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Simple high-performance liquid chromatographic method for the determination of acyclovir in pharmaceuticals

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Abstract

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 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 exipients present in the tablets did not interfere with the method.

Keywords: Acyclovir; High-performance liquid chromatography; Pharmaceutical product; Quality assessment

1. Introduction

Acyclovir, (9,2-hydroxyethoxy) methyl guanine, is an antiviral drug used extensively in the treatment of skin infections caused by hyper simplex virus [1]. It is official in European Pharmacopoeia [2], British Pharmacopoeia [3] and United States Pharmacopoeia [4]. The therapeutic interest in this compound justifies research to establish analytical methods for its determination in pharmaceutical preparations and biological samples, the latter being subjected to extensive study by an array of techniques, chromatography occupying the prominent position. Quantitation of acyclovir in body fluids like plasma [5–13], serum [14–16], serum and urine [17,18], and urine [19], has been carried out by high-performance liquid chromatography (HPLC) with ultraviolet and fluorescent detection because of the high sensitivity achievable. Methods based on radio immuno assay [20,21], liquid chromatography [22], high-performance capillary electrophoresis [23] and micellar liquid chromatography [24] are also devoted to biological samples like plasma [20,22], serum [21], urine [23], and serum and plasma [24].

There are also reports of determination of acyclovir in pharmaceuticals by HPLC [25–28] but the methods are less sensitive with the linear ranges being 50–200 μg ml^{-1} [27] and 0.1–1.0 mg ml^{-1} [28]. Recently, Caamano et al. [29] have reported a reversed-phase liquid chromatographic method for the assay of acyclovir in liposomal formulations. The results, although showed a high reproducibility in retention time value with RSD of 2.37%, the same based on peak area were poor with a RSD value of 9.64%. Reports on the spectrophotometric determination of acyclovir are scarce. The derivative spectrophotometric [30] and differential spectrophotometric [31] methods proposed are applicable for combined dosage forms only. The only visible spectrophotometric method reported by the present authors [32] is not very sensitive ($\varepsilon = 1.65 \times 10^2$ l mol^{-1} cm^{-1}).

A new method for the HPLC determination of acyclovir is described in this paper. The method is substantially simpler, faster and more sensitive than the previously reported HPLC [25–29] and spectrophotometric methods [30–32].

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2. Experimental

2.1. Apparatus

A HPLC (Agilent 1100 Series, Agilent Technologies, Waldbronn, Germany) equipped with an inbuilt solvent degasser, quarternary pump, and photodiode array detector with variable injector and auto sampler was used along with a reversed-phase column (5 μm Hypersil ODS C_{18}; 25 cm long and 4.6 i.d., Thermosil, USA).

2.2. Reagents and materials

Analytical reagent grade ammonium acetate (Thomas Baker, England), HPLC grade MeCN (Rankem, India), AR grade AcOH (S.d. Fine Chemicals, India) and distilled water filtered through a 0.45 μm filter (millipore) were used.

2.2.1. Ammonium acetate buffer

A 20 $mmol^{-1}$ solution was prepared by dissolving 3.08 g in 2 l of water, the pH was adjusted to 4.5 with AcOH, filtered through 0.45 μm filter.

2.2.2. Diluent solution

Prepared by mixing MeCN and water in the ratio 60:40.

2.2.3. Solvent system

The solvent system employed for chromatography consisted by MeCN–ammonium acetate buffer (40:60).

2.2.4. Acyclovir and its preparations

Pharmaceutical grade acyclovir was kindly provided by Cipla India Ltd., Mumbai, India and was used as received. A stock standard solution of acyclovir (198 μg ml^{-1}) was prepared in the diluent solution. Proprietary drugs containing acyclovir were purchased from local stores.

2.3. Procedures

2.3.1. Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a reversed-phase Hypersil ODS C_{18} column (25 cm × 4.6 mm i.d.) using a mobile phase consisting of MeCN–20 mM ammonium acetate buffer (40+60) at a flow rate 0.8 ml min^{-1}. The detector wavelength was set at 250 nm with a sensitivity of 0.2 a.u.f.s.

