2. LITERATURE REVIEW:

2.1 General Literature review from Journals for determination of Antiviral drugs:

Literature studies show various analytical methods reported for the estimation of individual drug substances of anti-viral drugs.

Bahrami G. et al. (2005)\textsuperscript{[7]} reported that a fast, simple and sensitive high performance liquid chromatographic (HPLC) method has been described for determination of acyclovir in human serum. Since acyclovir is a polar compound and soluble in aqueous medium and practically insoluble in most of organic solvents, its analysis in biological fluids in currently published HPLC methods, involve pre-treatment of acyclovir plasma sample including deproteinization or solid phase extraction. In present method liquid–liquid extraction of acyclovir and internal standard (vanillin) is achieved using dichloromethane-isopropyl alcohol (1:1, v/v) as an extracting solvent. Analysis was carried out on ODS column using methanol-phosphate buffer (0.05 M) containing sodium dodecyl sulfate (200 mg/L) and triethylamine (2 mL/L, v/v) as mobile phase (pH = 2.3; 5:95, v/v) at flow rate of 2 ml/min. The method was shown to be selective and linear into the concentration range of 10–2560 ng/mL. Accuracy and precision of the method were also studied. The limit of quantitation was evaluated to be 10 ng/mL. This method was applied in bioequivalence study of two different acyclovir preparations after administration of 400 mg in 12 healthy volunteers.

Basavaiah K. et al. (2003)\textsuperscript{[8]} reported that an assay method for the determination of acyclovir from pharmaceutical preparations has been developed for assessment of product quality utilising high-performance liquid chromatography. The chromatographic conditions comprised a reversed-phase C18 column (250 x 4.6 mm i.d.) with a mobile phase of acetonitrile : 20 mmol aqueous ammonium acetate buffer of pH 4.5 (40:60). The flow rate was 0.8 ml/min and UV detection was used at 250 nm. Calibration graph was linear in the range 1.98-59.4 mg/ml. The method has been validated according to current guidelines including assay of pharmacopoeial standard tablets. Recoveries ranged from 96.64 to 99.53%. The exipients present in the tablets did not interfere with the method.

Hung C. V. et al. (2002)\textsuperscript{[9]} reported that a sensitive plasma assay for acyclovir has been developed and validated. Acyclovir was separated from plasma components using Oasis HLB columns. Separation was obtained with no plasma interference using micellar electrokinetic chromatography (175 mM SDS) and hydroxypropyl-b-cyclodextrin (100 mM) in 90 mM borate
buffer (pH 8.8) containing 0.2% NaCl. High sensitivity was achieved by large volume sample introduction and stacking. The linear range was from 20 to 10000 ng/ml with a limit of quantitation of 20 ng/ml. This method is a viable alternative to HPLC because of its high separation and sensitivity, reproducibility, and adaptability to other nucleoside analogs.

**Emami J. et al. (2009)** [10] reported that a sensitive, accurate and rapid reverse phase HPLC method was described to quantitate levels of acyclovir in human plasma. The drug, internal standard (metronidazole) and phosphate buffer (0.05 M) were added to serum samples and vortexed for 30 sec. A mixture of isopropyl alcohol:dichloromethane (60:40) was then added and vortexed for 3 min. Samples were centrifuged and the supernatant layer was separated, evaporated to dryness under nitrogen gas stream, reconstituted in mobile phase and, an aliquot of 50 µl was analyzed on a µ-bondapack C18 (250 × 3.9 mm) column, with 3% acetonitrile in deionised water and 0.5% orthophosphoric acid, (pH 2.5) at 254 nm. The standard curve covering 100-1500 ng/ml concentration range, was linear, relative errors were within 0.79 to 17.4% and the CV% ranged from 3.81 to 18.2. The limits of quantitation and detection of the method were 100 ng/ml and 25 ng/ml respectively.

