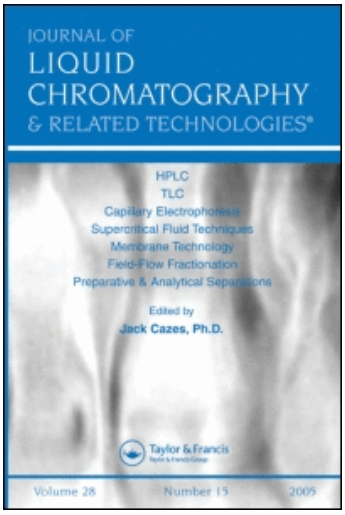

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Validation of an HPLC Method for the Determination of Valacyclovir in Pharmaceutical Dosage

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Abstract: A new analytical method was developed together with its validation study, by means of a high resolution liquid chromatography (HPLC) of reverse phase to quantify valacyclovir L-Valine, ester with 9-[(2-hydroxyethoxy)methyl]guanine hydrochloride in tablets. Determination was carried out by means of an ODS C_{18} column (Microsorb-MV^{TM}100 A, 10 μm, 25 cm × 4.6 mm); the mobile phase consisted of acetic acid in water (1 : 1000) : methanol (70 : 30). It was pumped through the chromatographic system at a flow rate of 1.0 mL/min.

The UV detector was operated at 254 nm. The validation study was carried out fulfilling the ICH guidelines in order to prove that the new analytical method meets the reliability characteristics, and these characteristics show the capacity of an analytical method to keep, throughout the time, the fundamental criteria for validation: selectivity, linearity, precision, accuracy, and sensitivity. The method is applied during the working day for the quality control of commercial valacyclovir tablets in order to quantify the drug and to check the uniformity content test.

Keywords: Valacyclovir, Acyclovir, HPLC method, stability indicating

INTRODUCTION

Valacyclovir, L-Valine, ester with 9-[(2-hydroxyethoxy)methyl]guanine hydrochloride, is a prodrug of the antiviral acyclovir that is used in the

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treatment of herpes zoster and herpes simplex infections of the skin and mucous membranes, including genital herpes. It is given by mouth as the hydrochloride doses, which are expressed in terms of valacyclovir[1] (Figure 1).

Because of the limited oral bioavailability of acyclovir (only 20%), it was replaced by its prodrug, valacyclovir, in the oral treatment of herpes simplex and varicella zoster virus infections.[2]

Some analytical methods for the analysis of valacyclovir in biological fluids and *in vitro* studies have been described.[2,3]

The target of this study is to develop a new, simple, and fast analytical method by HPLC to quantify valacyclovir and its degradation product in a final product, together with its latter validation study. The method was validated following the analytical performance parameters suggested by ICH.[4]

EXPERIMENTAL

Equipment

The HPLC system consisted of a dual piston reciprocating pump (KNK-500 G model), an UV-Vis detector (KNK-029-757 model), an integrator (SP 4600 model) (all from Konik), and a Rheodyne injector (7125 model).

Chemicals and Reagents

Methanol used in the mobile phase was HPLC grade. Acetic acid was AR grade. Solutions and mobile phase were prepared just before use and all solvents, distilled water, and solutions for HPLC analyses were filtered through a Micron Separations N04SP04700 nylon membrane filter (pore size 0.45 μm) and vacuum degassed before use.

Valacyclovir hydrochloride was kindly donated by Laboratorios Kampel Martian (Argentina) and used as working standard without further purification.

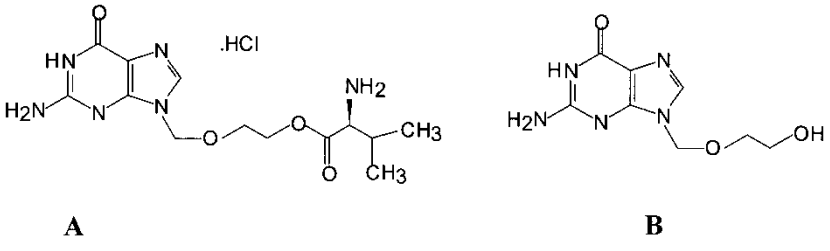

Figure 1. Structures A Valacyclovir, B Acyclovir.

A commercial local tablet formulation was used in this study. Its composition was: Valacyclovir hydrochloride 556.3 mg in a matrix of: microcrystalline cellulose, lactose, colloidal silicon dioxide, crospovidone, povidone, and magnesium stearate.

