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REVIEW OF ACYCLOVIR ANALYSIS IN PHARMACEUTICAL PREPARATIONS AND BIOLOGICAL MATRICES

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ABSTRACT

Acyclovir is an active antiviral drug for treating Herpes Simplex Virus (HSV) types 1 and 2. This drug is also useful in the treatment of infections due to varicella-zoster virus. Acyclovir preparations on the market are in the form of tablets, ointments, or intravenous injections. This review article discusses the acyclovir analysis method in pharmaceutical preparations and biological matrices. Several spectrophotometric, voltammetry, capillary electrophoresis analysis methods, and thin-layer chromatography are used to determine acyclovir in raw materials, and pharmaceutical preparations. Also, high-performance liquid chromatography (HPLC) and mass spectrophotometry (MS) methods can be used to analyze acyclovir in various biological matrices (eg plasma, urine).

KEYNOTES: Acyclovir, spectrophotometry, voltammetry, electrophoresis, HPLC, MS.

INTRODUCTION

Acyclovir has the chemical name 9- [(2-Hydroxyethoxy) methyl] guanine, with the chemical formula $C_8H_{11}N_5O_3$ and molecular weight 225.21. The structure of acyclovir can be seen in Figure 1. Acyclovir is crystal powder, white to almost white with solubility soluble in dilute hydrochloric acid, difficult to dissolve in water, insoluble in ethanol.[1]

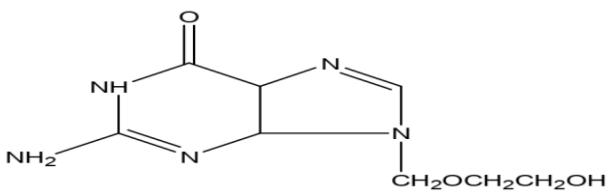

Figure 1: Structure of acyclovir.[1]

The partition coefficient value in n-octanol at 22 ^{o}C is -1.57, at 25 ^{o}C with a pH of 6.8 is -1.8 and the acyclovir pKa value at room temperature is 2.16 and 9.04 at 37 ^{o}C.[2] Acyclovir has pharmacological effects as an antiviral agent, especially treating herpes. The mechanism of action is analogous to the nucleoside that binds to the DNA polymerase virus.[3]

The activity of acyclovir is greatest against herpes 1 and herpes 2, less against varicella-zoster, still less against Epstein-Barr, and very little against cytomegalovirus. Acyclovir is an antiviral agent only after it is phosphorylated in infected cells by a viral-induced thymidine kinase. Acyclovir monophosphate is phosphorylated to diphosphate and triphosphate forms by cellular enzymes in the infected host cell where the drug is concentrated. Acyclovir triphosphate inactivates viral deoxyribonucleic acid polymerase. Acyclovir incorporation into the growing viral deoxyribonucleic acid chain causes its termination. The antiviral process has relatively little effect on normal, uninfected cells. An important toxic effect of acyclovir is its potential to cause obstructive nephropathy. The drug is excreted primarily by the kidney, which may require smaller doses in patients with decreased kidney function. Oral dosages of acyclovir as recommended for herpes simplex are probably not adequate for varicella-zoster infections.[4]

Several methods have been reported related to the analysis of acyclovir in both pure compounds and pharmaceutical preparations and biological fluids. To date, many analytical methods have been developed for quantitative determination related to acyclovir levels.

DATA COLLECTION

In compiling this review article, the technique used is to use a literature study by finding sources or literature in the form of primary data in the form of official books and international journals in the last 20 years (2000-2020). Also, in making this review article a data search using online media with keywords was acyclovir analysis in pharmaceutical preparations and biological matrices. The search for primary references used in this review article through a trusted web such as ScienceDirect, NCBI, Researchgate, Google Scholar, and other published and trustworthy journals.

ANALYTICAL METHODS

Ultraviolet spectrophotometric analysis

Several ultraviolet spectrophotometry methods have been used for acyclovir analysis, either as raw material or in pharmaceutical dosage forms (Table 1).

Table 1: Acyclovir analysis using ultraviolet spectrophotometry.

No	Sample	Solvent	Wavelength	Range of Concentration	Reference
1	Cream	H_2SO_4 0.5 M	255 nm		[1]
2	Capsule	Copper (II) in borax/sodium pH 9 hydroxide buffer	290 nm	112–1620 µg/mL	[5]
3	Capsule	Cobalt (II) in 1 % pyridine in methanol	287 nm	112–1620 µg/mL	[5]
4	Bulk and tablet	Distilled water	253 nm	2 – 20 µg/mL	[6]
5	Poly (n-butyl cyanoacrylate) (PBCA) nanoparticles	Methanol + Acetonitrile (8:2)	295.2 nm	1.25 to 40.0 µg/mL	[7]
6	Bulk	Distilled water, phosphate buffer pH 7.4 and pH 5	252.8 nm	1 – 20 µg/mL	[15]
7	Bulk and pharmaceutical preparation	Distilled water	254 nm	5 – 30 µg/mL	[16]

The determination of acyclovir levels in the cream is carried out as follows. Weigh carefully the amount of cream equivalent to 7.5 mg acyclovir, put in the appropriate separating funnel, add 50 mL of 0.5 M sulfuric acid and 50 mL of ethyl acetate, shake, leave to separate and gather a clear, lower layer of water. Wash the organic layer with 20 mL of 0.5 M sulfuric acid, dilute the laundry mixture, and the water layer with 0.5 M sulfuric acid to 100.0 mL. Mix and strain with Whatman GF/F paper, discard the first filtrate, and pipette as much as 10 mL of filtrate into a 50-mL volumetric flask add water to the mark. Measure the absorption at the maximum absorption wavelength of approximately 255 nm.[1]

A simple spectrophotometric method for the determination of some antiviral drugs, such asacyclovir and amantadine hydrochloride in pure and dosage forms have been developed. The proposed method is based on the reactions of the selected drugs and metals. Acyclovir was reacted with copper (II) and cobalt (II) using a borax/sodium pH 9 hydroxide buffer, and in a non-aqueous medium using 1 % pyridine in methanol, respectively. The formedcomplexes were absorbed maximally at A, 290 nm, and at X 287 nm. The method determined from 112–1620 µg/mL of acyclovir, with a mean percentage of recovery of 99.32 ± 0.63 and of 98.77 ± 0.70 for copper (II) and cobalt (III), respectively. The suggested method was applied to a *Novirus* capsule, and its validity was ascertained by the standard addition technique.[5]

