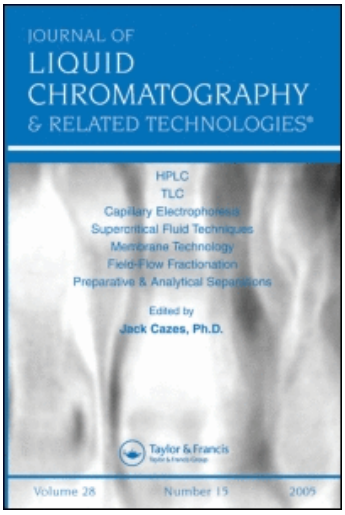

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Development and Validation of an RP-HPLC Method for the Determination of Valacyclovir in Tablets and Human Serum and Its Application to Drug Dissolution Studies[#]

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ABSTRACT

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

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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.

Key Words: Valacyclovir; HPLC; Determination; Pharmaceuticals; Human serum; Dissolution profile.

INTRODUCTION

Valacyclovir (VACL) is the *L*-valy ester prodrug of acyclovir. Valacyclovir is used for the treatment of the herpes simplex viruses and the varicella zoster virus. Valacyclovir is converted rapidly, and virtually completed, to acyclovir after oral administration in healthy adults. This conversion is thought to result from first-pass intestinal and hepatic metabolism through enzymatic hydrolysis. The relative bioavailability of acyclovir increases three-to-five fold to approximately 50% following VACL administration. Therefore, it is suitable to know the dissolution rate of VACL from the tablets for predicting in vivo drug bioavailability behavior.[1,2]

The determination of dissolution profiles for solid dosage forms of pharmaceuticals is required by pharmaceutical factories and research institutes. Dissolution testing is performed under precisely specified conditions of temperature, volume, and stirring rate, which may mimic processes in the human gastrointestinal tract. Because of differences in the content uniformity among samples of pharmaceutical formulations, parallel dissolution tests (at least six samples per batch) are required.

Typically, such assays are performed by drawing filtered aliquots of the sample, followed by determination by HPLC or spectrophotometric measurements. Drug dissolution testing is an integral part of pharmaceutical development and routine quality-control monitoring of drug release characteristics. The in vitro dissolution profiles obtained from dissolution rate studies, have also been used for the successful characterization of the in vivo behavior of drugs.[3–7] Among all tests that can be performed on drug solids, dissolution rate study is considered to be sensitive, reliable, and rational for predicting in vivo drug bioavailability behavior. Very few methods for the assay of VACL from the biological samples have been reported.[8–10] All of these methods

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involve time-consuming separation, which complicates routine analysis. It is of interest to mention, that the dissolution rate studies and related kinetic parameters of VACL and determination from tablet dosage forms of this compound, have not yet been reported.

This paper describes the development of the method that meets the proposed aim; a reliable, reproducible, fully validated, easy to perform, and low cost routine RP-HPLC method for the determination of VACL in raw material, pharmaceutical dosage forms, and human serum samples. The method appears to be suitable for quality control in the pharmaceutical industry and the therapeutic monitoring of levels of VACL in serum samples, due to its simplicity, sensitivity, selectivity, and lack of excipients and interference of endogeneous substances.

The developed method was also successfuly applied to the in vitro dissolution rate studies for tablet dosage forms.

EXPERIMENTAL

Apparatus

A HP chromatographic system (Hewlett Packard, Avondale, USA) consisting of a Model Agilent 1100 series with a Model Agilent series G-13158 DAD detector and a Model Agilent 1100 series G-1329 ALS auto sampler were used. The chromatograms were analyzed with Agilent Technologies HPLC 1100 software. The separation was carried out at ambient temperature, on a reversed-phase Waters spherisorb column (250×4.6 mm; 5 μm particle size). The chromatographic separation was performed using an isocractic mode.

The dissolution rate studies of VACL from tablets were performed on Caleva 7ST dissolution apparatus (G.B. Caleva Inc., UK).

