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Development and validation of new analytical methods for the estimation of Valacyclovir hydrochloride in pharmaceutical dosage form

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ABSTRACT

For the assay of Valacyclovir hydrochloride five new spectrophotometric methods have been developed in pharmaceutical dosage forms. The first method was developed in Sodium Acetate (Method A) showing absorption maxima at 251 nm, the second method Phosphate buffer pH 5.0 (Method B) was used (λ_{max} 251 nm), the third method phosphate buffer pH 7 (Method C) was used (λ_{max} 252 nm), the fourth method borate buffer pH 9.0 (Method D) was used (λ_{max} 253 nm) and the fifth method 0.1N NaOH (Method E) was used (λ_{max} 265 nm). Linearity was observed over the concentration range 1-80 µg/mL for all the methods A, B, C, D and E respectively with regression equations 0.032x + 0.0144, 0.0324x + 0.0044, 0.0322x + 0.0118, 0.0304x + 0.0013 and 0.0278x + 0.0063 for method A, B, C, D and E respectively. The proposed spectrophotometric methods were validated and can be applied for the determination of Valacyclovir Hydrochloride in pharmaceutical formulations.

Key words: Valacyclovir hydrochloride, Sodium acetate buffer, Phosphate buffer pH 5.0, Phosphate buffer pH 7.0, Borate buffer pH 9.0, 0.1N NaOH.

INTRODUCTION

Valacyclovir hydrochloride (Figure1) is an antiviral drug used in the management of herpes simplex, herpes zoster (shingles) and herpes B. It is a prodrug, being converted in vivo to acyclovir. Valacyclovir hydrochloride is an orally administered chemotherapeutic agent used in the treatment of HSV and VZV infections, it has shown promise as a treatment for infectious mononucleosis and is preventively administered in suspected cases of herpes B virus exposure. Valacyclovir hydrochloride is a prodrug intended to increase the bioavailability of acyclovir by increasing lipophilicity. Valacyclovir hydrochloride converted by esterase to active drug acyclovir via hepatic first pass metabolism. It inhibits viral DNA synthesis. Acyclovir that exhibits activity against herpes simplex virus types, 1(HSV–1) and 2(HSV–2) and vericellazoster virus. The mechanism of action of acyclovir involves the highly selective inhibition of virus DNA replication, via enhanced uptake in herpes virus-infected cells and phosphorylation by viral thymidine kinase. The substrate specificity of acyclovir triphosphate for viral, rather than cellular, DNA polymerase contributes to the specificity of the drug. Valacyclovir Hydrochloride is rapidly converted to acyclovir and further phosphorylated to acyclovir triphosphate. The incorporation of acyclovir tri phosphate into the growing chain of viral DNA results in chain termination. Chemically it is (S)-2-[(2-amino-6-oxo-6, 9-dihydro-3H-purin-9-yl) methoxy] ethyl-2-amino-3-methylbutanoate with empirical formula of $C_{13}H_{20}N_6O_4$ and the molecular weight of 324.336 g/mol [1-3].

Literature review reveals that few analytical methods have been evoked for the estimation of valacyclovir hydrochloride by spectrophotometric (UV) method [4-10]. Whereas method development and validation of valacyclovir hydrochloride assay by RP-HPLC in pharmaceutical dosage form (HPLC) [11-19], Development and validation of bioanalytical method for the determination of Valacyclovir HCl in human plasma by liquid chromatography (LC) [20] and high-performance thin-layer chromatographic method for the determination of

valacyclovir both in bulk drug and pharmaceutical dosage form was developed and validated (HPTLC) methods were reported [21-22].

In present study the authors were developed a sensitive, accurate and reliable method for the estimation of Valacyclovir Hydrochloride in bulk and pharmaceutical dosage forms. So, the authors have made an attempt to develop spectrophotometric methods in five different buffer solutions for the determination of valacyclovir hydrochloride in ophthalmic solutions and validated as per ICH guidelines.