A simple, sensitive, rapid, accurate, and precise spectrophotometric method has been developed for the estimation of acyclovir in bulk and pharmaceutical dosage forms. Acyclovir shows maximum absorbance at 253 nm with molar absorptivity of 1.3733 $\times 10^4$ L/mol×cm Beer's law was obeyed in the concentration range of 2-20 µg/ml. Results of the analysis were validated statistically and by recovery studies.[6]

A derivative spectrophotometric method was validated for the quantification of acyclovir in poly (*n*-butyl cyanoacrylate) (PBCA) nanoparticles. Specificity, linearity, precision, accuracy, recovery, detection (LOD) and quantification (LOQ) limits were established for method validation. First-derivative at 295.2 nm eliminated interferences from nanoparticle ingredients and presented linearity for acyclovir concentrations ranging from 1.25 to 40.0 µg/mL (r = 0.9999). Precision and accuracy data demonstrated good reproducibility. Recovery ranged from 99.3 to 101.2. LOD was 0.08 µg/mL and LOQ, 0.25 µg/mL. Thus, the proposed method proved to be easy, low cost, and accurate, and therefore, a useful alternative to quantify acyclovir in nanoparticles.[7]

Simple, precise, accurate, and economical UV spectrophotometric methods have been developed and validated for the routine estimation of acyclovir in bulk drug and pharmaceutical preparations. The drug shows maximum absorption at 252.8 nm with molar absorptivity of 1.5899 ×104 L/mol×cm and it obeyed Beer-Lambert's law in the concentration range of 1-20µg/mL. Correlation coefficients (R^2) were found to be 0.999, 0.998. 0.999 in distilled water, phosphate buffer pH 7.4 and 5 respectively.[15]

A new, economical, sensitive, simple, rapid UV spectrophotometric method has been developed for the estimation of Acyclovir in pure form and pharmaceutical formulation. This UV method was developed using distilled water as a solvent. In the present method, the wavelength selected for analysis was 254 nm. UV Visible double beam spectrophotometer (Systronic 2201) was used to carry out the spectral analysis. The ICH guidelines were used to validate the method. Results: The method was validated for linearity, range, accuracy, precision, robustness, LOD, and LOQ. Linearity was found in the range of 5 - 30 µg/mL. Accuracy was performed by using a recovery study. The amount of drug recovered was found to be in the range of 100.1-100.5 %. The % RSD value was found to be less than 2. The proposed UV spectroscopic method was found to be accurate, precise, stable, linear, specific, and simple for quantitative estimation of acyclovir in the bulk and pharmaceutical

dosage form. Hence the present UV spectroscopic method is suitable for the routine assay of acyclovir in bulk and pharmaceutical formulations.[16]

Visible spectrophotometric analysis

Several visible spectrophotometry methods have been used for acyclovir analysis, either as raw material or in pharmaceutical dosage forms (Table 2).

Table 2: Acyclovir analysis using visible spectrophotometry.

No	Sample	Reagent	Wavelength	Range of Concentration	Reference
1	Tablet, ophthalmic ointment and cream	MBTH and $FeCl_3$	616 nm	20–200 µg/mL	[8]
2	Tablet and cream	DMAB	404 nm	1.81-9.06 µg/mL	[9]
3	Dosage form	Ninhydrin-Ascorbic acid at pH 5	540 nm	Up to 30 µg/mL	[10]
4	Bulk drug and formulations	Folin–Ciocalteu (F–C) reagent in alkaline medium	760 nm	50–450 µg/mL	[18]

A simple and reliable spectrophotometric method has been developed for the determination of acyclovir in pharmaceutical formulations. The method is based on its oxidative coupling reaction with 3-methyl benzothiazoline-2-one hydrazone (MBTH) in the presence of $FeCl_3$ as an oxidant to produce deep-green colored species measurable at 616 nm. The absorbance–concentration plot is linear over the range of 20–200 µg/mL with minimum detectability of 1.06 µg/mL (4.71×10^{-6} M). The molar absorptivity was 9.41×10^{2} L mol^{-1} cm^{-1} with correlation coefficient (n = 7) of 0.9998. The different experimental parameters affecting the development and stability of the color were studied carefully and optimized. The proposed method was applied successfully to the determination of acyclovir in its dosage forms. The percentage recoveries ± SD (n=9) were 98.63 ± 0.34, 99.61 ± 0.58, 99.35 ± 0.58 and 99.72 ± 0.86 for tablets, ophthalmic ointment and cream, respectively.[8]

A new spectrophotometric method has been developed for the analysis of acyclovir in bulk and dosage forms. The method is based on the diazo coupling reaction between diazotized acyclovir and *p*-dimethylaminobenzaldehyde (DMAB). Spot tests and thin layer chromatographic analysis confirmed the formation of a greenish-yellow adduct which was stable in the laboratory environment for more than three hours. Critical factors affecting optimal detector response were identified and optimized. The optimal temperature and

coupling reaction time were established at 50 ^{o}C and 10 min. The azo adduct was determined at 404 nm where neither diazotized acyclovir nor DMAB has any significant absorptivity. Methanol was found as the best diluting solvent after coupling. The assays of acyclovir were linear over the range of 1.81-9.06 µg/mL with a correlation coefficient of 0.9998 and a limit of detection of 0.024 µg/mL. The method was accurate (error < 3 %) and precise (RSD < 2.7 %) over three days assessment. There was no interference from commonly used excipients. The method was successfully applied to the determination of acyclovir in tablets and creams with similar accuracy to the official USP spectrophotometric method. The method is rapid, simple, and cost-effective and could find application in the in-process quality control of acyclovir.[9]

A study is aimed at developing and validating a simple and rapid spectrophotometric method for the determination of acyclovir. The mechanism of the proposed method is based on the condensation/coupling reaction between Acyclovir and Ninhydrin-Ascorbic acid at pH 5. A purple-colored product with maximum absorption at 540 nm was assayed to quantitatively evaluate the drug content in the formulation. The calibration curve was found to be linear up to 30 µg/mL. Analyte recovery tests carried out by the proposed method gave recovery of between 96.9 – 102.0 %. Molar absorptivity and Sandells' sensitivity were determined to be 41,071.43 L mol^{-1} cm^{-1} and 1.84 µg cm^{-2} respectively. The precision was assessed by determining the inter-day and intra-day variation which ranged between 1.45 – 1.63 % and 0.81 – 1.12 % respectively. The results show that the reaction produced a stable product and the proposed method is cost-effective and possesses adequate accuracy, precision, and sensitivity. It can, therefore, be conveniently applied for the determination of acyclovir in dosage forms.[10]

