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SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR VALACYCLOVIR HYDROCHLORIDE MONOHYDRATE AND RITONAVIR IN BULK AND TABLET DOSAGE FORM USING ABSORPTION RATIO METHOD

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A simple, economic and accurate absorption ratio method was developed for the simultaneous estimation of valacyclovir (VC) hydrochloride monohydrate and ritonavir (RT) in bulk and tablet dosage form. 0.1M hydrochloric acid (HCl) was used as a diluent to dissolve VC and RT. One percent sodium lauryl sulphate (SLS) was used to enhance the solubility of the drugs in 0.1M HCl. The absorptions were observed at 237.52 nm and 256.75 nm, which were selected based on overlapping spectra of VC and RT. The linearity range was found to be 10–20 μg/mL at 237.52 nm (r^2=0.995) and 256.75 nm (r^2=0.994). The method was found to be simple, precise, accurate and rapid for the simultaneous determination of VC and RT in bulk and tablet dosage form using absorption ratio method.

Keywords: Valacyclovir, Ritonavir, Spectrophotometry

INTRODUCTION

Valacyclovir (VC) which is a prodrug, being converted in vivo to acyclovir, is an antiviral drug used in the management of herpes simplex, herpes zoster (shingles) and herpes B. Its molecular formula and molecular weight are $C_{13}H_{20}N_6O_4$ and 360.80 g/mol respectively. Acyclovir is converted into the active triphosphate form, acyclo-guanosine triphosphate (GTP), by cellular kinases. Acyclo-GTP is a very potent inhibitor of viral DNA polymerase (Umapathy, Ganapathy and Ganapathy 2004). The chemical structure of VC is shown in Figure 1(a). It is soluble in water at 174 g/L (Sweetman 2011; Moffat *et al.* 2004). Literature survey revealed that various analytical methods such as UV spectrophotometry (Srihari *et al.* 2013; Sudhakar Reddy *et al.* 2011; Sugumaran and Jothieswari 2010), HPLC, RP-HPLC (Jahnavi and Ashok 2013; Sultana, Agarwal and Khanam 2013; Rasool *et al.* 2012; Sugumaran *et al.* 2011; Rao *et al.* 2006) methods have been reported for the estimation of VC from its formulations and biological fluids. Ritonavir (RT) is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. The molecular formula is $C_{37}H_{48}N_6O_5S_2$ and the structural formula of RT is shown in Figure 1(b). RT is a yellow crystalline substance, practically insoluble in water but soluble in ethanol

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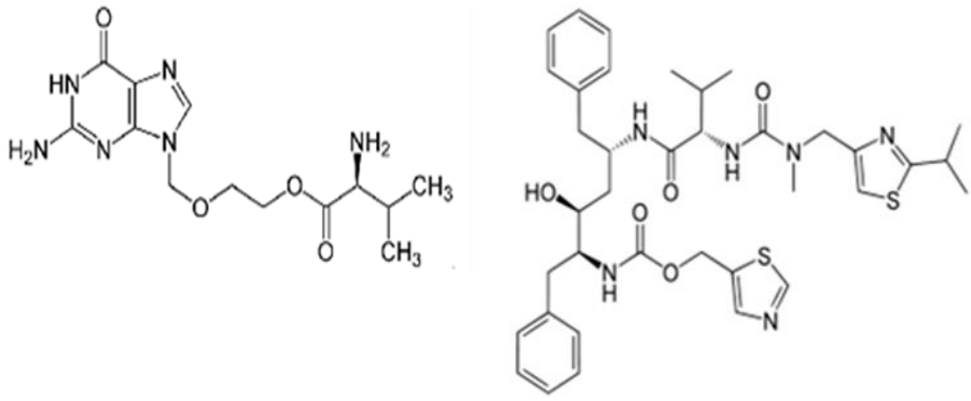
(Sweetman 2011, Moffat *et al.* 2004). It has a molecular weight of 720.95 g/mol. Detailed survey of literature for RT revealed several methods that have been reported for the assay of it either alone or in combined form in drug formulations. These analytical techniques include UV visible (Vis) spectrophotometry (Nagulwar and Bhusari 2012; Seetaramaiah *et al*. 2012; Behera *et al*. 2011; Dias, Bergold and Fröehlich 2009), RP-HPLC (Gowthami *et al.* 2012; Jagadeeswaran *et al.* 2012), HPLC and HPTLC (Mohammad *et al*. 2012).