2.3.2. Calibration graph

Working standard solutions containing 1.98–59.4 μg ml^{-1} of acyclovir were prepared by transferring 0.25–10.00 ml of stock standard solution (198 μg ml^{-1}) into separate 25 ml calibrated flasks and diluting to volume with the diluent solution. A 20 μl volume was injected automatically into the chromatograph in duplicate and chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area against acyclovir concentration.

2.3.3. Procedure for dosage forms

Five tablets, each labelled to contain 200, 400 or 800 mg acyclovir, were chosen at random from the total number of 20. They were weighed accurately and ground into a fine powder with agate pestle and mortar. An amount of the powdered tablets equivalent to approximately 20 mg of the drug was dissolved in the diluent solution and the resulting mixture was transferred quantitatively to a 100 ml calibrated flask and made up to volume with the diluent solution with thorough shaking. A small portion of this solution (≅ 10 ml) was withdraw and filtered through a 0.2 μm filter to ensure the absence of particulate matter. This filtered solution was appropriately diluted to give the final solution (198 μg ml^{-1} for analysis, and proceeded as described under Section 2.3.2.

A 5 ml aliquot of Ocuvir suspension equivalent to 400 mg of active component was quantitatively transferred in to a 100 ml calibrated flask and diluted to volume with the diluent solution and mixed well and proceeded further as described under tablets.

3. Results and discussion

3.1. Method development

HPLC methods using Hypersil ODS column and UV detection employing different mobile phases have earlier been reported for the determination of acyclovir in biological samples [5–7] and pharmaceuticals [27,28]. The methods utilising 0.1 mol l^{-1} potassium dihydrogen phosphate (pH 5.6) as the mobile phase yield narrow linear ranges of response (0.5–4.0 μg ml^{-1})[5,6]. The use of mobile phase consisting of 20 mmol l^{-1} potassium dihydrogen phosphate of pH 3.5 [7] although improved the range of determination (0.1–50.0 μg ml^{-1}) of acyclovir in human plasma, it failed to yield similar results when applied to pharmaceuticals. The procedure using the mobile phase of 5% acetonitrile in 0.01 mol l^{-1} potassium dihydrogen phosphate of pH 4.8 is not considered sensitive enough (50–200 μg ml^{-1}) for the assay of acyclovir in pharmaceuticals [27]. Recently, Kourany Lefoll and Cyr [28] have reported the determination of 0.1–1.0 mg ml^{-1} of acyclovir in authentic samples utilising the mobile phase consisting of 20 mmol l^{-1} ammonium acetate (pH 4.5)–methanol (19:1). In our attempt to improve the sensitivity of the above procedure, the composition of the mobile phase was changed by replacing methanol with acetonitrile and got

better results in terms of sensitivity and linear range of response (1.98–59.4 μg ml^{-1}). Further, acetonitrile was preferred to methanol because it has lower absorptivity at 250 nm (0.005 absorbance unit).

During the development of the method, a number of variations were tested. The pH, buffer concentration, acetonitrile concentration and flow rate were chosen to give retention times of <5 min and a symmetric peak with a good resolution. With a mobile phase consisting ammonium acetate buffer (pH 4.5)–acetonitrile (50+50), the peak symmetry was not adequate. Increasing the proportion of acetate buffer to 60% resulted in a well resolved and a symmetric peak (Fig. 1). Thus, a mobile phase consisting of acetonitrile–20 mmol l^{-1} ammonium acetate (pH 4.5) was found to be optimum with respect to peak shape, retention time and sensitivity.

Flow rates between 0.5 and 1.5 ml min^{-1} were studied. A flow rate of 0.8 ml min^{-1} gave an optimal signal-to-noise ratio with a reasonable separation time. Using a reversed-phase ODS column, the retention time was observed to be 3 min and the total time for analysis was less than 5 min.

The maximum absorption of acyclovir was at 250 nm and this wavelength was chosen for analysis.

The sample acyclovir chromatograms were identical with those of the standard acyclovir when they were run for a longer period of time. Such an observation suggests the absence of non-active ingredients or degradation products.