A simple and cost-effective spectrophotometric method is described for the determination of acyclovir in bulk drug and formulations. The method is based on the formation of a blue-colored chromogen when the drug reacts with Folin–Ciocalteu (F–C) reagent in an alkaline medium. The colored species has an absorption maximum at 760 nm and obeys Beer's law in the concentration range 50–450 µg mL^{-1}. 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 1.65×10^{2} L mol^{-1} cm^{-1} and 1.36 µg cm^{-2}, respectively. The slope and intercept of the equation of the regression line are 6.87×10^{-4} and 8.33×10^{-3}, respectively. The limit of

detection was 5.68 µg/mL and the limit of quantification was 18.95 µg/mL. The proposed method was successfully applied to the determination of acyclovir in pharmaceutical formulations. The reliability of the assay method was established by parallel determination by standard-addition method, and by recovery studies. The results demonstrated and the procedure is at least as accurate, precise, and reproducible (RSD < 2 %) as the official method, while being simple and less time-consuming. Statistical analysis indicated that there was no significant difference between the results obtained by the proposed procedure and those of the official method.[18]

High-Performance Liquid Chromatography (HPLC)

Some HPLC methods have been used for acyclovir analysis, either as raw material, in pharmaceutical dosage forms, and biological matrices (Table 3).

Table 3: Acyclovir analysis using high-performance liquid chromatography.

No.	Sample	Column	Mobile Phase	Detector	Chromatographic Condition	Ref.
1	Human plasma	Novaflex C	octane sulfonic acid buffer (pH 2.5) and methanol (92:08)	UV (254 nm)	Flow rate: 1.0 $mL.min^{-1}$ Temperature: room	[11]
2	Rat plasma, amniotic fluid, placental tissue, and fetal tissue	Agilent Eclipse XDB C_{18} column (150×2.1 mm, 5 µm)	10 m*M* acetate/citrate buffer–3.7 m*M* aqueous octane sulfonic acid (87.5:12.5, v/v)	UV (254 nm)	flow-rate of 0.2 mL/min	[12]
3	Percutaneous	Column 250 × 4 mm C_8 LiChrospher Select B	acetonitrile–ammonium acetate 0.05 M (1 : 99, v/v) pH 6.5	UV (252 nm)	Not available	[13]
4	Maternal plasma, amniotic fluid, fetal tissue, and placenta	Agilent Eclipse XDB C_{18} column (150×2.1 mm, 5 µm)	30 mM acetate/citrate buffer (pH 3) and methanol	UV (254 nm)	flow-rate of 0.2 mL/min	[14]
5	Rat plasma, amniotic fluid, placental tissue, and fetal tissue	Underivatized silica column	acetonitrile/formate buffer mobile phase (80:20)	Not available	Not available	[17]
6	Human plasma	RP-HPLC	Not available	UV (254 nm}	Not available	[19]
7	Tablet	C_{18} column (250×4.6 mm i.d.)	acetonitrile-20 mmol L^{-1} aqueous ammonium acetate buffer of pH 4.5	UV (250 nm)	flow-rate of 0.8 mL/min	[20]

			(40:60)			
8	Plasma	LiChrospher 100 RP-18	18 % acetonitrile, sodium dodecyl sulfate 5 m*M* and phosphate buffer at pH 2.6	UV (250-260 nm)	Not available	[21]
9	Human serum	ODS column	methanol-phosphate buffer (0.05 M) containing sodium dodecyl sulfate (200 mg/L) and triethylamine (2 mL/L, v/v)	Not available	the flow rate of 2 mL/min	[22]
10	Aqueous humor	Inertsil ODS-3 C_{18} column	methanol: water: acetic acid (5:95:0.1, *v/v*)	MS-ESI	flow-rate of 0.3 mL/min	[23]
11	Human plasma	Diamonsil-5 µm C_{18} column	methanol and 0.08 % aqueous trifluoroacetic acid solution	fluorescence detection at 260 nm (excitation) and 380 nm (emission)	Not available	[24]
12	Human serum	µ-bondapack C18 (250 × 3.9 mm) column	3 % acetonitrile in deionised water and 0.5 % orthophosphoric acid, (pH 2.5)	UV (254 nm)	Not available	[25]
13	Human plasma	Hypersil GOLD C18, 5 µm, 4.6 x 50 mm i.d.	acetonitrile and waters (80:20 %, v/v)	MS	the flow rate of 0.5 mL/min	[27]
14	Tablet	Novapak ODS C-18 (150 x 3.9 mm, 4 µm)	water: acetonitrile (78:22)	UV (254 nm)	the flow rate of 0.8 mL/min	[29]
15	Vitreous humor	C18 column	0.02 mol/L acetic acid/methanol (95:5)	UV (254 nm)	25 ^{o}C	[30]
16	Human plasma	Reversed-phase C8 column	0.1 % (*v/v*) triethylamine in water (pH 2.5)	UV (255 nm)	Not available	[31]
17	Topical formulations	Reversed-phase C18 column	20 mM ammonium acetate pH 3.5 in water and acetonitrile	time of flight mass spectrometry	Not available	[32]

A rapid, simple, and sensitive reversed-phase high-performance liquid chromatographic (HPLC) method has been developed for the measurement of acyclovir concentrations in human plasma and its use in bioavailability studies is evaluated. Unchanged acyclovir has

been quantified without the introduction of an internal standard using the present method. Human plasma proteins were selectively precipitated by the addition of 7% perchloric acid to spiked plasma samples or the plasma samples obtained after acyclovir administration to human volunteers and the mixture was spun at 1000 *g* for 10 min. The supernatant was directly injected into a Novaflex C column and detected at 254 nm. The mobile phase consisted of octane sulfonic acid buffer (pH 2.5) and methanol (92:08). The limit of quantitation for acyclovir in plasma was 20 ng/mL, which enabled the determination of the area under the curve (AUC) more precisely, that is, it is much closer to its extrapolated value. The present method has been successfully applied to samples from bioavailability studies.[11]