**Basavaiah K. et al. (2002)** [15] reported that a simple and cost effective spectrophotometric method is described for the determination of acyclovir in bulk drug and in formulations. The coloured species has an absorption maximum at 760 nm and obeys Beer’s law in the concentration range 50–450 g/ml. The absorbance was found to increase linearly with increasing concentration of acyclovir, which is corroborated by the calculated correlation coefficient value of 0.9998 (n=9). The apparent molar absorptivity and Sandell sensitivity were 1.65×102 l/mol/cm and 1.36 g/cm², respectively. The slope and intercept of the equation of the regression line are 6.87×104 and 8.33×103, respectively. The limit of detection was 5.68 g/ml and the limit of quantification was 18.95 g/ml.

**Jadhav A.S. et al. (2007)** [20] reported that 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-yI) methoxy] ethyl ester, an antiviral agent in bulk drug substance. The enantiomers of Valacyclovir were resolved on a Chiralpak AD (250mm×4.6 mm, 10µ) 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 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.

Uslu B. et al. (2006) \[21\] reported that electrochemical properties of valacyclovir, an antiviral drug, were investigated in pH range 1.8–12.0 by cyclic, differential pulse and squarewave voltammetry. For analytical purposes, a very resolved diffusion controlled voltammetric peak was obtained in Britton–Robinson buffer at pH 10.0 using differential pulse and square-wave modes. Limits of detection were $1.04 \times 10^{-7}$ and $4.60 \times 10^{-8}$ M for differential pulse and square-wave voltammetry, respectively. The applicability to direct assays of tablets, spiked human serum and simulated gastric fluid, was described.

Aswani Kumar C. et al. (2010) \[22\] reported that a new, simple and sensitive spectrophotometric method for the determination of valacyclovir and cefotaxime has been developed. The method is based on the condensation of valacyclovir and cefotaxime with 1, 2-napthaquinone-4-sulfonic acid sodium (NQS) in alkaline media to yield orange colored products respectively. Valacyclovir and cefotaxime showed maximum absorbance at 495 nm and 475 nm with linearity was observed in the concentration range of 20-120 µg/ml and 20-140 µg/ml respectively. The relative standard deviations of 0.363% for valacyclovir and 0.66% for cefotaxime were obtained. The recoveries of valacyclovir and cefotaxime injections are in the range 96.01±0.52 and 98.12±0.96 respectively.

Granero G. et al. (2006) \[24\] reported that the absolute bioavailability of the prodrug valacyclovir, the l-valyl ester of acyclovir, after oral administration is 54.5%. The stability of the prodrug was found to be dependent on pH. This prodrug is chemically stable along the acidic pH side (under 4), while the prodrug degrades in alkaline medium through a base-catalyzed pseudo-first-order kinetics. The degradation of the prodrug valacyclovir progressed faster in intestinal fluid than in phosphate buffer at the same pH. There was no appreciable release of Valacyclovir neither in the human and dog stomach contents nor in phosphate buffers at pHs fewer than 4, although its degradation was fastest in the human and dog stomach contents.

Ganesh M. et al. (2009) \[25\] reported that 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 with 1.0910 x104 mol/cm, the slope, intercept, correlation coefficient, detection and quantitation limits were also calculated. 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.

**Pradeep B. et al. (2010)**[^27] reported that acyclovir a specific and selective inhibitor of herpes virus has been used safely and effectively. The bioavailability of the drug is low results in poor absorption of drug. Valacyclovir is the L-valyl ester prodrug of Acyclovir. It is used in the treatment of Herpes simplex virus and Varicella zoster virus. After oral administration it is rapidly converted to acyclovir in the Gastro intestinal tract and liver, which increases the bioavailability of acyclovir three to five times that of acyclovir alone.