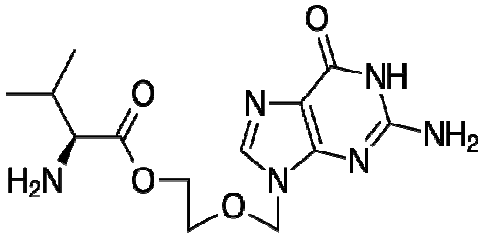

Figure 1. Chemical Structure of Valacyclovir hydrochloride

MATERIALS AND METHODS

Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Shimadzu).

Reagents

Analytical grade methanol (Merck), disodium phosphate (Na_2HPO_4) (Merck), monopotassium phosphate (KH_2PO_4) (Merck), boric acid, sodium hydroxide, glacial acetic acid were used. Valacyclovir hydrochloride was obtained as gift sample from Mylan limited was used.

Preparation of sodium acetate buffer pH 4.0

For preparation of sodium acetate, 2.86 mL of glacial acetic acid and 1.0 mL of 50% w/v solution of Sodium hydroxide was added in a 100mL volumetric flask and made up the volume with HPLC grade water.

Preparation of phosphate buffer pH 5.0

For preparation of phosphate buffer, 50.0 mL of 0.2 M potassium hydrogen phthalate in a 200 mL volumetric flask, add the specified volume of 0.2 M sodium hydroxide 22.6 mL and the volume was made up with distilled water.

Preparation of phosphate buffer pH 7.0

For preparation of phosphate buffer, 0.5 gm of Na_2HPO_4 and 0.301gm of KH_2PO_4 was added in a 1000mL volumetric flask and the volume was made up with distilled water.

Preparation of borate buffer pH 9.0

For preparation of borate buffer, 6.2 gm of boric acid was dissolved in 500 mL of water and the pH was adjusted to 9.0 with 1M sodium hydroxide (about 41.5 mL) and diluted with water in a 1000 mL volumetric flask.

Preparation of 0.1N NaOH

For preparation of 0.1N NaOH, 4 gm of NaOH is diluted with 1000mL of distilled water.

Preparation of stock and sample solutions

The standard solution of Valacyclovir hydrochloride was prepared by dissolving accurately about 25 mg of the valacyclovir hydrochloride with methanol in a 25 mL volumetric flask.

The stock solution was further diluted with sodium acetate buffer pH: 4.0, phosphate buffer pH 5.0, phosphate buffer pH 7.0, and borate buffer pH 9.0, 0.1N NaOH. For method A (1-80 µg/mL), method B (1-80 µg/mL), method C (1-80 µg/mL), method D (1-80 µg/mL) and method E (1-80 µg/mL) respectively as per the requirement.

Procedure for preparation of calibration curve

The drug solutions were scanned (200-400 nm) against their reagent blank that is for method A sodium acetate, Phosphate buffer pH 5.0 for method B, Phosphate buffer pH 7.0 for method C, Borate buffer pH 9.0 for method D,

and 0.1N NaOH for method E (Figure 2, 3, 4, 5 and 6) and the absorption spectra were recorded. The absorption maximum (λ_{max}) was observed at 251 nm, 251nm, 252 nm, and 253nm and 265 nm for method A, B, C, D and E respectively and the absorbance was recorded against each concentration.

A graph was drawn by taking the concentration of the drug solutions on the x-axis and the corresponding absorbance values on the y-axis for method A, B, C, D and E (Figure 7A, 7B, 7C, 7D and 7E).

Assay procedure for the commercial formulations of valacyclovir hydrochloride

Valacyclovir hydrochloride is available as tablets with brand names VALAVIR (500mg; CIPLA limited, India.), VALCIVIR (1000 mg; CIPLA Ltd, India) and procured from the local pharmacy store. The contents of each brand of Valacyclovir Hydrochloride equivalent to 10 mg was extracted with methanol, sonicated and made up to volume with methanol in a 10 mL volumetric flasks (1 mg/mL) and filtered. The dilutions were made from this stock as per the requirement for method A, B, C, D and E and the percentage recovery was calculated.