A fast and reproducible HPLC method for the determination of the highly polar acyclovir in maternal rat plasma, amniotic fluid, placental tissue, and fetal tissue has been developed and validated. Plasma and amniotic fluid samples were prepared by protein precipitation using 2 *M* perchloric acid and syringe filtering. Tissue samples were homogenized in distilled water, centrifuged, and extracted using a C solid-phase extraction method before analysis. Baseline resolution was achieved for acyclovir and the internal standard gancyclovir, the anti-viral of similar structure to acyclovir, using an Agilent Eclipse XDB C_8 column (150×2.1 mm, 5 µm). The mobile phase used for the plasma and amniotic fluid was 10 m*M* acetate/citrate buffer–3.7 m*M* aqueous octane sulfonic acid (87.5:12.5, v/v) at a flow-rate of 0.2 mL/min. The mobile phase used for the tissue samples was 30 m*M* acetate/citrate buffer with 5 m*M* octane sulfonic acid–acetonitrile (99:1, v/v). Both aqueous mobile phase portions were pH adjusted to 3.08. All separations were done using an Agilent 1100 Series HPLC system with UV detection of 254 nm. The assay was validated for each matrix over a range of 0.25–100 µg/mL over 3 days using five replicates of three spiked concentrations. The relative standard deviation and percent error for each validation data set was < 15 % for middle and high-quality control (QC) points and < 20 % for all low QC points. All calibration curves showed good linearity with an $R > 0.99$. The extraction efficiency for recovery of acyclovir from all matrices was > 80 %.[12]

A study was aimed to develop a direct, simple, and rapid high performance liquid chromatographic (HPLC) method for the determination of acyclovir (ACV) after in vitro percutaneous permeation studies. Samples were chromatographed on a reversed-phase encapped column 250 × 4 mm C_8 LiChrospher Select B. The phase mobile was a mixture of acetonitrile–ammonium acetate 0.05 M (1: 99, v/v) pH 6.5. Detection was at 252 nm and the

run time was 12 min. The limit of detection was 0.006 µg/mL. The detector response was found to be linear in the concentration range of 0.05 to 10 µg/mL. This assay is a selective, sensitive, and reproducible method for the quantification of ACV in skin layers and the receptor compartment of Frank-type diffusion cells after percutaneous permeation studies.[13]

The gradient HPLC assay aids in the quantitation of these drugs from the matrices associated with pregnancy (maternal plasma, amniotic fluid, fetal tissue, and placenta). The mobile phase consists of 30 mM acetate/citrate buffer (pH 3) and methanol. The plasma and amniotic fluid samples are prepared using a combination of protein precipitation and filtration, while the more complex tissues are prepared with the use of solid-phase extraction (SPE). The method was validated in the calibration range of 0.1–100 µg/mL and showed precision (% Relative Standard Deviation; % RSD) and accuracy (% Error) of less than 15 % for all matrices over three days. The assay was applied to a pharmacokinetic study involving the coadministration of ACV in the pregnant rat.[14]

Reversed-phase chromatography is the most common means of separation for small drug molecules. However, polar drugs may suffer from poor retention and peak shape in reversed-phase high-performance liquid chromatography (RP-HPLC). Hydrophilic interaction liquid chromatography (HILIC) provides a viable alternative to RP-HPLC and is an excellent way to separate polar compounds. This paper describes a HILIC/ESI-MS/MS assay for the determination of acyclovir from rat plasma, amniotic fluid, placental tissue, and fetal tissue. The isocratic separation utilizes an underivatized silica column with an acetonitrile/formate buffer mobile phase (80:20). The method is validated over a range of 50 ng/mL to 50 µg/mL with % error and % relative standard deviation of <15% over 3 days. All samples are prepared by acetonitrile protein precipitation, which yields high recovery (> 84% for acyclovir). This assay can be applied to the pharmacokinetic study of the placental transfer of acyclovir.[17]

A simple high-performance liquid chromatographic method was developed for the simultaneous determination of the therapeutic levels of acyclovir and ganciclovir in human plasma. After precipitation of plasma proteins with 6% perchloric acid, acyclovir and ganciclovir were simultaneously determined by reversed-phase chromatography with spectrophotometric detection at 254 nm. The peak heights for acyclovir and ganciclovir were linearly related to their concentrations ranging from 0.063 to 2.080 µg/mL. The recovery was

100.48–102.84 % for acyclovir and 99.26–103.07 % for ganciclovir. The intra- and inter-day relative standard deviation values were in the range 0.186–8.703 % for acyclovir and 0.137–6.424 % for ganciclovir. The detection limits for both compounds were 0.01 µg/mL determined as the signal-to-noise ratio of 3. The present method applies to therapeutic monitoring during antiviral medication.[19]

An assay method for the determination of acyclovir from pharmaceutical preparations has been developed for the assessment of product quality utilizing high-performance liquid chromatography. The chromatographic conditions comprised a reversed-phase C_{18} column (250×4.6 mm i.d.) with a mobile phase of acetonitrile-20 mmol L^{-1} aqueous ammonium acetate buffer of pH 4.5 (40:60). The flow rate was 0.8 mL min^{-1} and UV detection was used at 250 nm. Calibration graph was linear in the range 1.98–59.4 µg mL^{-1}. The method has been validated according to current guidelines including assay of pharmacopoeial standard tablets. Recoveries ranged from 96.64 to 99.53 %. The excipients present in the tablets did not interfere with the method.[20]

A high-performance liquid chromatography (HPLC) method was developed for the determination of acyclovir (ACV) in plasma. The plasma samples, recharged with acyclovir and in presence of 5′-*N*-methylcarboxyamidoadenosine (MECA) as an internal standard, were purified using a solid-phase extraction technique with Waters Oasis HLB columns. The separation of the components from the extract was carried out in a LiChrospher 100 RP-18 column for further ultraviolet detection at a wavelength range of 250–260 nm. The mobile phase composition was 18 % acetonitrile, sodium dodecyl sulfate 5 m*M*, and phosphate buffer at pH 2.6 with an analysis time of 13 min per sample. The average retention time for acyclovir was 5.0 min and for the internal standard 11.2 min. The calibration curve was linear ranging between 0.05 and 1.80µg/ml. The detection limit was 0.006 µg/ml with a quantification limit of 0.020 µg/mL. The ACV recuperation percentage for 250 µl of plasma was between 94.7 and 109.7% with a coefficient of variation not higher than 5.2 %. This method was developed and validated for use in bioavailability and bioequivalence studies.[21]