Chromatographic Conditions

The mobile phase consisted of a mixture of acetonitrile : methanol : 0.067 M KH_2PO_4 (27 : 20 : 53, v/v/v) adjusted to pH 6.5 with 3 M NaOH and delivered at a flow rate of 0.75 mL min^{-1}. The UV detector was set at a wavelength of 244 nm. An injection volume of 20 μL was used. Etodolac was used as an internal standard.

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Chemicals and Reagents

Valacyclovir and its pharmaceutical dosage forms were kindly provided by Glaxo-Smith-Kline Pharm.Ind. (Istanbul, Turkey) and internal standard Etodolac was kindly supplied from Mustafa-Nevzat Pharm.Ind (Istanbul, Turkey).

Methanol and acetonitrile were of HPLC grade, purchased from Merck (Darmstadt, Germany). All other chemicals were of commercial analytical reagent grade. Doubly distilled water was used for preparing mobile phase solutions.

Standard Solutions and Calibration Curves

The standard VACL and internal standard (IS) etodolac were prepared by dissolving 10 mg of compounds with 10 mL of mobile phase in a 10 mL volumetric flask. The concentration of VACL was varied in the range of 5–20,000 ng mL^{-1} and the concentration of IS was maintained at a constant level of 5000 ng mL^{-1}. The calibration curve for HPLC analysis was constructed by plotting the ratio of the peak area of the drug to that of internal standard against the drug concentration.

The ruggedness and precision were checked at different days; within day ($n = 5$) and between days ($n = 5$) for two different concentrations. The relative standard deviations were calculated to check the ruggedness and precision of the method.

Analysis of Tablets

Ten tablets were weighed, crushed, and combined. An amount of powder equivalent to about 10 mg VACL was accurately weighed, transferred into a 10 mL volumetric flask, diluted with mobile phase, sonicated for 10 min, and then completed to volume with the same solution. After filtration, appropriate solutions were prepared by taking suitable aliquots of clear filtrate and adding the appropriate IS solution, diluting them with mobile phase in order to obtain a final solution.

The amount of VACL per tablets was calculated from the related linear regression equation.

Recovery Studies

In order to establish the reliability and suitability of the proposed method, recovery experiments were carried out by the standard addition method. The known amounts of the pure drug and internal standard, at a constant level,

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were added to VACL pharmaceutical formulation and the mixtures were analyzed by the proposed method. After four repeated experiments, the recoveries were calculated.

Recovery Studies in Spiked Human Serum Samples

After gentle thawing 1 mL aliquots of serum samples were spiked with 100 μg mL^{-1} of VACL and (dissolved in mobile phase), 1 mL methanol (for precipitation of proteins). The tubes were vortexed for 5 min and then centrifuged for 5 min at 5000 *g*. The supernatant was taken carefully. The concentration of VACL was varied in the range of 5–20,000 ng mL^{-1} in human serum samples and the concentration of IS was maintained at a constant level of 5000 ng mL^{-1}. Serum samples were injected into the column. The amount of VACL in spiked human serum samples was calculated from the related linear regression equation.

In Vitro Dissolution Studies

Drug dissolution studies were carried out according to the USP 24[11] dissolution procedure for the single entity products, with use of a paddle-stirrer type of apparatus in 900 mL of 0.1 M HCl (pH 1.2, gastric medium), at a stirring rate of 75 rpm. The temperature of the cell was maintained at $37 \pm 0.5^{\circ}C$ by use of a thermostatic bath. At each sample time interval, an exact volume of sample was withdrawn from each flask and immediately replaced with an identical volume of fresh medium. At predetermined time intervals (5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 90, 120 min) the concentrations of VACL in the dissolution medium were determined from linear regression equations of the proposed method.

RESULTS AND DISCUSSION

Initial experiments were carried out by using a mobile phase comprising of acetonitrile, methanol and 0.067 M KH_2PO_4 in different proportions and at different pH values.

Mobile phase composition of acetonitrile : methanol : 0.067 M KH_2PO_4 (27 : 20 : 53, v/v/v) adjusted to pH 6.5 was finally optimized to give retention times of 5.13 and 7.89 min for VACL and IS, respectively. This mobile phase composition was found to be optimal for good peak shape, as well as to achieve minimal background current.