Precision and Accuracy

The precision and accuracy studies were performed as per the ICH guidelines.The absorbance of six replicates (20 µg/mL) for Method A, B, C, D and E was noted and the % RSD was calculated.

Accuracy was evaluated as per the ICH guidelines by the percent recovery studies by the addition of 80%, 100%, and 120% of pure sample solution to the pre-analysed formulation solution. For the present study 5 µg/mL of valacyclovir hydrochloride solution extracted from the formulation was taken and 80%, 100%, and 120% of pure drug solution (i.e. 4, 5 and 6 µg/mL) were added and the % RSD was calculated.

Table-1: Spectral characteristics of Valacyclovir Hydrochloride

Parameters	Method A	Method B	Method C	Method D	Method E
Beer-Lambert's limits (µg /mL)	1-80	1-80	1-80	1-80	1-80
λ_{max} /Amplitude range (nm)	251	251	252	253	265
Molar extinction coefficient (Liter/mol.cm)	12.3247×10^3	13.6221×10^3	10.70308×10^3	11.02742×10^3	10.70308×10^3
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.02631	0.023809	0.030303	0.0294117	0.030303
Slope	0.032	0.0324	0.0322	0.0304	0.0278
Intercept	0.0144	0.0044	0.0118	0.0013	0.0063
Correlation coefficient	0.9996	0.9999	0.9997	0.9991	0.9999

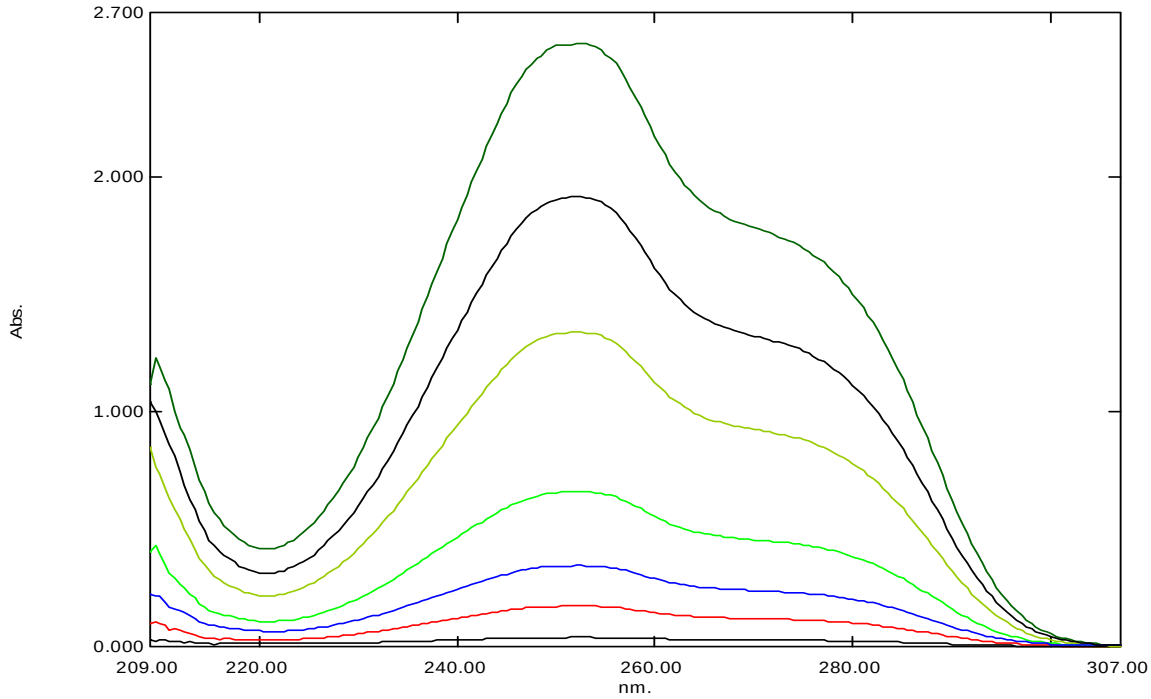

Figure 2. Absorption spectrum of Valacyclovir hydrochloride in sodium acetate pH 4.0