A fast, simple, and sensitive high-performance liquid chromatographic (HPLC) method has been described for the determination of acyclovir in human serum. Since acyclovir is a polar compound and soluble in aqueous medium and practically insoluble in most of the organic solvents, its analysis in biological fluids in currently published HPLC methods, involves pre-treatment of acyclovir plasma sample including deproteinization or solid-phase extraction. In

the 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 10ng/mL. This method was applied in a bioequivalence study of two different acyclovir preparations after administration of 400 mg in 12 healthy volunteers.[22]

A specific, sensitive and precise liquid chromatographic assay method was established using LC-MS for the determination of acyclovir (ACV) in aqueous humor (AH), which was directly injected onto an Inertsil ODS-3 C_{18} column without any pretreatment. The Agilent 1100 series LC-MS system was operated under the electrospray ionization mode (ESI). The analyte was separated from endogenous substances with a mobile phase of methanol: water: acetic acid (5:95:0.1, *v*/*v*) at a flow-rate of 0.3 mL min^{-1}. A linear response was observed over the concentration range from 5 to 50 ng mL^{-1} (r=0.9993). Intra- and interday coefficients of variation were in the ranges 5.2–9.0 % and 5.8–8.2 %, respectively. The recovery was > 91.0 %, and the limit of detection was approximate 1 ng mL^{-1}. The pharmacokinetics of topically applied eye-drop and thermosetting gel were compared in rabbits utilizing the present method, the results demonstrated that LC-MS was a powerful tool for the detection of ACV in AH.[23]

A fast, simple and selective HPLC method has been developed for the assay of acyclovir, ganciclovir, and penciclovir in human plasma by coupling HPLC with fluorescence detection. 200 µL plasma, with guanosine 5-monophosphate as an internal standard, was subjected to protein precipitation with a 7 % [v/v] aqueous perchloric acid solution. The 40 µL supernatant was injected into a Diamonsil-5 µm C18 column. Acyclovir, ganciclovir, and penciclovir, with solvents composed of methanol and 0.08 % aqueous trifluoroacetic acid solution, were analyzed by fluorescence detection at 260 nm (excitation) and 380 nm (emission) using a gradient elution program. The calibration curves of all three analytes were linear between 20 and 2000 ng/mL. The mean absolute recoveries of acyclovir, ganciclovir, and penciclovir were 93.91 ± 1.20 %, 97.42 ± 0.75 %, and 99.01 ± 3.30 %, respectively. The mean inter-day CVs for acyclovir, ganciclovir, and penciclovir, were within 1.29–7.30 %,

1.00–5.53 %, and 1.19–3.54 %, respectively. The intra-day bias for acyclovir, ganciclovir, and penciclovir ranged from -2.01 to 6.33 %, 1.81 to 7.37 %, and 1.42 to 6.91 %, respectively. The method has been validated and applied in pharmacokinetic studies in Chinese adult renal transplant patients.[24]

A sensitive, accurate, and rapid reverse phase HPLC method was described to quantitate levels of acyclovir in human serum. 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 the mobile phase and, an aliquot of 50 µL was analyzed on a µ-bondapack C18 (250 × 3.9 mm) column, with 3 % acetonitrile in deionized 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. The method was suitable for bioavailability and pharmacokinetic studies of acyclovir in humans and applied in a randomized, two-way cross over bioequivalence study of two different acyclovir preparations with twelve subjects and with a one-week washout period.[25]

We developed a novel, sensitive high-performance liquid chromatography assay with ultraviolet detection for measuring acyclovir, ganciclovir and (*R*)-9-[4-hydroxy-2-(hydroxymethyl)butyl] guanine in human plasma to identify quantitative relationships between *in vitro* anti-EBV activity and therapeutic response. Characteristics of the assay include a low plasma volume (200 µL), perchloric acid protein precipitation, use ofpenciclovir as the internal standard, run times less than 8 min, and a 50 ng/mL lower limit of quantification. The within- and between-assay variability are 0.7–4.8 and 1.0–7.9 %, respectively. Accuracy for all three drugs ranges from 89.5 to 106.4 % for four qualitycontrols (50, 100, 1000, and 10,000 ng/mL). This assay supports pharmacokinetic and pharmacodynamic studies of candidate anti-EBV drugs in children and adults with EBV infections.[26]

The objective of this study was to validate a simple, specific, accurate, and precise solid-phase high performance liquid chromatographic method with the Tandem Mass

Spectrometry-Waters Quattro Premier XE method for the determination of acyclovir in human plasma using Ganciclovir as Internal Standard (IS). The precision and accuracy data have to fulfill the requirements for quantification of the analytes in biological matrices to generate data for bioequivalence, bioavailability, pharmacokinetic or toxicology investigations. A Hypersil GOLD C18, 5 µ column having 4.6 x 50 mm internal diameter in binary gradient mode with flow rate was 0.5 mL/min of mobile phase containing ammonium acetate and acetonitrile were used. The chromatographic separation was achieved by using elution solution consisting of acetonitrile and waters (80:20 %, v/v), diluent solution of methanol and water (50:50 %, v/v)] were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring (MRM) mode. The method was validated over the concentration range 5.0-5000.0 ng/mL, by using 500 µL plasma samples. Limit of detection and limit of quantification were found 5.0 ng and 30.0 ng respectively. The retention time for Acyclovir and Internal Standard was 1.24 min and 1.65 min respectively and overall chromatography run time was 2.24 minutes. The mean recovery of Acyclovir (89.09 %) and IS (98.84 %) from spiked plasma samples was consistent and reproducible. The method was validated for linearity, accuracy, precision, specificity, the limit of detection, the limit of quantification, and robustness. The intra- and inter-day precision and accuracy values were found to be within the assay variability limits as per the FDA guidelines. The developed assay method was applied to a clinical pharmacokinetic study in human volunteers.[27]