(a) (b)

Fig. 1: Structures of drugs (a) VC and (b) RT (images are derived from http://en.wikipedia.org).

The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes. A survey of literature revealed that simultaneous analytical methods are not available for the drug combination VC and RT, even though very few methods of individual estimation of these drugs are available. Hence it is proposed to develop new methods for the assay of VC and RT in pharmaceutical dosage forms adapting UV visible spectrophotometry. The objective of the study was to develop a simple and accurate method for the determination of VC and RT simultaneously using absorption ratio method by UV-spectrophotometry in pharmaceutical dosage forms.

METHODS

VC and RT obtained from Matrix Laboratories (Hyderabad, India) were of analytical grade. A commercial sample of VC and RT tablets were procured from the local market and used within their shelf-life period. Hydrochloric acid (HCl) (S. D. Fine Chemical Limited, Mumbai) and sodium lauryl sulphate (SLS) (LobaChemie Laboratory, Mumbai) were of pharmaceutical or analytical grades.

Quantitative estimation was performed on Labindia UV 3000+ (Maharashtra, India) and Elico SL 210 (Andhra Pradesh, India) double beam UV visible spectrophotometers with matched 1 cm path-length quartz cells. Absorption spectra was

recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto. Labindia UVWin (Maharashtra, India) software was used along with quartz cuvette for the λ_{max} prediction. To develop a suitable and robust absorption ratio method for the determination of VC and RT, different diluents like methanol, 0.1M HCl etc. were tried based on the solubility and functional group present in the compound. Finally 0.1M HCl in 1% SLS solution, prepared by adding 8.5 mL HCl to a 1000 mL volumetric flask and making it up to the mark with 1% SLS solution, was selected due to its reproducible results. Absorbances were measured at selected λ_{max} (237.52 nm and 256.75 nm) based on the overlap spectrum of both drugs. The data were collected and analysed with a software (LabIndia UVWin, Maharashtra, India) in a computer system.

Preparations

Stock solution of VC (200 µg/mL) was prepared by dissolving 10 mg of drug in a 50 mL volumetric flask containing 20 mL of 0.1M HCl in 1% SLS . The solution was sonicated for about 15 minutes and then made up to volume with the solvent. From the stock solution, 1 mL was pipetted out and transferred into the 10 mL volumetric flask to get 20 µg/mL concentration. The same procedure was followed for RT standard. The final solutions of both standard drugs solutions were scanned and the spectra obtained were overlapped. From the overlapping spectra, two wavelengths were selected. Among the two, 256.75 nm is the λ_{max} of VC and 237.52 nm is an isobestic point (the wavelength at which both the drugs show same absorbance). Then the absorbance was measured at 237.52 nm and 256.75 nm, and the absorptivity, from the formula $\boldsymbol{\varepsilon = A/cl}$ where A is absorbance; c is concentration; l is path length, was calculated.

Preparation of Standard Mixture

From 200 µg/mL of VC and RT standard stock solutions, 1 mL was pipetted out individually and mixed in 10 mL volumetric flask and was made up to the mark with 0.1M HCl in 1% SLS. Absorbance were measured at selected λ_{max} (237.52 nm and 256.75 nm).

Preparation of Tablet Mixture

20 tablets were weighed and powdered. The amount of powder equivalent to 25 mg of VC and 10 mg of RT were weighed and transferred into the 100 mL of volumetric flask containing 20 mL of 0.1M HCl in 1% SLS. The solution was sonicated for about 20 minutes and then made up to volume with 0.1M HCl in 1% SLS. The solution was filtered using 0.25 µ filter paper and vacuum-associated filtration unit. From the filtrate, 1 mL was pipetted out and transferred into the 10 mL volumetric flask and made up to the mark with 0.1M HCl in 1% SLS. The amount of drug present in pharmaceutical formulation was calculated (Beckett and Stenlakke 2007) using the following formula:

Cy = (A1/ax1)–Cx
Cx = [(Qm–Qy)/Qx–Qy)] (A1/ax1)

where, Cy is the concentration of RT in mixture; Cx is the concentration of VC in mixture; Qx (absorption ratio of VC) = ax2/ax1; Qy (absorption ratio of RT) = ay2/ay1; Qm

(absorption ratio of mixture) = A2/A1; A1 is absorption at 237.52 nm in mixture; A2 is absorption at 256.75 nm in mixture; ax1 and ax2 are absorptivities of VC at 237.52 nm and 256.75 nm respectively; ay1 and ay2 are absorptivities of RT at 237.52 nm and 256.75 nm, respectively.