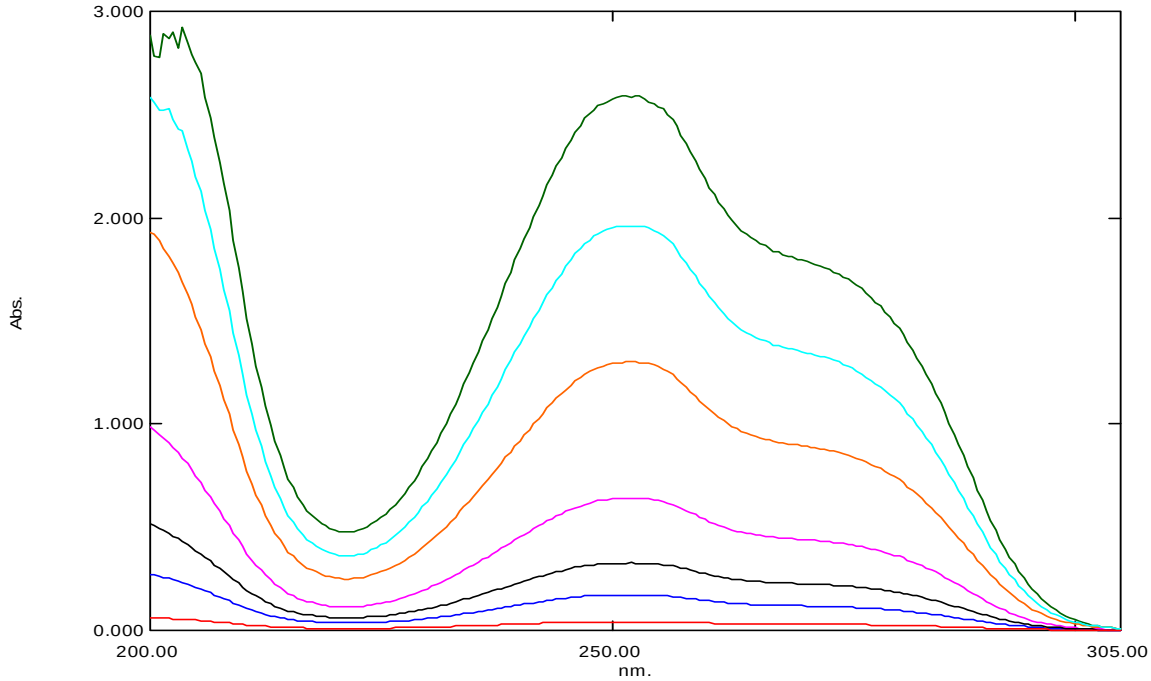

Figure 3. Absorption spectrum of Valacyclovir hydrochloride in phosphate buffer pH 5.0