Chromatographic Conditions

Chromatographic separation of the active, related substance (synthesis impurity) and its degraded products was performed using a Variant Microsorb-MV^{TM}100 A, 10 μm, 25 cm × 4.6 mm, made of stainless steel. The mobile phase chosen was acetic acid in water (1 : 1000) : methanol (70 : 30). All analyses were performed under isocratic conditions at a 1.0-mL/min flow rate, and 12-min run time, at room temperature. In these conditions acyclovir retention time (t_R) was roughly 2 minutes and valacyclovir hydrochloride retention time was 9 minutes. Detector sensitivity was set at 2 a.u.f.s. and eluents monitored at 254 nm. The volume of each injection was 50 μL.

Preparation of Solutions

Solutions were prepared on a weight basis and volumetric flasks used as suitable containers in order to minimize solvent evaporation.

Reference stock solution of valacyclovir was prepared at a concentration of 1 mg/mL, dissolving the appropriated amount of raw material in water. The reference preparation was obtained by diluting the reference stock solution with water to yield a concentration of 100 μg/mL.

This reference solution will be used to quantify the active ingredient in the final product. Based on this solution and by means of an adequate dilution for acyclovir, a reference stock solution of 0.1 mg/mL was prepared in 0.1N OHNa. The reference preparation was obtained by diluting the reference stock solution with water to yield a concentration of 1 μg/mL.

Resolution Solution

Ten milliliters of valacyclovir reference stock solution and one milliliter of acyclovir reference stock solution were transferred into a 100 mL volumetric flask and diluted to volume with water.

Procedure

Prior to injecting solutions, the column should be equilibrated for at least 30 minutes with mobile phase flowing through the system. Quantitation was

accomplished by using an external standard method. Each solution was injected in triplicate and the relative standard deviation (RSD) was required to remain below 1.5% on a valacyclovir hydrochloride peak area basis. Standards were interspersed with the samples if a large number of analyses were to be performed.

Validation Study

Specificity

A study was carried out to check the absence on interference by the excipients, which take part in the pharmaceutical preparation (placebo solution).

Within the study of specificity, a series of degradation studies were carried out where the samples were subjected to different degrees of stress. Samples were prepared by transferring approximately 50 mg of sample into 50 mL volumetric flasks. Intentional degradation was attempted using 10 mL of HCl 1N, NaOH 1N, H_2O_2 100 vol, water and refluxing for at least 15 minutes. Valacyclovir hydrochloride was subjected to thermal (in an open container in an oven at 110°C, 24 h) and photochemical degradation (a solution was transferred in a container exposed to daylight for 24 h). After degradation treatments were completed, samples were allowed to cool to room temperature and diluted to the same concentration as the standard preparation after being neutralized with acid-base, if required. After the stress assays, the samples were analyzed as shown in the chromatographic conditions.

Linearity

To carry out this study for valacyclovir, six levels of concentration within the range 40–160% of the work-concentration (100 μg/mL) were prepared. The standard curve for the assay of acyclovir covered the range of concentrations from 50–150% of the work-concentration (1 μg/mL). Each of the levels of concentration were injected in triplicate. The experimental results were represented graphically, obtaining a calibration graph and carrying out the corresponding statistic study.

Precision

For the precision study three different test were carried out. The first one consisted of checking instrumental system precision, where a sample corresponding to a concentration of 100 μg/mL was injected 6 times, consecutively, into the chromatograph. The second test consisted of checking the

precision of the method, six individual samples were prepared and the relative standard deviation (R.S.D.) was studied for the response factor obtained. Lastly the intermediate precision was studied. The samples were prepared according to the precision of the method and studying the variability, which takes place when different analysts work on different days with the same lot of one commercial formulation.

Accuracy (Recovery Method)

The recovery method was studied at concentration levels of 80%, 100%, and 120% (three samples each). Twenty tablets from the same lot of a commercial formulation were weighed and finely powered. The amount of valacyclovir recovered in relation with the results obtained in the intermediate precision study were calculated.

RESULTS AND DISCUSSION

System Suitability

The analytical column was equilibrated with the eluting solvent system used. After an acceptable stable baseline was achieved, the standards and then the samples were analyzed. System suitability results were calculated according to the USP 27 <621> from typical chromatograms.[5] Instrument precision, as determined by six successive injections of the standards preparations, should provide a relative standard deviation (RSD) below 1.0% for both drugs. Peak asymmetry or tailing factor, T, was calculated as $T = W_{0.05}/2f$; where $W_{0.05}$ in the distance from the leading edge to the tailing edge of the peak, measured at 5% of the peak height from the baseline and f is the distance from the peak maximum to the leading edge of the peak. The tailing factor did not exceed 1.8. The resolution between valacyclovir and acyclovir should be greater than 2.4 (Figure 2).