Validation

The described method has been validated for the assay of VC and RT using the following parameters [International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) 1995]. Linearity was studied to find out the relationship of concentration with absorbance. Six different concentrations of VC and RT drug mixtures (10 to 20 µg/mL of each drug in the mixture) were employed i.e., 10, 12, 14, 16, 18 and 20 µg/mL. All solutions were scanned and absorbance measured at 237.52 nm and 256.75 nm. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/mL) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of VC and RT (20 µg/mL) on the same day. On different days, the same solutions were scanned using different Instruments (Elico SL 210, Labindia UV 3000+) and ruggedness is determined. The precision of each method was ascertained separately from the absorbance obtained by actual determination of six replicates of a fixed amount of drug (20 µg/mL). Precision and ruggedness were done on the same day and the different day respectively, and the percentage of relative standard deviation (% RSD) was calculated for each. The accuracy of the method was shown by analysing the model mixtures containing 20, 25 and 30 µg/mL of sample solution of VC and 8, 10 and 12 µg/mL of sample solution of mixture of RT and along with 10 µg/mL of bulk standard solutions of VC and RT. After the measurement, the amount found, amount added for VC and RT and individual recoveries were calculated. Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope.

RESULTS

An absorption ratio method procedure was proposed as a suitable method for the analysis of the drugs VC and RT in dosage forms. A typical overlap spectrogram of standard VC and RT and their mixture is shown in Figure 2 (data in Table 1). The λ_{max} was found to be 237.52 nm and 256.75 nm. The regression equation for the method at 237.52 nm was found to be y = 0.02519x+0.00202 (r=0.995) and linear over Beer's range 10–20 µg/mL. The regression equation for the method at 256.75 nm was found to be y = 0.03939x+0.00655 (r=0.994) and linear over Beer's range 10–20 µg/mL. The linearity graph of VC and RT mixtures is shown in Figure 3. A typical overlap spectrogram of different concentration of mixture of VC and RT is shown in Figure 4.

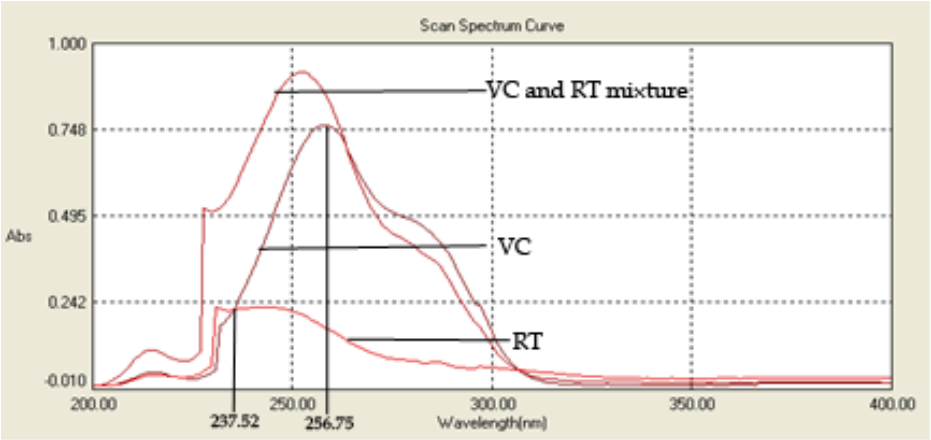


Fig. 2: Overlap spectrogram of standard drugs VC, RT and their mixture.