For HPLC methods, precision and accuracy can often be enhanced by the use of an appropriate internal standard, which also serves to correct for

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fluctuations in the detector response. The chemical structure of etodolac is not similar to VACL structure. However, it was chosen as the internal standard because it showed a shorter retention time with better peak shape and better resolution, compared to other potential internal standards. For both compounds, sharp and symmetrical single peaks were obtained with good resolution.

System suitability tests are an integral part of HPLC methods.[11] System suitability was checked by evaluating different parameters (retention time, tailing factor, capacity factor, resolution, and selectivity). System suitability tests were carried out on freshly prepared standard stock solutions of VACL. Tailing and capacity factors were obtained as 1.16 and 2.42 for VACL and 1.04 and 4.26 for IS, respectively. Resolution and selectivity factors for this system were 4.80 and 1.76, respectively. The retention times of VACL standard sample, tablets, and serum samples were 5.13, 5.14, and 5.22 min, respectively. The variation in retention time among five replicate injections of VACL reference solution was very little in raw material, tablets, and serum samples, giving relative standard deviations (RSD%) of 0.61%, 0.93%, and 0.66%, respectively. Linearity of response was studied by running the standard curve of VACL.

The plot of peak area ratio vs. VACL concentration in mobile phase and spiked serum samples were found to be linear in the concentration range 5–20,000 ng mL^{-1}. The correlation coefficient was found to be always greater than 0.999 for both media. Table 1 shows the calibration characteristics and validation parameters of VACL.

Accuracy, precision, and reproducibility of the proposed method were assessed by performing replicate analysis of the standard solutions. Two different concentrations within calibration range were prepared in mobile phase and serum samples, and analyzed with related calibration curves to

Table 1. Characteristics of the linear regression analysis of VACL.

	Mobile phase	Human serum
Linearity range (ng mL^{-1})	5–20,000	5–20,000
Slope	3.22×10^{-4}	3.26×10^{-4}
Intercept	-8.08×10^{-4}	−0.013
Correlation coefficient (r)	0.999	0.999
RSD% of slope	0.82	0.47
RSD% of intercept	0.62	0.77
Detection limit (ng mL^{-1})	0.38	0.14
Quantification limit (ng mL^{-1})	1.25	0.47

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Table 2. Intra-day and inter-day precision of VACL standards.

Theoretical concentration (ng/mL)	In mobile phase				In serum samples			
	Intra-day measured concentration (ng/mL)[a]		Inter-day measured concentration (ng/mL)[b]		Intra-day measured concentration (ng/mL)[a]		Inter-day measured concentration (ng/mL)[b]	
	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%
100	99.66	0.75	98.30	1.37	99.94	1.12	100.13	1.35
750	749.87	0.98	747.00	1.29	750.61	0.49	745.91	0.71

[a]Mean values represent five different VACL standards for each concentration.
[b]Between-day reproducibility was determined from five different runs over a two weeks period.

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determine intra-day and inter-day variabilities. The intra and inter-day precision were determined as the RSD%. Precision, accuracy, and reproducibility results shown in Table 2 demonstrate good precision, accuracy, and reproducibility.

The stability studies of VACL in mobile phase indicated no significant changes in sample concentrations upon storage of samples for one week at 4°C in refrigerator.

On the basis of above results, the proposed method was applied to the direct determination of VACL content in marketed product. The assay showed the drug content of this product to be accordance to the labeled claim. The mobile phase resolved the two compounds very efficiently into two distinct sharp peak of VACL and etodolac from the tablets as shown in Fig. 1.

In order to check the accuracy and precision of the developed method, the recovery studies were carried out using by standard addition technique. The results obtained from the analysis of tablets and their recovery studies are summarized in Table 3. This recovery result shows that there is no interference from the excipients present in the tablets.