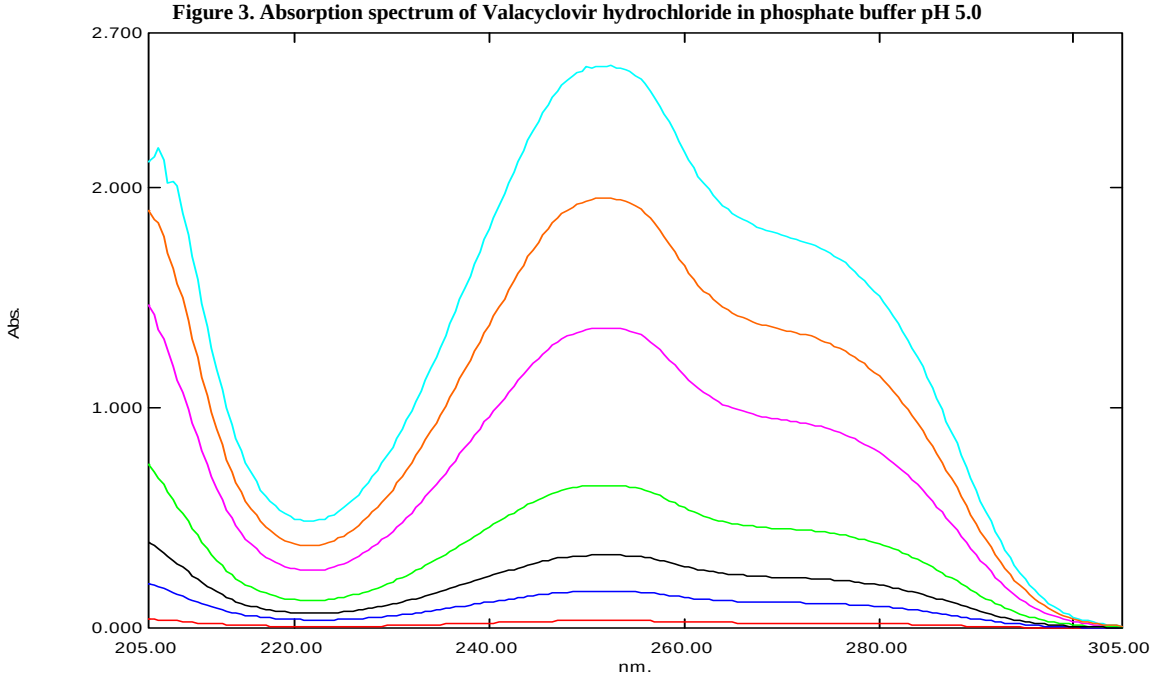

Figure 4. Absorption spectrum of Valacyclovir hydrochloride in phosphate buffer pH 7.0

Table-2: Assay of commercial formulations

Brand	Labeled Amount (mg)	*Amount obtained (mg)					% Recovery*				
		Method					Method				
		A	B	C	D	E	A	B	C	D	E
Valcivir	1000	999.45	994.45	996.15	995.26	994.54	99.89	98.89	99.23	99.05	99.49
Valavir	500	497.45	495.15	499.12	498.45	497.68	99.49	99.03	99.82	99.69	99.52

**Each value is average of three determinations*

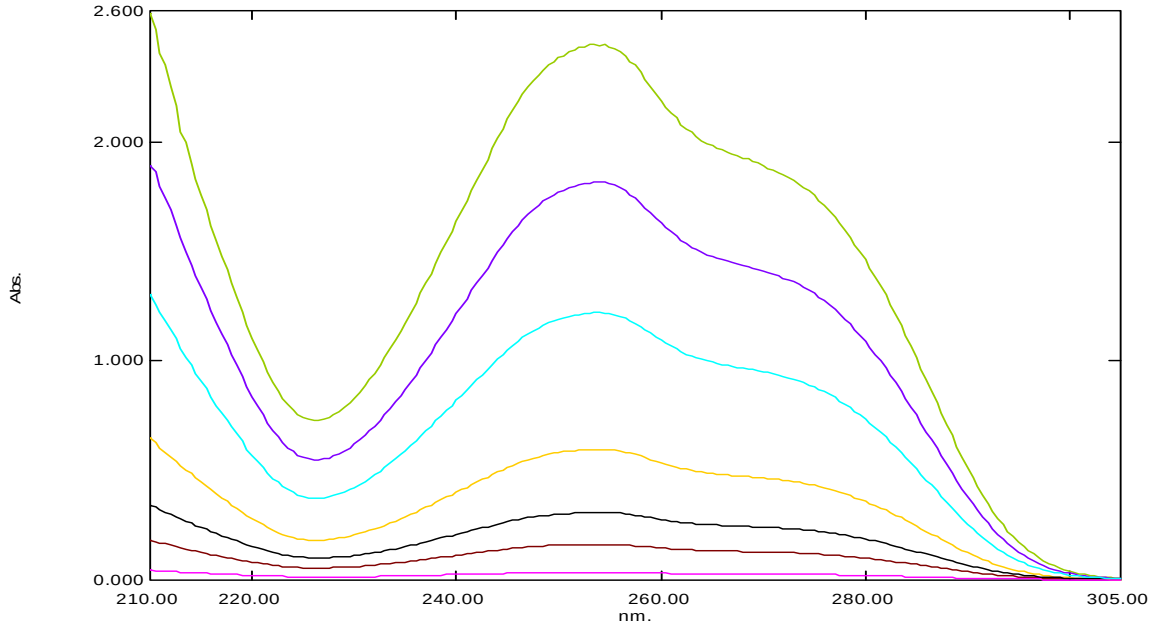

Figure 5. Absorption spectrum of Valacyclovir hydrochloride in borate buffer pH: 9.0

