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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR VALACYCLOVIR HYDROCHLORIDE

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ABSTRACT

Analytical method development and Validation are the continuous and inter-dependent task associated with the research & development, quality control and quality assurance departments. Literature survey reveals that the analytical methods based on UV spectrometry, and HPLC for the determination of Valacyclovir. The developed methods are simple, sensitive and reproducible and can be used for the analysis of Valacyclovir in bulk and Tablet dosage form. Valacyclovir hydrochloride, an antiviral prodrug of acyclovir, is widely used in the treatment of herpes simplex and varicella-zoster infection. The results demonstrated that the proposed UV spectroscopic method is suitable for routine analysis of valacyclovir in quality control laboratories due to its simplicity, cost-effectiveness, and reliability.

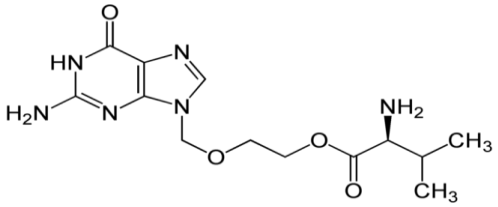
KEYWORD: Valacyclovir, UV Visible Spectrometer, Validation of the method was performed following ICH guidelines.

INTRODUCTION

Valacyclovir, approved for medical use in 1995, is marketed by GlaxoSmithKline under the brand names Valtrex and Zelitrex. Generic versions of the drug have been available in the United States since November 25, 2009.[1] Valacyclovir (VCV), chemically known as L-valine- 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl ester, is the L-valyl ester prodrug of the antiviral agent acyclovir. It is active against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), as well as varicella-zoster virus (VZV).[2] Valacyclovir hydrochloride is an orally administered antiviral agent used to treat infections caused by herpes simplex virus (HSV) and varicella-zoster virus (VZV). It has also shown potential in the treatment of infectious mononucleosis and is used prophylactically in suspected cases of herpes B virus exposure. After oral administration, valacyclovir hydrochloride is converted by esterases to its active form, acyclovir, through hepatic first-pass metabolism. Acyclovir works by inhibiting viral DNA synthesis.[3] Following oral administration, valacyclovir (VCV) is efficiently absorbed from the gastrointestinal tract and rapidly metabolized in the liver to produce acyclovir (ACV) and the essential amino acid L-valine. The bioavailability of acyclovir from valacyclovir is approximately 3 to 5 times higher than that of orally administered acyclovir, allowing for less frequent dosing.[5]

DRUG PROFILE

- **Generic name:** e.g., Valacyclovir Hydrochloride
- **Structure**



- **Chemical name (IUPAC):** 2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl) methoxy]ethyl L-valinate hydrochloride.[4]

REVIEW OF LITRATURE

1 Rohini A et al., (2013) A gradient reversed phase liquid chromatographic (RP-LC) method has been developed and subsequently validated for the determination of valacyclovir and its process-related impurities (noted as: impurity G & E). Separation was achieved with a Hypersil BDS C18 column and buffer: acetonitrile 90:10 (pH adjusted to 3) as eluent, at a flow rate of 0.8 mL/min. UV detection was performed at 254 nm. The described method was linear over a range of 0.254-0.762 µg/mL for impurity G, 0.2615-0.7845 µg/mL for impurity E. The accuracy of the method has been demonstrated at 3 concentration levels in the range of 50,100,150% of the specification limit and the recovery of impurities was found to be in the range of 90–110%. The method is simple, rapid, selective, and accurate and

it is useful in the quality control of bulk manufacturing and pharmaceutical formulations.

2 Bhavya Sri K et al,. (2025) Valaciclovir is an antiviral drug that requires accurate and sensitive analytical methods for quantification in pharmaceutical formulations and biological matrices. This study presents the development and validation of two analytical techniques in accordance with ICH Q2(R2) and M10 guidelines: a reversed- phase high-performance liquid chromatography (RP-HPLC) method and a novel chromogenic UV–visible spectrophotometric method extended to bio samples.

3 Jadhav AS, et al,. (2007) A chiral high performance liquid chromatographic method was developed and validated for the enantiomeric resolution of Valacyclovir, l-valine 2-[(2- amino-1,6-dihydro-6-oxo-9h-purin-9-yl) methoxy] ethyl ester, an antiviral agent in bulk drug substance. The enantiomers of Valacyclovir were resolved on a Chiralpak AD (250 mm × 4.6 mm, 10 µm) column using a mobile phase system containing *n*-hexane: ethanol: diethylamine (30:70:0.1, v/v/v). The resolution between the enantiomers was found not less than four. The developed method was extensively validated and proved to be robust. The limit of detection and limit of quantification of (d)-enantiomer were found to be 300 and 900 ng/ml, respectively, for 20 µL injection volume. The percentage recovery of (d)-enantiomer was ranged from 97.50 to 102.18 in bulk drug samples of Valacyclovir. Valacyclovir sample solution and mobile phase were found to be stable for at least 48 h. The proposed method was found to be suitable and accurate for the quantitative determination of (d)-enantiomer in bulk drugs substance. It can be also used to test the stability samples of Valacyclovir.