We report a rapid, sensitive, and robust method for the determination of acyclovir in human plasma and its validation towards evaluating the bioequivalence of drug formulations. After a simple liquid-liquid extraction from plasma, acyclovir is quantified using ultra-high-performance liquid chromatography - heated electrospray ionization - tandem mass spectrometry (UHPLC-HESI-MS/MS). The assay has a total analysis time is 5 min, a linear range of 1.0 - 2000 ng/mL, a lower limit of detection of 0.5 ng/mL, and a lower limit of quantification of 1.0 ng/mL. Intra- and inter-day precision is no more than 10.3 % and intra- and inter-day accuracy was within 13 % at various concentrations in human plasma. Validation according to FDA guidelines for bioanalysis indicates that the described UHPLC-HESI-MS/MS method provides rigorous quantification of acyclovir in human plasma and representative data demonstrates successful application towards the determination of pharmacokinetic profiles as part of an evaluation of drug formulation bioequivalence.[28]

A simple, economic, accurate reverse phase isocratic RP-HPLC method was developed for the acyclovir (200 mg) in the tablet dosage form. A Novapak ODS C-18 (150 x 3.9 mm, packed with 4 microns) in an isocratic mode with mobile phase water: acetonitrile (78:22) was used with flow rate (0.8 mL/min) and monitored at 254 nm. The retention times were 2.453 min for acyclovir. The linearity range was found to be 1 - 60 mg/mL. The procedure was validated as per ICH rules for accuracy, precision, linearity, and reproducibility. The method has been successfully used to analyze commercial solid dosage containing Acyclovir with good recoveries and proved to be robust.[29]

An HPLC-UV method was developed and validated for the determination of acyclovir in the vitreous humor. The method was carried out in isocratic mode using 0.02 mol/L acetic acid/methanol (95:5) as mobile phase, a C18 column at 25 °C and UV detection at 254 nm. The method was linear ($r > 0.99$) over the range of 35–700 µg/mL, precise (RSD < 5 %), accurate (recovery ranged from 98.18 to 99.64 %), robust, selective regarding of the vitreous humor, and robust remaining unaffected by deliberate variations in relevant parameters. The validated HPLC-UV method can be successfully applied to determine acyclovir directly injected into the vitreous cavity of rabbits' eyes.[30]

To evaluate the pharmacokinetic study of acyclovir, a method for the quantitative determination of acyclovir in human plasma should be simple, rapid, and reproducible. Therefore, the method is developed, validated, and applied for the analysis of acyclovir in plasma samples obtained from healthy volunteers. High performance liquid chromatographic (HPLC) method with UV detection for the determination of acyclovir in human plasma is presented. This method involves protein precipitation with 20 % (*v/v)* perchloric acid. The chromatographic separation was accomplished on a reversed-phase C8 column with a mobile phase composed of 0.1 % (*v/v*) triethylamine in water (pH 2.5). No internal standard is required. UV detection was set at 255 nm. The method was successfully applied for the evaluation of pharmacokinetic profiles of acyclovir tablets in 24 healthy volunteers. The validation results show that the proposed method is rugged, precise (RSDs for intra- and inter-day precision ranged from 1.02 to 8.37 %) and accurate (relative errors are less than 6.66%). The calibration curve was linear in the concentration range of 0.1-2.0 µg/mL and the limit of quantification was 0.1 µg/mL. The C_{max}, $_{Tmax}$, and AUCs for the two products were not statistically different ($p > 0.05$), suggesting that the plasma profiles generated by Zovirax were comparable to those produced by acyclovir manufactured by Jaka 80 company. Good

precision, accuracy, simplicity, sensitivity, and shorter time of analysis of the method make it particularly useful for the processing of multiple samples in a limited period for the pharmacokinetic study of acyclovir.[31]

A simple, rapid, and accurate stability-indicating HPLC assay was developed for the determination of acyclovir and lidocaine in topical formulations. Chromatographic separation of acyclovir and lidocaine was achieved using a reversed-phase C18 column and a gradient mobile phase (20 mM ammonium acetate pH 3.5 in water and acetonitrile). The degradation products of acyclovir and lidocaine in the samples were analyzed by ultra-performance liquid chromatography-time of flight mass spectrometry. The HPLC method successfully resolved the analytes from the impurities and degradation products in the topical formulation. Furthermore, the method detected the analytes from the human skin leachables following the extraction of the analytes in the skin homogenate samples. The method showed linearity over wide ranges of 5–500 and 10–200 µg/mL for acyclovir and lidocaine in the topical product, respectively, with a correlation coefficient (r^2) > 0.9995. The relative standard deviations for precision, repeatability, and robustness of the method validation assays were < 2 %. The skin extraction efficiency for acyclovir and lidocaine was 92.8 ± 0.7% and 91.3 ± 3.2 %, respectively, with no interference from the skin leachables. Thus, the simultaneous quantification of acyclovir and lidocaine in the topical formulations was achieved.[32]

Electrochemistry

Many electrochemistry methods have been used for acyclovir analysis, either as raw material or in pharmaceutical dosage forms. The following will explain several methods of analysis of acyclovir based on electrochemistry.

Multi-wall carbon nanotubes (MWNTs)-dihexadecyl hydrogen phosphate (DHP) film-coated glassy carbon electrode (GCE) was fabricated, and the electrochemical behaviors of acyclovir on the MWNTs-DHP film-coated GCE were investigated by using cyclic voltammetry (CV), linear sweep voltammetry (LSV), electrochemical impedance spectroscopy (EIS) and chronocoulometry (CC). The oxidation peak current of acyclovir increased significantly and the peak potential shifted negatively at the MWNTs-DHP film-modified GCE, compared with that at a bare GCE. The results showed that this nanostructured film electrode exhibited excellent enhancement effects on the electrochemical oxidation of acyclovir. Consequently, a simple and sensitive electroanalytical method was developed for the determination of acyclovir. The oxidation peak current was proportional to the concentration of acyclovir from

8.0 × 10 to 1.0 × 10 mol/L. The detection limit was about 3.0 × 10 mol/L for 60 s accumulation at 0.00 V. The proposed method was demonstrated by using acyclovir tablets and the result was satisfying.[33]

An electroanalytical method was developed for the direct quantitative determination of Acyclovir (Acy) in spiked human urine base on its oxidation behavior. The electrochemical oxidation and determination of Acy were easily carried out on ultra-trace graphite electrode (UTGE) and glassy carbon electrode (GCE) using a variety of voltammetric techniques. The electrochemical measurements were carried out on these electrodes in various buffer solutions in the pH range of 3.66 to 9.08 by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The best results for the quantitative determination of Acy were obtained by the DPV technique in 0.2 M acetate buffer (pH = 4.66). In this acidic medium, one irreversible anodic peak was observed. The anodic peak current and peak potential depend on pH and the scan rate was studied. The diffusion-controlled nature of the peak was established. Acy was determined in the concentration range from 4×10^{-6} to 7×10^{-5} mol L^{-1} for UTGE and 2.0×10^{-6} to 1.0×10^{-4} mol L^{-1} for GCE by the applied electroanalytical procedure.[34]