**Chuong P. et al. (1999)**[^30] reported that a rapid high-performance liquid chromatographic assay with isocratic elution is developed for the simultaneous quantification of valaciclovir (VACV) prodrug and its active converted compound, acyclovir (ACV), in biological fluids of treated patients. For serum, the samples are deproteinized with perchloric acid in presence of 1-methylguanosine as the internal standard (IS). For urine and dialysis liquid, the samples are diluted with a mobile phase containing the IS, then filtered. VACV, ACV and the IS are separated on a SymmetryShieldE RP-8 column with acetonitrile–ammonium phosphate buffer as the mobile phase and detected at 254 nm. The chromatographic time is about 12 min. The relative standard deviations (RSD) of VACV and ACV standards are between 0.5 and 3.5%. Most endogenous nucleosides and their metabolites, psychotropic drugs and drugs of abuse are shown not to interfere with this technique.

**Anil Kumar T. et al. (2011)**[^31] reported that a simple, sensitive spectrophotometric methods are presented for the assay of acyclovir and valacyclovir in bulk drug and in tablets. The methods employ N-bromosuccinimide (NBS) as the oxidimetric reagent and dye, methyl orange used as a spectrophotometric reagent. The method involves adding a measured excess of NBS to acyclovir and valacyclovir in acid medium followed by determination of residual NBS by reacting with a fixed amount of methyl orange and measuring the absorbance at 508 nm. Acyclovir and valacyclovir showed maximum absorbance at 508 nm with linearity was
observed in the concentration range of 1-5 µg/mL and 5-10 µg/mL respectively. The relative standard deviations of 0.024 % for acyclovir and 0.018 % valacyclovir were obtained. The accuracy and reliability of the methods were further ascertained by performing recovery tests via standard-addition method. The recoveries of acyclovir and valacyclovir tablets are in the range 99.26 ±0.52, 99.47±0.96 respectively.

**Ibrahim A. et al. (2005)** [32] reported that a simple and sensitive fluorimetric method for determination of antiviral drugs: ribavirin, acyclovir, and amantadine hydrochloride has been developed. The method was based on the oxidation of these drugs by cerium(IV) in presence of perchloric acid and subsequent monitoring the fluorescence of the induced cerium(III) at kexcitation 255 and kemission 355 nm. Under the optimum conditions, linear relationships with good correlation coefficients (0.9978–0.9996) were found between the relative fluorescence intensity and the concentrations of the investigated drugs in the range of 50–1400 ng/ml. The assay limits of detection and quantitation were 20–49, and 62–160 ng/ml, respectively. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 1.58%. No interference could be observed from the excipients commonly present in dosage forms. The proposed method was successfully applied to the analysis of the investigated drugs in pure and pharmaceutical dosage forms with good accuracy and precision; the recovery percentages ranged from 99.2 to 101.2 ± 0.48–1.30%.

**Similarly some other methods are also referred they are as follows:**

- **Smith J. et al. (2010)** [12] reported that a Pharmacokinetics of Acyclovir and Its Metabolites in Cerebrospinal Fluid and Systemic Circulation after Administration of High-Dose Valacyclovir in Subjects with Normal and Impaired Renal Function.

• **Sinha V. et al. (2007)** [18] reported that a method on Stress Studies of Acyclovir.

• **Fernandeza M. et al. (2003)** [19] reported that a technique for validation by liquid chromatography for the determination of acyclovir in plasma.

• **Wang L. et al. (1996)** [23] reported that a Pharmacokinetics and Safety of Multiple-Dose Valaciclovir in Geriatric Volunteers with and without Concomitant Diuretic Therapy.

• **Kasiari M. et al. (2008)** [26] reported that a Selective and rapid liquid chromatography/negative-ion electrospray ionization mass spectrometry method for the quantification of valacyclovir and its metabolite in human plasma.

• **Patil G. et al. (2009)** [28] reported that a validated Specific Reverse Phase Liquid Chromatographic Method for the Determination of Valacyclovir in the Presence of its Degradation Products in Bulk Drug and in Tablet Dosage Form.

• **Srinivas K. et al. (2011)** [29] reported that a RP-HPLC method development and validation for estimation of Valacyclovir in bulk and tablet dosage forms.


• **Bomgaars L. et al. (2008)** [34] reported that a method on Valacyclovir and Acyclovir Pharmacokinetics in Immunocompromised Children.