4 an antiviral agent in bulk drug substance. The enantiomers of Valacyclovir were resolved on a Chiralpak AD (250 mm × 4.6 mm, 10 µm) column using a mobile phase system containing *n*-hexane: ethanol: diethylamine (30:70:0.1,v/v/v). The developed method was extensively validated and proved to be robust. The limit of detection and limit of quantification of (d)-enantiomer were found to be 300 and 900 ng/ml, respectively, for 20 µL injection volume. The calibration curve showed excellent linearity over the concentration range of 900 ng/ml (LOQ) to 6000 ng/ml for (d)-enantiomer. The percentage recovery of (d)-enantiomer was ranged from 97.50 to 102.18 in bulk drug samples of Valacyclovir. Valacyclovir sample solution and mobile phase were found to be stable for at least 48 h. The proposed method was found to be suitable and accurate for the quantitative determination of (d)-enantiomer in bulk drugs substance. It can be also used to test the stability samples of Valacyclovir.

5 Özkan CK et al,. (2002) A specific, sensitive, simple, and rapid HPLC method has been developed for the determination of valacyclovir (VACL) in raw material, pharmaceutical dosage forms, and human serum, in order to carry out drug dissolution studies from tablets. The chromatographic separation was achieved with acetonitrile:methanol:0.067 M KH_2PO_4 (27:20:53, v/v/v) adjusted to pH 6.5 with 3 M NaOH as mobile phase, a Waters Spherisorb C18 column, and UV detection at 244 nm. Etodolac was used as an internal standard. Linearity range was 5–20,000 ng mL^{-1}. Limit of detection obtained was 0.38 and 0.14 ng mL^{-1} in mobile phase and spiked human serum samples, respectively. The described method can be readily applied, without any interferences from the excipients, for the determination of the drug in tablets, human serum samples, and drug dissolution studies.

6 Ganesh M et al,. (2009) A simple, sensitive, highly accurate UV spectrophotometric method has been developed for the determination of valacyclovir in bulk and tablet dosage form. Solution of valacyclovir in 0.1N HCl shows maximum absorbance at 255 nm. Beer's law was obeyed in the concentration range of 5-25 mcg mL-1with 1.0910x104 mol-1 cm-1, the slope, intercept, correlation coefficient, detection and quantitation limits were also calculated. The proposed method has been applied successfully for the analysis of the drug in pure and in its tablets dosage forms. Result of percentage recovery and placebo interference shows that the method was not affected by the presence of common excipients. The percentages assay of valacyclovir HCl in tablet was 99.82%. The method was validated by determining its sensitivity, accuracy and precision which proves suitability of the developed method for the routine estimation of valacyclovir in bulk and solid dosage form.

7 Sugumaran M et al,. (2010) A simple, rapid and sensitive method has been developed for the quantitative estimation of valacyclovir hydrochloride in bulk and tablet. The wavelength 252 nm was selected for the concentration range 4-24µg/ml. The accuracy of the method was assessed by recovery studies and was found between 99.61-101.28%. The method was statistically validated for the linearly, precision, accuracy repeatability. LOD, LOQ and ruggedness. The method was successfully applied for routine analysis of this drug in bulk and formulations.

8 Bhavar GB et al,. (2014) A chiral high performance liquid chromatographic method was developed and validated for the separation of Valacyclovir drug substance and its related substances V1 (guanine), V2 (acyclovir) and V3 unknown impurity). The Valacyclovir and its impurities were resolved on 150 x 4.0 mm (i.d.), stainless steel, packed with 5µm Daicel Chiral Phase Crown pack CR (+) column at 15°C using 0.1% aqueous Phosphoric acid (85%): M ethanol (90:10 V/ V) as a mobile phase. A PDA detector set at 254 nm was used for detection. The linearity for related substances was obtained within concentrations ranging from 0.3 to 6µg/mL. The correlation coefficient of Valacyclovir was 0.9997. Relative standard deviations of peak areas from six measurements were always less than 2%. The proposed method was found to be suitable and accurate for the quantitative determination of Valacyclovir in bulk drug substance and tablet formulation. Validation parameters showed that the method is specific, accurate, precise and reproducible. The method can be used for routine quality control and stability analysis of

Valacyclovir drug substance.

9 Morgan EM et al,.(2022): Valaciclovir hydrochloride (VAL) is an essential antiviral prodrug used to cure various types of herpes. Analysis of VAL by different analytical techniques demonstrates a persuasive aspect that is favourable in quality control application. Objective: This study describes a comparison between colorimetric and chromatographic (RP-high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC)-densitometric) methods concerning selectivity and specificity for the determination of VAL in all possible degradation products (alkali- and acid- induced degradation products, namely aciclovir [ACI] and guanine [GUA], respectively) in their synthetic mixture and pharmaceutical formulations.