In order to check the applicability of the proposed method to the human serum samples, the linearity range studies and recovery studies were

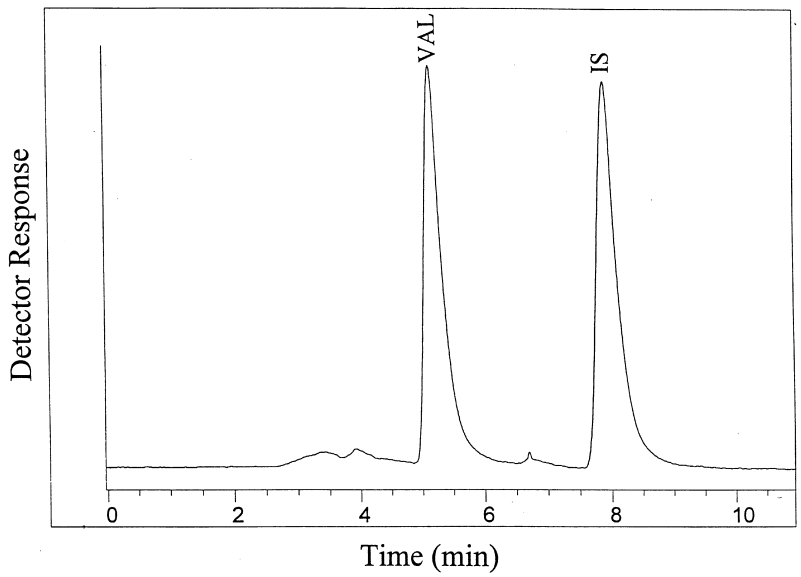

Figure 1. Chromatogram obtained from tablet dosage form containing 1000 ng mL^{-1} VACL and 5000 ng mL^{-1} etodolac (IS).

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Table 3. Results of the assay and the recovery analysis of VACL in tablets and spiked human serum samples.

	Tablets (mg)	Serum samples ($ng\,mL^{-1}$)
Labeled claim (mg per tablets)	500	
Mean of amount found[a]	494.91	
RSD% of amount found	0.61	
Added	20.0	50.00
Recovered[b]	19.77	49.41
Recovery%	98.84	98.81
RSD% of recovery	0.74	1.35

[a]Each value is the mean of six experiments.
[b]Each value is the mean of four experiments.

performed in human serum samples. Calibration graph parameters were shown in Table 1. Necessary validation parameters and recovery study results for human serum samples were given in Table 2 and Table 3. Figure 2(a) shows a typical chromatogram of an extract of fresh blank serum sample and Fig. 2(b) shows a chromatogram obtained when the method was applied to spiked human

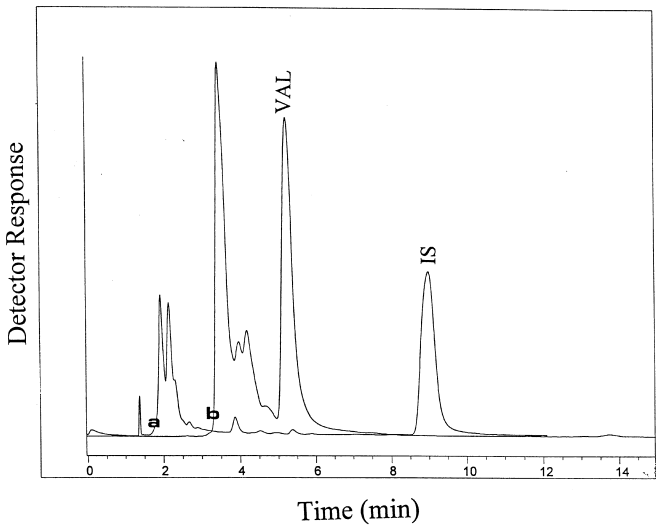

Figure 2. Chromatogram of blank serum (a) and serum spiked with 10,000 $ng\,mL^{-1}$ VACL and 5000 $ng\,mL^{-1}$ etodolac (IS) (b).

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serum samples. There are no extraneous peaks in chromatograms obtained for serum samples.

Further, the developed method was used for the quantitative determination of VACL in in-vitro dissolution test samples obtained during the drug release studies of tablets. It is essential to consider the in-vitro dissolution tests as an important criteria for the quality of the pharmaceutical dosage formulations if obtained from various sources and can judge the suitability of this formulation to deliver the required active substance properly to the patient.