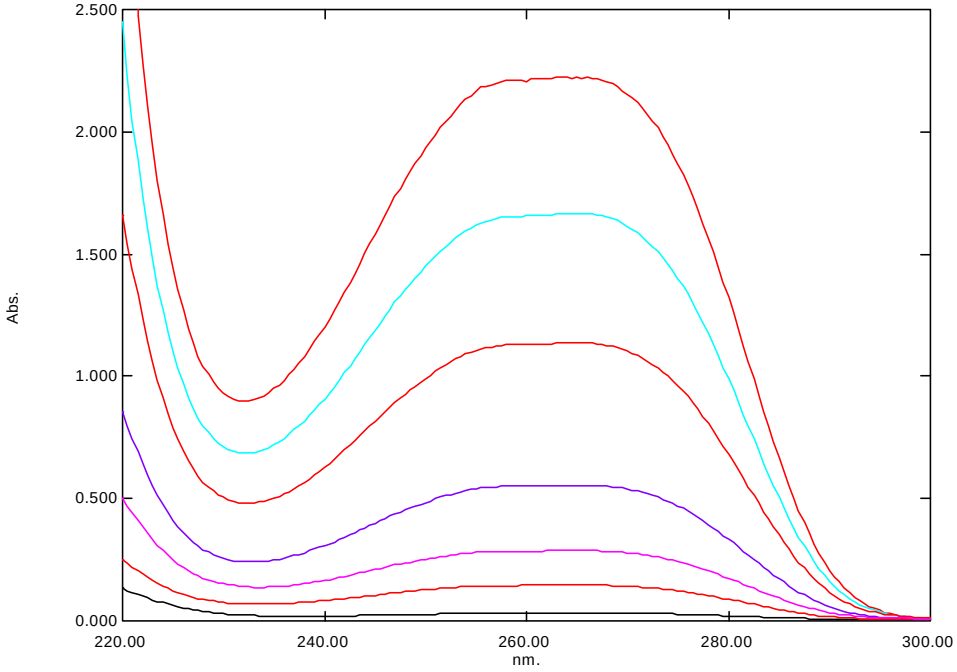

Figure 6. Absorption spectrum of Valacyclovir hydrochloride in 0.1N NaOH

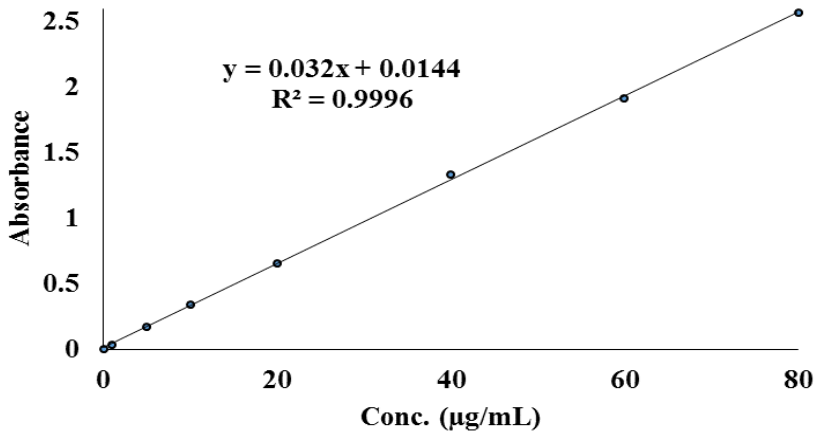

Figure 7 (A). Calibration curves of Valacyclovir hydrochloride in sodium acetate pH 4.0