3.2. Assay validation

3.2.1. Linearity

For quantitative determinations, a linear calibration graph ($Y = -20.47 + 80.83X$; $r = 0.9998$; $n = 6$ where Y and X are mean peak area and concentration, respectively) was obtained over the working concentration range 1.98–59.4 μg ml^{-1} (Fig. 2). The limit of detection was determined by reducing the concentration until a signal-to-noise ratio of 3 was obtained, and this was found to be 0.80 μg ml^{-1}. The limit of quantification was similarly determined by reducing the concentration until a signal-to-noise ratio 9 was reached and this was calculated to be 1.50 μg ml^{-1}.

3.2.2. Precision

The within-day precision of the method was determined for both peak area and retention time by repeat analysis (seven identical injections) of the standard solution containing the drug at three concentration levels. The RSD for retention time ranged from 0.08 to 0.13% and that for peak area ranged from 0.44 to 0.91% (Table 1). The between day precision was established by performing the analysis over a 5-day period on solution prepared freshly each day. The low RSD values indicate the ruggedness of the method (Table 2).

3.2.3. Accuracy

Accuracy of the proposed method for the determination of acyclovir was established by assaying the solution of known concentrations as done for determining the within-day precision i.e. solutions of known concentration in three levels were prepared and subjected to replicate analyses ($n = 7$). The concentrations of the drug were then calculated. The results obtained (Table 1) showed the method to be satisfactorily accurate (% error <3) and precise (RSD $<1\%$).

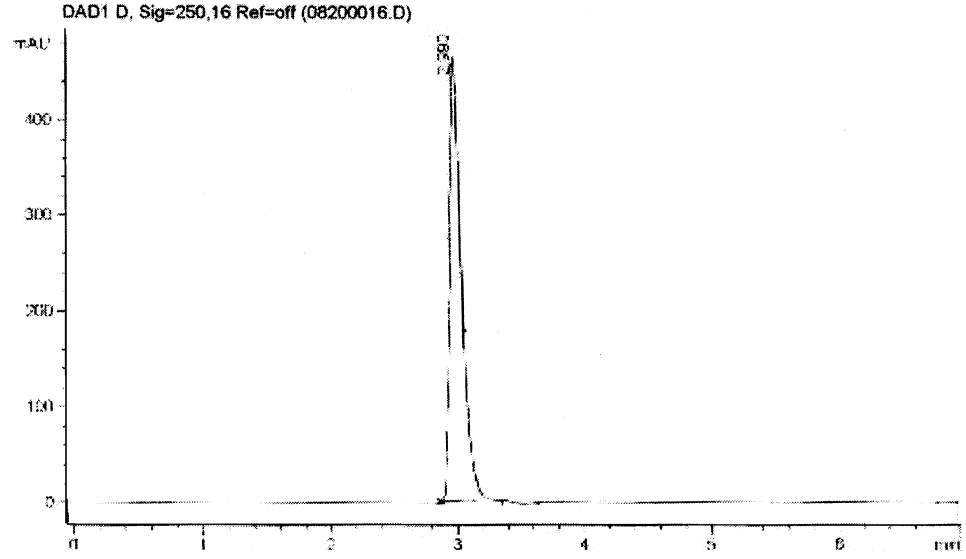

Fig. 1. Typical chromatogram of acyclovir (39.6 μg ml^{-1}).