Validation study

Specificity

Typical chromatograms from valacyclovir and acyclovir are shown in Figure 2.

Forcing degradation of valacyclovir was used to demonstrate the stability indicating properties of the method. Degradation was indicated in the stressed sample by a decrease of the expected value of the drug and increased levels of

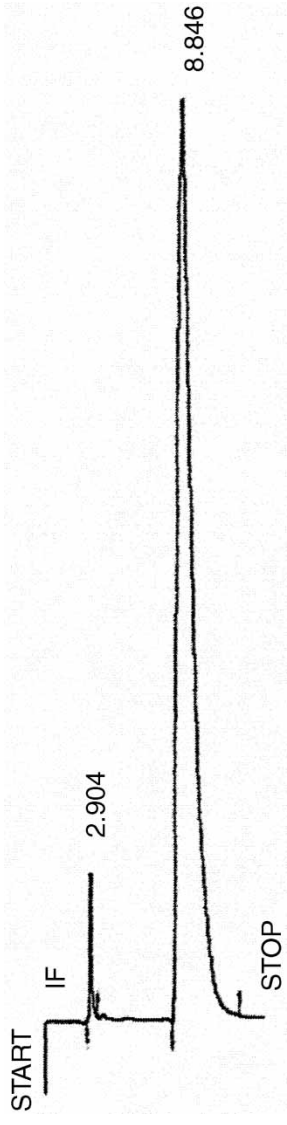

Figure 2. Typical chromatogram of valacyclovir and acyclovir.

degradation products. The results of the stress study are presented in Table 1. To conclude, it can be stated that none of the peaks that could be generated by the stress treatment interfere with the peak corresponding to the active, therefore showing it was a selective method and suitable for routine work (Figure 3).

Table 1. Selectivity: degradation conditions of valacyclovir

Condition	Time (h)	Recovery (%)	RRT of degradation products
Heat dry, 110°C	24	94.2	0.34; 0.43; 0.56
Water, refl.	0.5	84.5	0.25; 0.33; 0.40; 0.47
Day light	24	95.1	0.34
Acid 1N HCl, refl.	0.25	39.6	0.24; 0.30
Base 1N NaOH refl.	0.25	1.8	0.24; 0.30
Hydrogen peroxide 100 vol, refl.	0.5	89.9	0.33

Ref, refluxed.
RRT, relative retention time.

Range

Assay method range was set at 80 to 120% of the finished product label claim, since the method proved precise, accurate, and linear within these limits.

Linearity

The equation of the regression curve obtained (with all the values) for valacyclovir relating to the tested concentrations and the response obtained correspond to $Y = 11111388 + 362481X$, with a standard error ($S_{x,y}$) of 928143 and a correlation coefficient (r) of 0.9995, while intercept values were not significantly different from zero, ($p = 0.05$) (Table 2) (Figure 4). The equation of the regression curve obtained for acyclovir relating to the tested concentrations and the response obtained correspond to $Y = 15423980 + 171636X$ and a correlation coefficient (r) of 0.9974, while intercept values were not significantly different from zero, ($p = 0.05$) (Figure 5).

Precision

In the study of the instrumental system precision a R.S.D. of 0.4% for the area was obtained.

The precision of the method ($n = 6$ analyses), the inter-day study carried out, showed a R.S.D. of 1.0% for the area obtained. In all these cases the R.S.D. obtained was below 1%, the limit percentage set for the precision