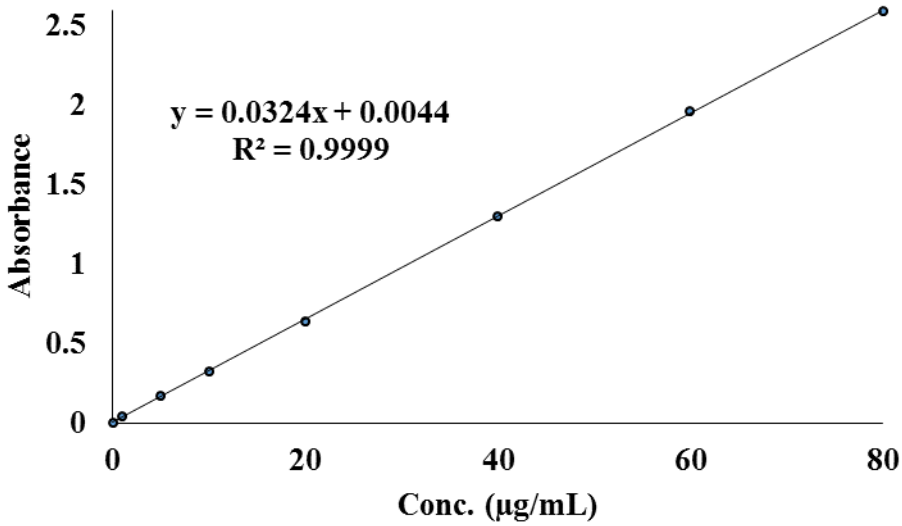

Figure 7 (B). Calibration curves of Valacyclovir HCl in phosphate buffer pH 7.0

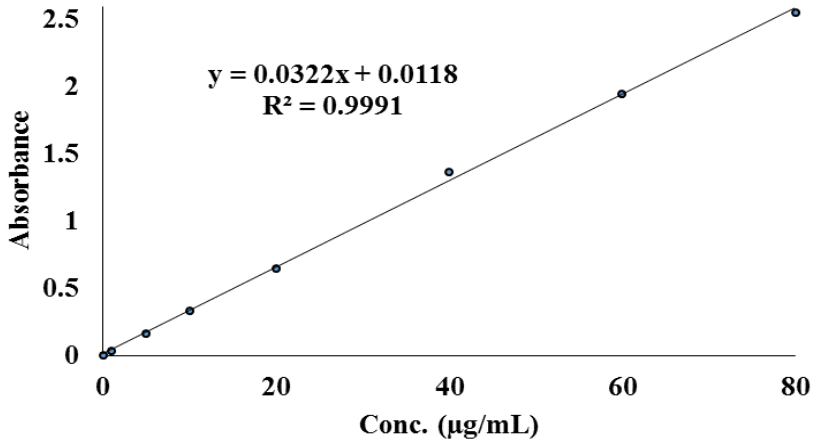

Figure 7 (C). Calibration curves of Valacyclovir HCl in phosphate buffer pH 7.0

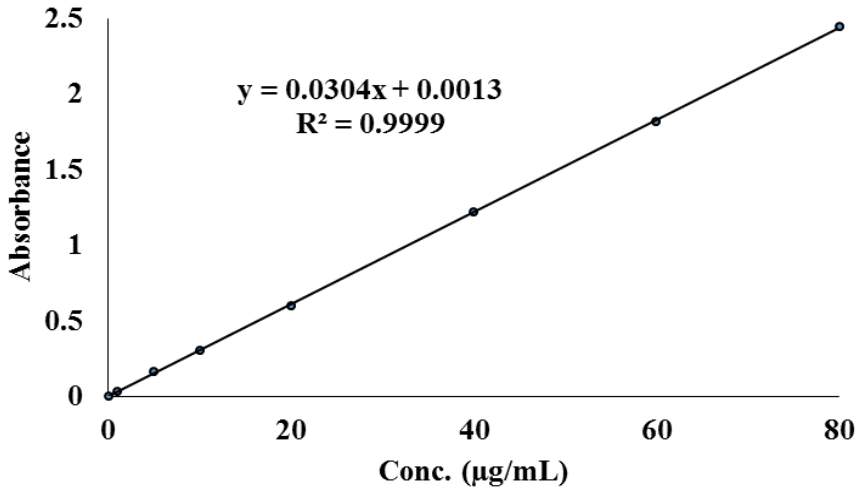

Figure 7 (D). Calibration curves of Valacyclovir hydrochloride in borate buffer pH 9.0