10 Deshpande MM et al,. (2015) Valacyclovir hydrochloride (2-[(2-Amino-1,6-dihydro-6- oxo-9H-purin-9-yl) methoxyl]ethyl ester-lvaline monohydrochloride), is an antiviral prescription medicine approved by the U.S. Food and Drug Administration (FDA) to prevent genital herpes outbreaks in adults infected with HIV. A simple method is described for the quantitation of Valacyclovir HCl in plasma by liquid chromatography. Chromatographic separation was achieved on a reversed phase Hypersil ODS C18 (150mm * 4.6mm, 5.0 µm) column, using isocratic elution (acetonitrile-water (85:15) at a flow rate of 0.2–1.2 mL min-1. Valacyclovir hydrochloride were measured using UV detection at 265 nm. The total chromatographic run-time was 10 min with Valacyclovir hydrochloride eluting at 4.19 min. Limit of quantification was 50 ng mL- The linearity range of the method was 50-2000 ng mL-1 (r2 = 0.9987). Mean recoveries from plasma were 105.13%. Intra-batch and inter-batch precision was 0.857 and 0.842, respectively. The Freeze and Thaw Stability, Short-Term Temperature Stability, Long- Term Stability, Stock Solution Stability evaluation indicated no evidence of degradation of Valacyclovir hydrochloride. The validated method is simple, selective and rapid and can be used for pharmacokinetic study.

Table 1: Summary of literature review on various analytical method reported for Valacyclovir.

Analytical Technique	Sample Matrix	Description	Reference
Analytical Method Development And Validation Of Valacylovir Hydrochloride By Uv And Hplc	Bulk drug and pharmaceutical dosage form**.**	**Model;** Agilent 1100 **Solvent;** Methanol :water **Wavelength;** 252nm **Linearity;** 10µgm/ml-50µgm/ml	[1]
Method Development And Validation Of Valacyclovir Hydrochloride And Ritonavirin Tablet Dosage Form Using Reverse Phase High Performance Liquid Chromatography	Tablet Tablet dosage form.	**Model:** AGILENT 1120 **Mobile Phase:** Methanol, acetonitrile and water. **Flow rate:** 1.3 mL/min **Wavelength:**222nm **Injection volume:**20 µ	[14]
Selective and Validated Spectrophotometric Method for Determination of Acyclovir and Valacyclovir using N-Bromo succinimide	bulk drug and in tablets	**Model:** Shimadzu 1700 **Solvent;** Methyl orange **Wavelength:** 508 nm **Linearity:**5–10µg mL^{-1}	[2]
Development and validation of new analytical methods for the estimation of Valacyclovir hydrochloride in pharmaceutical dosage form	Bulk drug and pharmaceutical dosage form**.**	**Model:** Shimadzu 1700 JAPAN **Method A:** **Solvent:** Sodium Acetate **Wavelength:**251nm **Linearity:** 1-80µgm/ml **Method B** **Solvent:** Phosphate Buffer(Ph 5.0) **Wavelength:**251nm **Linearity:**1-80µgm/ml **Method C** **Solvent:** Phosphate Buffer(Ph 7.0) **Wavelength:**252nm **Linearity:**1-80µgm/ml **Method D:** **Solvent:** 0.1 N NaOH **Wavelength:** 265nm **Linearity:**1-80µgm/ml	[3]
Method Development and Validation of Valacyclovir Related Compounds G & E in Valacyclovir by RP-HPLC	Bulk drug and pharmaceutical dosage form	**Stationary Phase;** Hypersil BDS C18 column **Flow Rate:** 0.8mL/min. **Wavelength:**252nm **Injection volume**:20 µl	[6]
UV Spectrophotometric Method for the Estimation of Valacyclovir HCl in Tablet Dosage Form	Tablet dosage form.	**Model:** PerkinElmer Lambda 35 **Solvent:** 0.1N HCL **Wavelength:**255nm **Linearity:**5–25 mcg/mL	**[**10]
Development and Validation of RP- HPLC M ethod for the Determination of Valacyclovir Hydrochloride and its Related Substances in	Tablet dosage form.	**Stationary Phase:** Daicel Chiral Phase Crownpak CR(+) column (5 µm, 150 × 4.0 mm)	[11]

Tablet Formulation		**Mobile Phase**: 0.1% aqueous phosphoric acid : Methanol (90:10 v/v) **Wavelength**: 254nm	
Development and validation of bioanalytical method for the determination of Valcyclovir HCl in human plasma by liquid chromatoghraphy	Bulk drug and pharmaceutical dosage form	**Stationary Phase:** Hypersil ODS C18 column (150 mm × 4.6 mm, 5.0 µm) **Mobile Phase:** Acetonitrile:Water (85:15 v/v) **Flow Rate** 0.2–1.2 mL/min	[13]

RESULT

The analysis data published is for the method development and validation by various analytical method for the Valacyclovir Hydrochloride bulk drug and pharmaceutical dosage form. According to the literature review, the drug Valacyclovir Hydrochloride was validated for various parameters as per ICH guidelines, and statistical analysis proved their reproducibility and selectivity. This review carried out an overview of the current state-of-art analytical methods for the determination of Valacyclovir Hydrochloride by UV Spectrometry and HPLC, which will be supportive for further research. The methods are also helpful for in-process evaluation during the manufacturing of API.

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