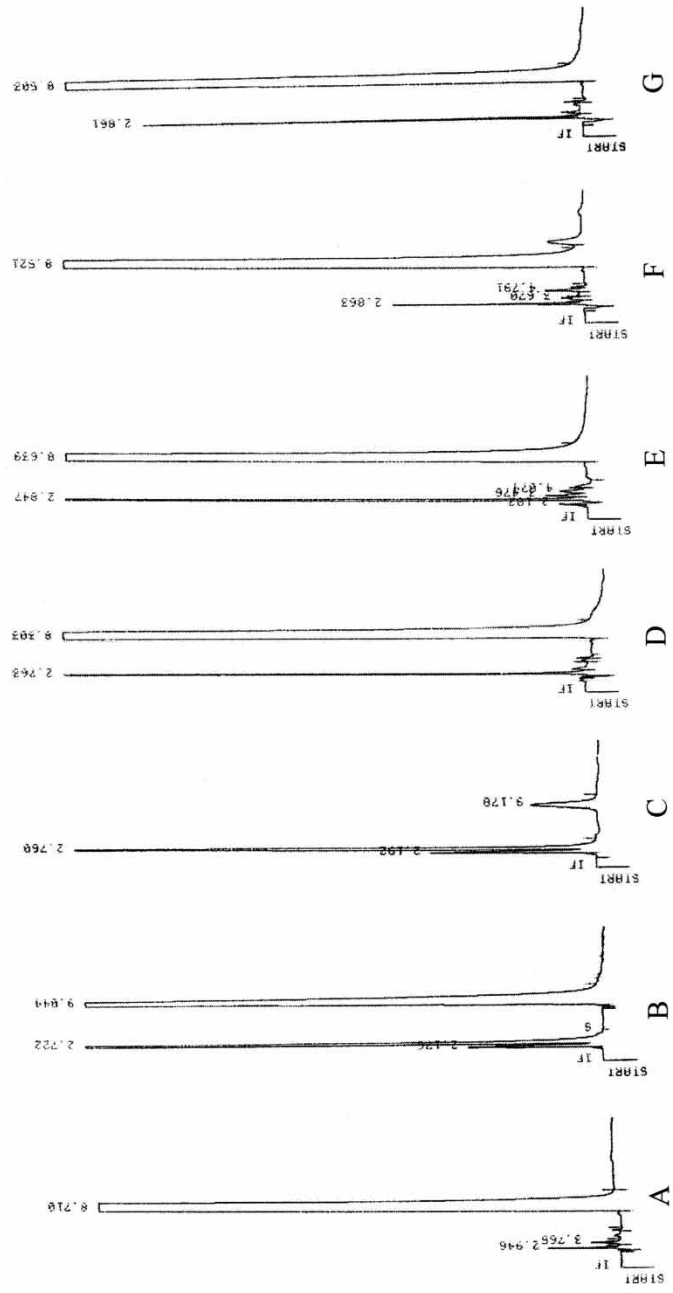

Figure 3. Chromatograms of valacyclovir. (A) valacyclovir standard, (B) acid degradation, (C) alkaline degradation, (D) oxidative degradation, (E) water degradation, (F) heat dry degradation and (G) photolysis.

Table 2. (a) Linearity data of valacyclovir. (b) Linearity data of acyclovir

% w/w	Injected (μg)	Average peak area response	RSD (%)
(a)			
40	1.996	23,118,181	0.3
60	2.994	33,342,011	0.4
80	3.992	45,145,547	0.3
100	4.990	55,197,877	0.3
120	5.988	65,948,096	0.5
160	7.984	89,919,787	1.0
Slope[a]	11,111,388 ± 534,460		1.7
Intercept[b]	362,481 ± 2,702,283		
(b)			
50	0.026	581,390	0.8
100	0.051	925,372	0.6
150	0.077	1,368,013	0.3
Slope[a]	15,423,980 ± 14,191,057		
Intercept[b]	171,635 ± 781,732		

[a]Confidence limits of the slope (p = 0.05).
[b]Confidence limits of the intercept (p = 0.05).
RSD, relative standard deviation.
Valacyclovir: $Y = 1.11 \times 10^7 X + 3.62 \times 10^5$.
Acyclovir: $Y = 1.54 \times 10^7 X + 1.71 \times 10^5$.

study of the instrumental system, thus showing that the equipment used for the study worked correctly for the developed method, and was highly repetitive.

For the intermediate precision, a study was carried out by the same analyst working on different days (n = 6 number of analysis per day). The results were given both individually and as a whole. For each precision assay the results were as follows: mean values 99.3 and 99.8%, standard deviations 1.0 and 1.4, and R.S.D 1.0% and 1.4%. Test "*t*" comparing two sample with 95% confidence for 10 degrees of freedom disclosed that both results were not significantly different, inter se ($t_{n-2, \alpha:0.05}$) = 2.23 (Table 3).