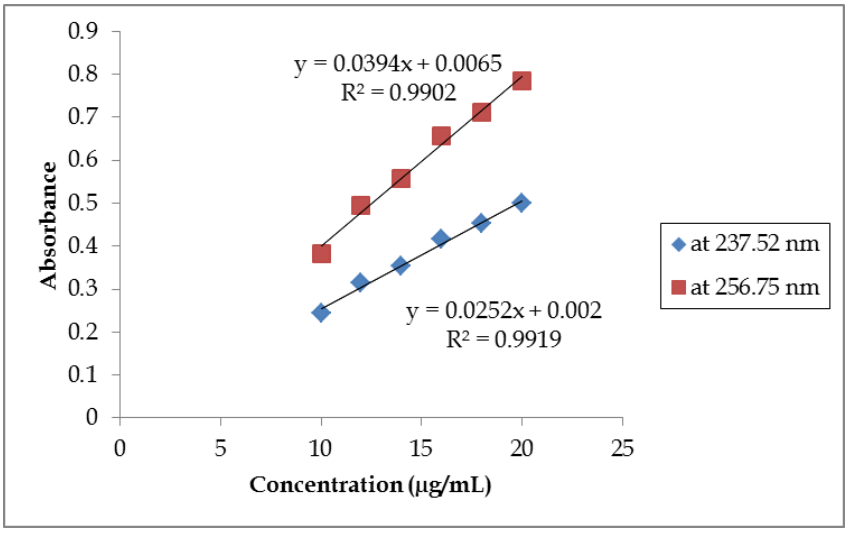


Fig. 3: Linearity graph of VC and RT mixture.

The percentage of purity of VC and RT in tablet dosage form was 108.3% and 108.5% respectively. The precision of the spectrophotometer system was determined using the % RSD of the absorbance for six replicate preparations of the drug. The % RSD was less than 2. Precision data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analysing model mixtures containing 80%, 100% and 120% of sample solution of drug VC and drug RT along with 10 µg/mL of bulk standard solution within the linearity ranges. The mean percentage recoveries were found to be 92.78%, 95.09% and 98.84% w/w for 80%, 100% and 120% respectively. Accuracy data are presented in Table 3. LOD for VC and RT was found to be

0.635 µg and 0.595 µg respectively. LOQ for VC and RT was found to be 1.924 µg and 1.804 µg respectively.

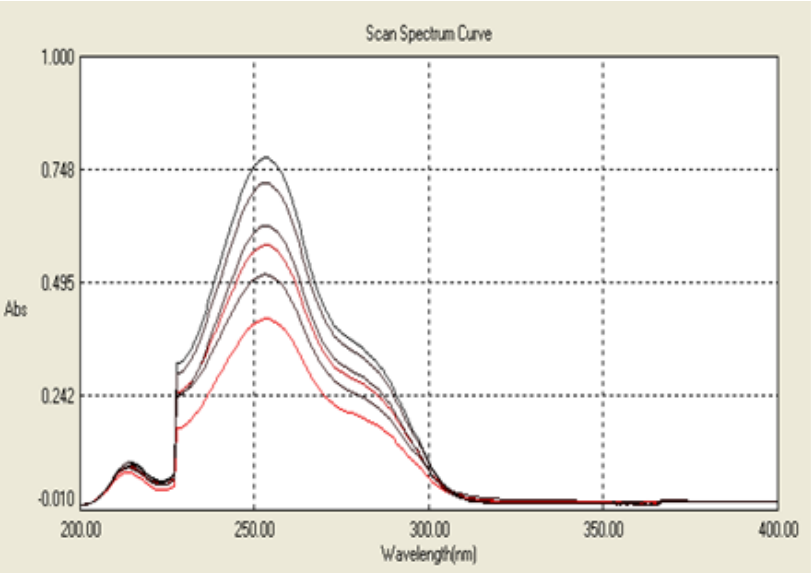


Fig. 4: Spectrogram of VC and RT mixture linearity.

Table 1: Data of VC and RT.

	Absorbance at 237.52 nm	Absorbance at 256.75 nm	Absorption ratio
VC	0.263	0.606	2.304183
RT	0.263	0.22	0.836502

Table 2: Data for precision of VC and RT.