A stripping method for the determination of acyclovir at the sub-micromolar concentration level is described. This method is based on the controlled adsorptive accumulation of acyclovir at a thin-film mercury electrode, followed by a linear cyclic scan voltammetry measurement of the surface species. Optimal experimental conditions include a NaOH solution of 2.0×10^{-3} mol L^{-1} (supporting electrolyte), an accumulation potential of -0.40 V, and a scan rate of 100 mV s^{-1}. The response of acyclovir is linear over the concentration range 0.02 to 0.12 ppm. For an accumulation time of 4 minutes, the detection limit was found to be 0.42 ppb (1.0×10^{-9} mol L^{-1}). More convenient methods to measure the acyclovir in presence of the didanosine, efavirenz, nevirapine, nelfinavir, lamivudine, and zidovudine were also investigated. The utility of this method is demonstrated by the presence of acyclovir together with adenosine triphosphate (ATP) or DNA.[35]

A novel voltammetric sensor based on glassy carbon electrode (GCE) modified with a thin film of multi-walled carbon nanotubes (MWCNTs) coated with an electropolymerized layer of iron-doped polypyrrole was developed and the resulting electrode was applied for the determination of acyclovir (ACV). The surface morphology and property of the modified electrode were characterized by field emission scanning electron microscopy and electrochemical impedance spectroscopy techniques. The electrochemical performance of the modified electrode was investigated using linear sweep voltammetry (LSV). The effect of

several experimental variables, such as pH of the supporting electrolyte, drop size of the cast MWCNTs suspension, several electro-polymerization cycles, and accumulation time was optimized by monitoring the LSV response of the modified electrode toward ACV. The best response was observed at pH 7.0 after accumulation at the open circuit for 160 s. Under the optimized conditions, a significant electrochemical improvement was observed toward the electrooxidation of ACV on the modified electrode surface relative to the bare GCE, resulting in a wide linear dynamic range (0.03–10.0 μM) and a low detection limit (10.0 nM) for ACV. Besides high sensitivity, the sensor represented high stability and good reproducibility for ACV analysis and provided satisfactory results for the determination of this compound in pharmaceutical and clinical preparations.[36]

A new selective and sensitive voltammetric procedure for the determination of acyclovir (ACV) was proposed using a disposable electrode, pencil graphite electrode (PGE). Cyclic and differential pulse voltammograms of ACV were recorded in Britton–Robinson buffer solution containing 0.10 M KCl with a pH of 4.0 at PGE. The PGE displayed a very good electrochemical behavior with significant enhancement of the peak current compared to a glassy carbon electrode (GCE). Under experimental conditions, the PGE had a linear response range from 1.0 μM to 100.0 μM ACV with a detection limit of 0.3 μM (based on 3 S_b). Relative standard deviations of 4.8 and 3.6 % were obtained for five successive determinations of 10.0 and 50.0 μM ACV, respectively, which indicate acceptable repeatability. This voltammetric method was successfully applied to the direct determination of ACV in real pharmaceutical samples. The effect of various interfering compounds on the ACV peak current was studied.[37]

The antiviral drug acyclovir had been analyzed by employing a voltammetric method that permits its quantification sensitively and reliably in pharmaceutical preparations. This investigation consisted of optimizing the voltammetric parameters and involved the chemical modification of the electrode. Among Au, Pt, and GC electrodes, the latter showed the best behavior, which was modified using electrochemical and chemical pretreatments, using 0.10 M $LiClO_4$ as supporting electrolyte, 20.0 mM pyrrole, and 1.0 mM acyclovir as templating species. Polypyrrole was then overoxidized in 0.10M phosphate buffer at 0.95 V vs Ag/AgCl. This approach enhanced the limit of quantification, stability, and sensitivity. The glassy carbon electrode coated with molecularly imprinted, overoxidized polypyrrole (OPPy) behaves as a cation exchanger due to dedoping and loss of conjugation. Square wave voltammetry allowed determining acyclovir in 0.10 M phosphate buffer, pH 4.00. The pulse height and frequency were 40 mV and 50 Hz. The limit of detection was 0.20 M. Its

quantitation in pharmaceutical preparation by multiple additions of standard, a content of (206.0 ± 4.2) mg was determined, R^2 was 0.9986; the amount of Acy was 200 mg. The voltammetric method is of easy application, less expensive, and as sensitive as HPLC.[38]

Electrophoresis

Several electrophoresis methods have been used for acyclovir analysis either as raw materials, in pharmaceutical preparations, or biological matrices. Some of these methods will be discussed below.

The separation of acyclovir (ACV) by high-performance capillary electrophoresis (HPCE) with on-column amperometric detection using α-amino-5-mercapto-3,4-dithiazole (AMD) as internal standard is described. The calibration line was linear in the range of 0.5—20 mg/L of ACV. The detection limit was 0.15 mg/L of ACV. Its recovery ranged from 98 to 101 % with relative standard deviations (RSDs) from 1.9 to 3.2 % (*n* = 5). This method was successfully used for determining ACV in some pharmaceuticals and human urine. Comparable results with HPCE with ultraviolet (UV) detection and amperometric detection were obtained.[39]

Two Capillary Zone electrophoresis (CZE) methods were developed, one using an acidic buffer (sodium citrate pH = 2.5) and the other using a basic buffer (sodium tetraborate pH = 9.8). The two methods were compared based on repeatability and reproducibility of results and the CZE method developed with the basic buffer was then selected for further studies. The method was fully validated in terms of repeatability [RSD for migration time and peak area of acyclovir at 0.05 mg (nominal concentration) were 0.3-1.0 % (x=10), and 1.5-2.6 % (n=3), respectively], reproducibility (RSD of peak area = 2.54 %, n = 6), linearity over two ranges of acyclovir concentration (0.01-0.07 and 0.05-0.3 mg/mL which resulted in y = 2.007x + 1.300 and y = 0.234x + 0.82, respectively) , limits of detection and quantitation (1×10-4 mg/mL and 3×10^{-4} mg/mL, respectively), ruggedness and robustness. The method was applied for the determination of the drug in a commercial tablet preparation (mean recovery value 100.2% w/w) and a commercial injection solution. The method proved to be fast and reliable for the quantitative analysis of acyclovir and its related substance in bulk and pharmaceutical dosage forms.[40]

