (mg)	% Recovery estimated* (mean ±S.D.)	Standard error
Commercial Tablet+15%urea, 8% propylene glycol and 7% PEG 6000 (UPEG)	200	10	102.0±0.9048	0.6610
	200	20	101.4±0.4679	0.5691
	200	30	101.2±0.7398	0.7112

***** Average of six determinations

Table 3: Optical characteristics data and validation parameters

Parameters	Values of Acyclovir in 15%urea, 8% propylene glycol and 7% PEG 6000 (UPEG)
Working λmax (nm)	251nm
Beer's law limit (µg/ml)	2-20
Molar Absorptivity	14.5×10^3
Correlation coefficient*	0.989
Intercept*	0.0045
Slope*	0.055
LOD* (µg/ml)	0.2720
LOQ* (µg/ml)	0.8180
Intra-day* (precision) (Co-eff. of variation)	0.3280
Inter-day*(precision) (Co-eff. of variation)	0.2275
Robustness	Robust

***** Average of 6 determinations

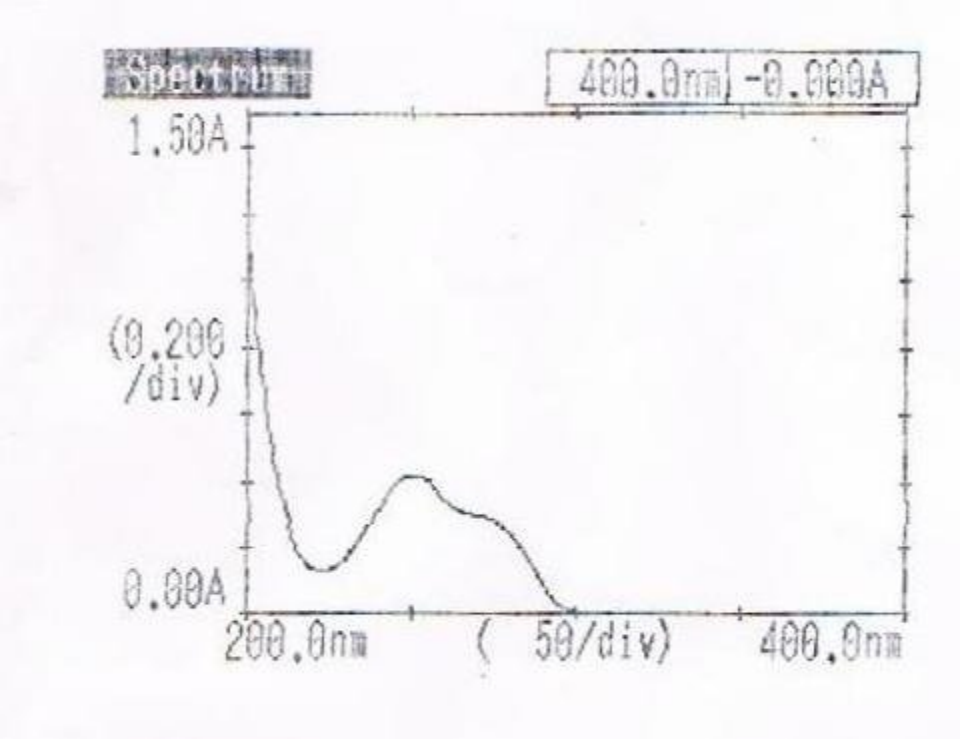

Figure 1: UV-spectra of Acyclovir in UPEG

REFERENCES

1. Maheshwari RK, "Solubilisation of ibuprofen by mixed-solvency approach", The Indian Pharmacist, 2009, 8: 81-84.
2. Maheshwari RK, "Analysis of frusemide by application of hydrotropic solublization phenomenon" The Indian Pharmacist, 2005, 4:55-58
3. Maheshwari RK, "New application of hydrotropic solublization in the spectrophotometric estimation of ketoprofen in tablet dosage form" The Pharma Review, 2005, 3:123-125
4. Maheshwari RK, "Novel application of hydrotropic solubilisation in the spectrophotometric analysis of tinidazole in dosage form" Asian J Chem, 2006,18: 640-644.
5. Jain NK, Singhai AK, et al. "Hydrotropic solubilization of ketoprofen" Pharmazie1996, 51: 236-239
6. Maheshwari RK, Chaturvedi SC, et al "Application of hydrotropy in the spectrophotometric determination of pharmaceutical dosage forms" Indian Drugs,2005, 42: 760-763.
7. Maheshwari RK, "Novel application of hydrotropic solubilization in the spectrophotometric analysis of piroxicam in solid dosage form" Indian Drugs, 2006, 8:683-685.
8. Maheshwari RK, "A novel application of hydrotropic solubilization in the spectrophotometric estimation of frusemide in tablets" The Pharma Review, 2006, 4:148-149.
9. Maheshwari RK, "Application of hydrotropic solubilization phenomenon in spectrophotometric estimation of norfloxacin in tablets" Indian J Pharm Edu Res, 2006, 40:237-240.
10. Maheshwari RK, "Mixed hydrotropy in spectrophotometric analysis of aceclofenac" The Indian Pharmacist, 2007, 6: 67-69.
11. Maheshwari RK, "Mixed-Solvency – A novel concept for solubilization of poorly water-soluble drugs" (JTES) Delving: J Tech Engg Sci, 2009, 1: 39-44.
12. Maheshwari RK, "Potentiation of solvent character by mixed-solvency concept: A novel concept of solubilisation" J of Pharm Res, 2010, 3(2): 411-413.
13. Martindale, The complete drug reference, 34th edition, Pharmaceutical Press : 626-627.
14. Anish Vinnakota SN, Deveswaran R, et al "Application of mixed hydrotropic solubilization in spectrophotometric estimation of aceclofenac in tablets" Current Pharma Res, 2011, 1(3): 223-226.

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