	Absorbance at 237.52 nm	Absorbance at 256.75 nm	Absorption ratio	Concentration of VC	Concentration of RT
Mixture 1	0.489	0.771	1.576687	1.875392	1.84324
Mixture 2	0.485	0.758	1.562887	1.825371	1.862842
Mixture 3	0.49	0.761	1.553061	1.819244	1.906992
Mixture 4	0.491	0.769	1.566191	1.85636	1.87748
Mixture 5	0.485	0.754	1.554639	1.804645	1.883567
Mixture 6	0.481	0.759	1.577963	1.847889	1.809906
Mean	0.486833	0.762	1.565238	1.83815	1.864004
SD	0.003817	0.006633	0.01058	0.026293	0.033975
%RSD	0.783971	0.870505	0.6759	1.430425	1.822677

Notes: SD = Standard deviation; %RSD = Percentage of relative standard deviation

Table 3: Data for accuracy of VC and RT.

Accuracy (%)	Abs at 237.52 nm	Abs at 256.75 nm	Abs ratio	Conc. of VC	Conc. of RT	% recovery of VC	% recovery of RT
1 (80)	0.5855	1.0265	1.75319	2.780975	1.671497	92.69916	92.86093
2 (100)	0.687	1.2145	1.76784	3.315146	1.909189	94.71846	95.45943
3 (120)	0.817	1.4395	1.76211	3.917503	2.295426	97.93756	97.80972

Note: Abs = absorbance; Conc. = concentration

DISCUSSION

The developed method can be used for routine analysis because the linearity found in VC and RT is nearing 1 that is 0.995 and 0.994 respectively which shows the good regression for linearity. Maximum recovery is obtained by this developed method and the mean percentage recovery for each component is nearing 100%. Therefore this method can be used for the routine analysis and one most important reason is that the developed method does not involve the use of expensive reagents. The spectrophotometric assay methods employed in our study indicated less interference from excipients used in formulation by the percent recoveries values. Most of the existing methods (Srihari *et al.* 2013; Nagulwar and Bhusari 2012; Siva Ramakrishna *et al.* 2012; Reddy *et al.* 2011; Seetaramaiah *et al.* 2011; Sudhakar Reddy *et al.* 2011; Aswani Kumar *et al.* 2010) consumed expensive reagents for individual drug analysis. But the method we developed involves chemicals like HCl, SLS and distilled water, which are very simple, economical and also easily available. Also, our proposed method requires less time for the determination of VC and RT simultaneously compared to other methods (Siva Ramakrishna *et al.* 2012; Reddy *et al.* 2011; Sudhakar Reddy *et al.* 2011) and these methods also required reagents which are costly and time consuming.

CONCLUSION

The presented method was found to be precise, sensitive and accurate. This method has simple sample preparation. The good recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of VC and RT in pharmaceuticals.

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REFERENCES

ASWANI KUMAR, C. H., ANIL KUMAR, T., GURUPADAYYA, B. M., NAVYA SLOKA, S. & RAHUL REDDY, M. B. (2010) Novel spectrophotometric determination of valacyclovir and cfotaxime using 1,2-napthaquinone-4-sulfonic acid sodium in bulk and pharmaceutical dosage form, *Archives of Applied Science Research*, 2: 278–287.

BECKETT, A. H. & STENLAKE, J. B. (2007) *Practical pharmaceutical chemistry*, vol. 2, 4th edition, pp. 300–306 (New Delhi: CBS Publishers & Distributors).

BEHERA, A., MOITRA, S. K., SI, S. C., MEHER, A. K. & GOWRI SANKAR, A. P. D. (2011) Method development, validation and stability study of ritonavir in bulk and pharmaceutical dosage form, *Spectrophotometric Method*, 2: 161–167.

DIAS, C. L., BERGOLD, A. M. & FRÖEHLICH, P. E. (2009) UV-derivative spectrophotometric determination of ritonavir capsules and comparison with LC method, *Analytical Letters*, 42, 1900–1910.

GOWTHAMI, K., FATIMA, G., FARHEEN, M., YASMEEN, F., AFREEN, S., SAILAJA, M. *et al.* (2012) Sensitive analytical method development and validation of ritonavir bulk drugs by RP-HPLC, *Journal of Scientific Research in Pharmacy* 1: 20–22.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE (ICH). (1995) *Draft guideline on validation procedure, definition and terminology federal register*, pp. 11260 (Rockville, MD, USA: Food and Drug Administration USA).

JAGADEESWARAN, M., GOPAL, N., PAVANKUMAR, K., & SIVA KUMAR, T. (2012) Quantitative estimation of lopinavir and ritonavir in tablets by RP-HPLC method, *Pharmaceutica Analytica Acta,* 3: 1–3.