A simple MEKC with UV detection at 254 nm for analysis of acyclovir in plasma and in cerebrospinal fluid (CSF) by direct injection without any sample pretreatment is described. The separation of acyclovir from the biological matrix was performed at 25 °C using a BGE consisting of Tris buffer with SDS as the electrolyte solution. Several parameters affecting the separation of the drug from the biological matrix were studied, including the pH and

concentrations of the Tris buffer and SDS. Using dyphylline as an internal standard, the linear ranges of the method for the determination of acyclovir in plasma and CSF all exceeded the range of 2–50 μg/mL; the detection limit of the drug in plasma and CSF (S/N = 3; injection 3.45 kPa, 5 s) was 1.0 μg/mL. The applicability of the proposed method for determination of acyclovir in plasma and CSF collected at 8 h after intravenous administration of 500 mg acyclovir (Zovirax®) in two patients with herpes simplex encephalitis was demonstrated.[40]

A micellar electrokinetic chromatography (MEKC) method for the simultaneous determination of the antiviral drugs acyclovir and valacyclovir and their major impurity, guanine, was developed. The influences of several factors (surfactant and buffer concentration, pH, applied voltage, capillary temperature, and injection time) were studied. Using tyramine hydrochloride as an internal standard, the analytes were all separated in about 4 min. The separation was carried out in reversed polarity mode at 28 °C, 25 kV, and using hydrodynamic injection (15 s). The separation was effected in a fused-silica capillary 100 μm × 56 cm and a background electrolyte of 20 mM citric acid–1 M Tris solution (pH 2.75), containing 125 mM sodium dodecyl sulfate and detection at 254 nm. The method was validated to linearity, the limit of detection and quantification, accuracy, precision, and selectivity. Calibration curves were linear over the range 0.1–1 μg/mL (guanine) and from 0.1 to 120 μg/mL for both valacyclovir and acyclovir. The relative standard deviations of intra- and inter-day migration times and corrected peak areas were less than 5.0 %. The proposed method was successfully applied to the determination of the analytes in tablets and creams. From the previous study, it is concluded that the stability-indicating method developed for acyclovir and valacyclovir can be used for the analysis of the drug in various stability samples.[41]

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Flow Injection

Some flow injection methods have been used for acyclovir analysis either as raw materials, in pharmaceutical preparations, or biological matrices. Some of these methods will be discussed below.

A new simple, rapid, and cost-effective flow injection (FI) electrochemiluminescence (ECL) method was described for the determination of acyclovir (9,2-hydroxyethoxy) methyl guanine. In the presence of acyclovir, the luminol electrochemiluminescence generated by a mini on-line galvanic cell could be greatly sensitized in an alkaline medium. The relative electrochemiluminescence intensity was found to increase linearly with increasing concentration of acyclovir, which was corroborated by the calculated correlation coefficient value of 0.9994 (n = 7). The limit of detection was 1.6×10^{-7} mol L^{-1} and the limit of quantification was 7.9×10^{-7} mol L^{-1}. The proposed method was applied to the determination of acyclovir in pharmaceutical formulations. The reliability of the assay method was established by parallel determination and by the standard-addition method. Experiment results demonstrated the described mini analysis system while being simple and less time consuming, was accurate, precise, and reproducible (R.S.D. = 1.6 %, recoveries = 99 – 103 %). Further experiments indicated that there was no significant difference between the results obtained by the proposed and official methods.[42]

A rapid and sensitive flow-injection chemiluminescence (FI-CL) method, which is based on the CL intensity that generated from the redox reaction of Ce(IV)-rhodamine B in H_2SO_4 medium, for the determination of acyclovir and gancyclovir is described. For acyclovir, the determination range is 3×10^{-8} g mL^{-1} – 7×10^{-5} g mL^{-1}, with 1.56×10^{-8} g mL^{-1} as its determination limit. During 11 repeated measurements for 1×10^{-6} g mL^{-1} acyclovir, the relative standard deviation was 2.08%. For gancyclovir, the determination range was 5×10^{-8} g mL^{-1} – 7×10^{-5} g mL^{-1}, with 2.35×10^{-8} g mL^{-1} as its determination limit. The relative standard deviation is 2.83% with 11 repeated measurements of 1×10^{-6} g mL^{-1} gancyclovir. This method can be successfully used to determine the content

of acyclovir and gancyclovir in injections, acyclovir in eye drops, and, maybe, also for other ciclovirs.[43]

The chemiluminescence (CL) reaction of acyclovir (ACV)–potassium permanganate, with formaldehyde as an enhancer, was investigated by the flow-injection system, and a new method is reported for the determination of ACV based on the reaction. The method is rapid, effective and simple for the determination of acyclovir in the range 0.2–80 mg/L, with a limit of detection of 0.06 mg/L (3 S: N), a relative standard deviation (RSD) of 3.7 % for the determination of 1.0 mg/L acyclovir solution in 11 repeated measurements. The method has been applied to the determination of acyclovir in pharmaceuticals, with satisfactory results.[44]

Thin Layer Chromatography (TLC)

A simple and rapid TLC method has been developed for the determination of acyclovir in pharmaceutical preparations. After extraction of the analyte with a 9:1 (v/v) mixture of 96 % alcohol and 0.05 M H_2SO_4 the extracts were applied to precoated silica gel TLC plates which were then eluted with n-butanol–glacial acetic acid–water, 15 + 9 + 6 (v/v). Quantitative evaluation was performed by measuring the absorbance–reflectance of the analyte spots at λ = 277 nm. This TLC–densitometric method is selective, precise, and accurate and can be used for routine analysis of pharmaceutical preparations in pharmaceutical industry quality-control laboratories.[45]

CONCLUSION

Overall, various analytical methods have been used to determine acyclovir levels. Spectrophotometry, voltammetry, capillary electrophoresis, flow injection, and thin-layer chromatography methods are simple and easy to apply. However, the HPLC analysis method is often used in research because it can detect samples with low concentrations. The HPLC method combined with mass spectrometry has specific advantages and sensitivity for acyclovir analysis in biological matrices.

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