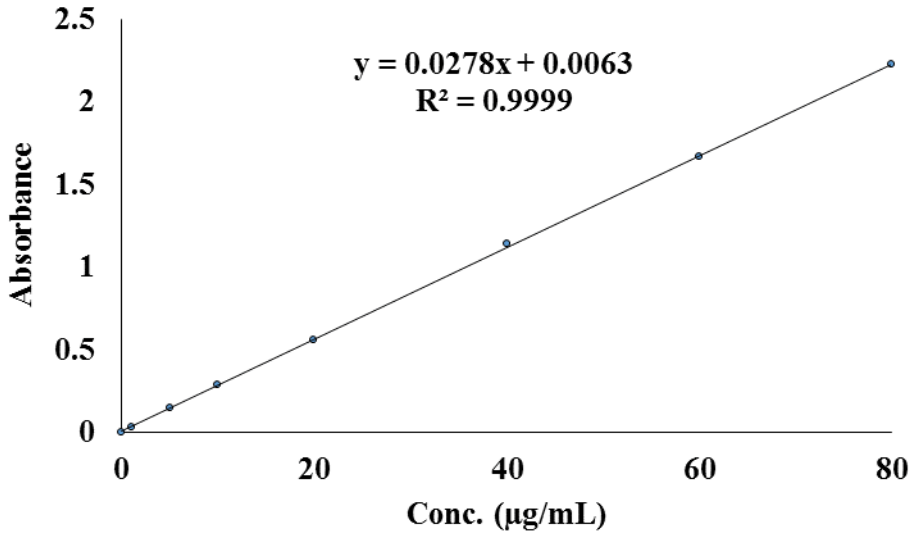

Figure 7 (E). Calibration curves of Valacyclovir hydrochloride in 0.1N NaOH

RESULTS AND DISCUSSION

Beer's law was obeyed in the concentration range of 1-80 µg/mL, 1-80 µg/mL, 1-80 µg/mL, 1-80 µg/mL and1-80 µg/mL for the methods A, B, C, D and E respectively. The linear regression equations were found to be 0.032x + 0.0144, 0.0324x + 0.0044, 0.0322x + 0.0118, 0.0304x + 0.0013 and 0.0278x + 0.0063 for method A, B, C, D and E respectively with correlation coefficient 0.9996, 0.9999, 0.9991, 0.9999 and 0.9999 for the five methods (Table 1). The % RSD values in precision studies were found to be 0.342, 0.145, 0.485, 0.152 and 0.124 for method A, B, C, D and E respectively (RSD <2%) indicating that the method is more precise. The % RSD values in accuracy studies were found to be 0.221, 0.324, 0.534, 0.548 and 0.144 for method A, B, C, D and E respectively (RSD <2%) indicating that the method is more accurate.

The percentage recovery (Table 2) was found to be in the range of 98.89-99.89, 98.04-99.03, 98.49-99.82, 98.84-99.69 and 99.98-99.49 for method A, B, C, D and E respectively (RSD <1.0 %) indicating that the proposed methods can be applied for the determination of pharmaceutical formulations successfully.

CONCLUSION

The proposed spectrophotometric methods are simple, rapid, accurate, precise, and economic. From the above data it was observed that all validation parameters meet the predetermined acceptance criteria and were validated in terms of linearity, accuracy, precision and reproducibility. Thus it has been concluded that the method is validated for the analysis on Valacyclovir Hydrochloride in pure and formulated microspheres dosage form.

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