JAHNAVI, B. & ASHOK, G. (2013) Method development and validation of valacyclovir hydrochloride assay by RP-HPLC in pharmaceutical dosage form, *International Journal of Advanced Research in Pharmaceutical and Bio Sciences*, 3: 33–41.

MOFFAT, A. C., OSSELTON, M. D., WIDDOP, B. & WATTS, J. (2004) *Clarke's analysis of drugs and poisons,* vol. 2, 3rd edition, pp. 1538, 1690 (London: Pharmaceutical Press).

MOHAMMAD, H. A., AZZA, A. G., RASHA, A. S., & HEBA, K. A. (2012) Validated stability-indicating HPLC and HPTLC methods for the determination of ritonavir in bulk powder Andin capsules, *Journal of Food and Drug Analysis*, 20: 963–973.

NAGULWAR, V. P. & BHUSARI, K. P. (2012) Development of UV spectrophotometric first order derivative method for the simultaneous estimation of ritonavir and lopinavir in combined tablet dosage form, *International Journal of Pharmaceutical Sciences and Research*, 3: 2317–2320.

RAO, P. N., RAJA RAJESWARI, K., RAO, V. J. & RAO, J. V. L. N. S. (2006) RP-HPLC estimation of valacyclovir in tablets, *Asian Journal of Chemistry*, 18: 2552–2556.

RASOOL, S. K., NAIK, D. V., PRASAD BABU, D. & NALLURI, B. N. (2012) RP-HPLC method for the estimation of valacyclovir in bulk and pharmaceutical formulations, *International Journal of Pharmacy and Pharmaceutical Sciences*, 4: 214–218.

REDDY S, A., CHAKRABORTY, R., SEN, S. & PARAMESHAPPA, B. (2011) Spectrophotometric determination and validation of acyclovir, *Archives of Applied Science Research*, 3: 328–332.

SEETARAMAIAH, K., SMITH, A. A., RAMYATEJA, K., ALAGUMANIVASAGAM, G. & MANAVALAN, R. (2012) Spectrophotometric determination of ritonavir in bulk and pharmaceutical formulation, *Scientific Reviews and Chemical Communications*, 2: 1–6.

SIVA RAMAKRISHNA, V., RAVI KUMAR, D., MALLESWARA RAO, N. V. N. & RAM BABU, C. (2012) Method development and determination of valacyclovir HCl in pharmaceutical dosage forms by visible spectrophotometry, *International Journal of Pharmtech Research,* 4: 1009–1014.

SRIHARI, G., RAMI REDDY, N., NAGARAJA SETTY, K. & CHAKRAVARTHI, I. E. (2013) Spectrophotometric determination of valacyclovir in pharmaceutical formulations, *Chemical Science Transactions*, 2: 61–64.

SUDHAKAR REDDY, J., MAQSOOD AHMED, M. S., CHAKRAVARTH, I. E. & PRABHAVATHI, K. (2011) Spectrophotometric estimation of valacyclovir in pharmaceutical preparations, *Journal of Chemical and Pharmaceutical Research*, 3: 773–776.

SUGUMARAN, M., BHARATHI, V., HEMACHANDER, R. & LAKSHMI, M. (2011). RP-HPLC method for the determination of valacyclovir in bulk and pharmaceutical formulation, *Der Pharma Chemica*, 3: 190–194.

SUGUMARAN, M. & JOTHIESWARI, D. (2010) Development and validation of spectroscopic method for estimation of valacyclovir in tablet dosage form, *Oriental Journal of Chemistry*, 26: 163–165.

SULTANA, Y., AGARWAL, N. K. A. & KHANAM, P. S. (2013) Development and validation of stability indicating rp-hplc method for estimation of valacyclovir in pharmaceutical dosage forms, *International Journal of Pharmaceutical and Clinical Research*, 5: 7–12.

SWEETMAN, S. C. (ED.). (2011) *MARTINDALE: The complete drug reference*, 34th edition, pp. 653, 656 (London: Pharmaceutical Press).

UMAPATHY, N. S., GANAPATHY, V. & GANAPATHY, M. E. (2004) Transport of amino acid esters and the amino-acid-based prodrug valganciclovir by the amino acid transporter ATB, *Pharmaceutical Research,* 21: 1303–1310.