Accuracy

The results obtained for the accuracy study (recovery method) from 9 samples studied (n = 3 for 80%, 100% and 120%) indicated that the mean

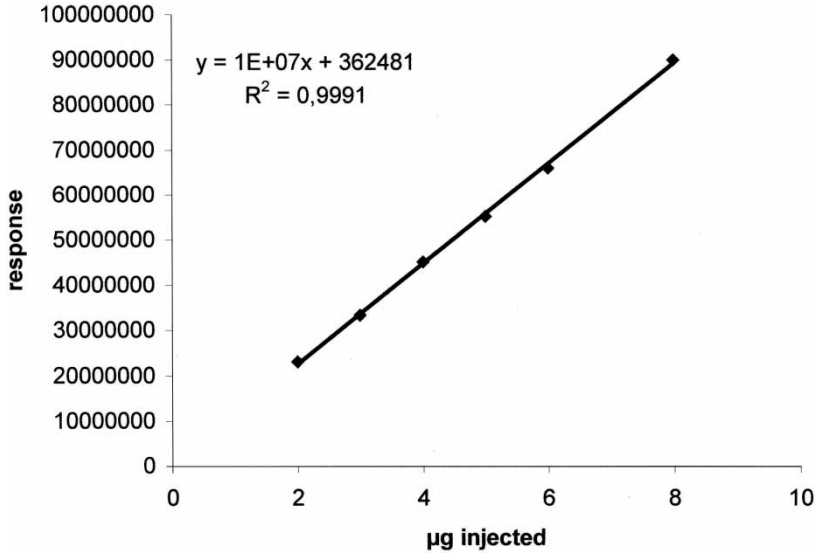

Figure 4. Peak area response versus μg injected of valacyclovir.

recovery was 99.2% and R.S.D. was 1.5%. The experimental t of the recovery percentage of which the value was 1.6, being far below the 2.306 established in the tabulated t (95% level of probability, 8 d.f), was also studied (Table 4).

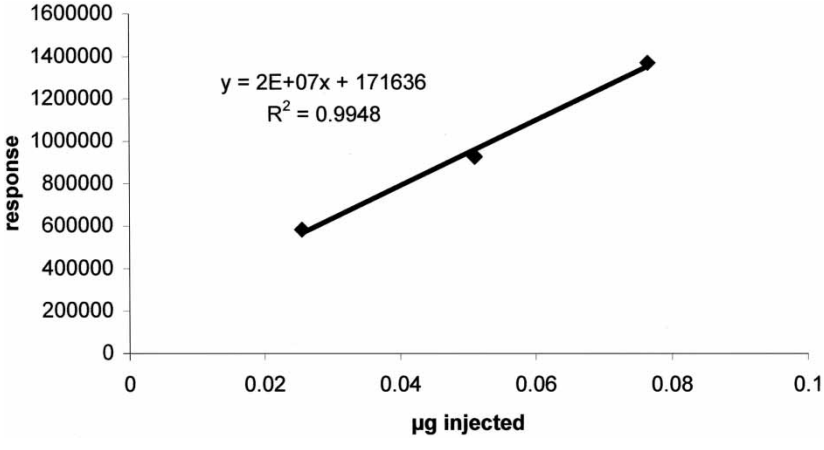

Figure 5. Peak area response versus μg injected of acyclovir.

Table 3. Precision of the assay method

	Analyst 1		Analyst 2	
Sample No.	Amount determined (%)	RSD	Amount determined (%)	RSD
1	97.6	0.5	100.6	0.3
2	99.7	0.4	102.0	0.6
3	100.5	0.4	98.4	0.8
4	99.0	0.0	99.5	0.1
5	99.4	0.1	98.2	0.5
6	99.5	0.7	100.0	0.7
Means	99.3	1.0	99.8	1.4

CONCLUSION

The method proposed by HPLC to determine valacyclovir in tablets has been proven in a linear, precise, accurate, and selective manner to be applied in routine and in quality control of valacyclovir tablets. It has been proven that it was selective, linear between 40 and 160% of the work concentration (100 μg/mL) for valacyclovir and between 50 and 150% of the work concentration (1 μg/mL) for acyclovir, with a correlation coefficient higher than 0.9995 for valacyclovir, precise, accurate, and robust regarding the wavelength, flow rate, mobile phase, and injection volume.

Table 4. Recovery analysis

% w/w	Amount added (mg)	Amount determined (mg)	Amount recovered (%)	Average recovered (n = 3)	RSD (%)
80	80.04	80.51	100.6	100.5	1.1
	80.63	81.94	101.6		
	79.40	78.81	99.3		
100	99.83	98.92	99.1	98.5	0.8
	99.83	98.70	98.9		
	101.31	98.85	97.6		
120	119.21	119.51	100.2	98.5	1.5
	120.10	116.98	97.4		
	120.80	118.19	97.8		
Overall recovery (n = 9)				99.2	1.5

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