The release rate profile was drawn as the percentage drug dissolved from the tablet vs. time. As can be seen from Fig. 3, more than 90% drug dissolved in media within 30 min. The dissolution data were fitted according to the different models, namely zero order, first order, Hixson-Crowell, Weibull distribution (RRSBW)[12,13] function, and Peppas equation.[14–16] All the kinetic and their related rate constants and parameters are summarized in Table 4. In order to understand the magnitude of the diffusional exponent n of drug from tablets, dissolution data were fitted to the Peppas equation where n is a factor, which indicates the mechanism of the release. For instance $n : 0.5$ for square root of time and $n : 1$ for zero order release. The values of n being greater than 0.5 indicate anomalous diffusion. Exponent value was found 0.67 (Table 4). According to the Peppas equation, the results were not in agreement with $Q\sqrt{t}$ and zero order kinetics.

The best compliance according to the highest determination coefficient and lowest sum of weighed squared deviation values for VACL dosage form

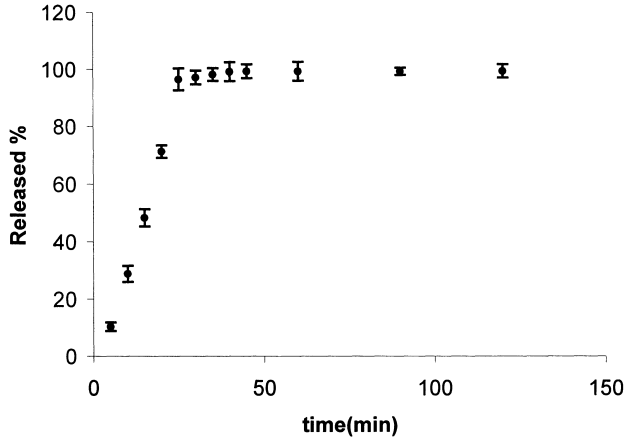

Figure 3. In vitro dissolution profiles of VACL tablets by proposed RP-HPLC method.

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Table 4. Kinetic parameters of release data of VACL tablets.

Zero order	kr^0	174.59
	r^2	0.384
	SWSD	0.21
First order	kr	2.72
	r^2	0.589
	SWSD	0.50
Hixson-Crowell	k	3.11
	r^2	0.489
	SWSD	0.34
Weibull distribution (RRSBW)	$T_{(\min)}$	17.34
	β	1.304
	r^2	0.836
	SWSD	0.066
Peppas equation	kp	0.070
	n	0.668
	r^2	0.728

Note: kr, release rate constant of first order kinetic; kr^0, release rate constant of zero order kinetic; k, release rate constant of Hixson-Crowell kinetic; kp, release constant of Peppas equation; r^2, determination coefficient; SWSD, sum of weighed squared deviations; β, shape factor; $T_{(\min)}$, value stands for the time for 63.2% release of the drug; n, diffusional exponent.

was found to be Weibull distribution (RRSBW) for releasing profile. The release of active material from tablets was attained to 63.2%, at the end of 17.34 min. When the shape factor (β) value, which is one of the parameters of Weibull distribution, being bigger than 1, it is characteristic for a slower initial rate followed by an accelerated approach to the final plateau, i.e., an initial upward curvature and sigmoid overall appearance. The value obtained was bigger than 1. The release of VACL from the tablets tested was completed within 30 min in the proposed method.

CONCLUSION

The proposed RP-HPLC method is simple, accurate, precise, and rapid for the determination of VACL in pharmaceutical dosage forms and for monitor-

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ing its concentration in in-vitro dissolution studies. Hence, it can be easily adopted for the routine quality control analysis of VACL in tablet dosage forms. The developed HPLC method was fully validated by evaluation of the validation parameters. High percentage recovery shows that the method is free from the interferences of the commonly used excipients and additives in the formulations of the drug. The aim of this paper is not to study the pharmacodynamic properties of VACL, because only healthy volunteers were used for sample collection. It only shows that the possibility of monitoring this drug makes the method useful for the pharmacokinetic and pharmacodynamic purposes. The results obtained show that the proposed method may be useful to determine VACL in human serum at the levels obtained after the administration of normal clinical doses, and it would be a method of choice for monitoring this substance in patients. The proposed method used a simple serum deproteination step instead of extraction. No interferences from endogenous substances were observed in serum samples.

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