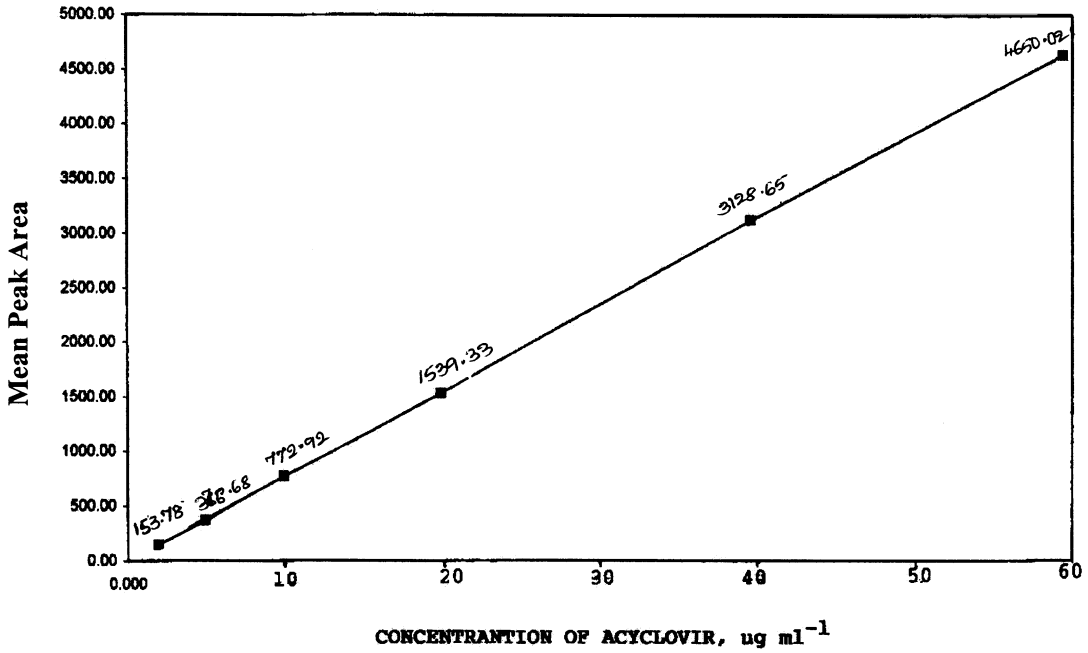

Fig. 2. Calibration curve.

Table 1
Results of accuracy and precision studies

Acyclovir taken (μg ml^{-1})	Acyclovir found (μg ml^{-1})	Error (%)	RSD [a] (%) ($n = 7$)	RSD [b] (%) ($n = 7$)
10.15	9.86	2.86	0.91	0.08
20.30	20.03	1.33	0.30	0.13
40.45	39.51	2.32	0.44	0.12

SD, standard deviation; RSD, relative standard deviation.
[a] Based on peak area.
[b] Based on retention time.

3.3. Application to acyclovir tablets

Commercially available acyclovir tablets were analysed by the described HPLC method. The results obtained are summarised in Table 3. As can be seen, the data for Acivir and Ocuvir tablets and Ocuvir suspension were in agreement with the labeled amounts. The results obtained by the HPLC method were compared with those obtained by the official method [3] by applying Student's t-test and F-test at the 95% confidence level. The calculated t- and F-values did not exceed the tabulated values of 2.77 and 6.39, respectively, indicating that the proposed HPLC method is as accurate and precise as the official method.

In order to demonstrate the validity and applicability of the proposed method, recovery studies were performed via standard-addition technique. To a fixed and known amount of drug in the pre-analysed tablet extracts and suspension solution, pure acyclovir (standard) was added at three levels, and the total amount was found by the proposed method. The experiment at each level was repeated three times. The percent recoveries of the pure drug added which are compiled in Table 4 reveal that the commonly added excipients such as lactose, talc, starch, gum acacia, sodium alginate and magnesium stearate did not interfere in the assay method. This is amply demonstrated by a single peak due to acyclovir in the chromatogram of the sample solution (Fig. 3).

In conclusion, a method has been developed and appropriately validated for the assay of acyclovir in pharmaceutical preparations for the purpose of product quality assessment. The method does not require extensive sample treatment and involves a HPLC system

Table 2
Between-day precision of the method

Acyclovir taken (μg ml^{-1})	RSD [a] (%)	RSD [b] (%)
5.08	2.01	0.16
15.25	1.25	0.20
30.50	0.63	0.15

[a] Based on peak area.
[b] Based on retention time.

Table 3
Results of analysis acyclovir containing tablets

Dosage forms and brand name	Label claim (mg)	Found (mg±SD) *		Student's *t*-value (2.78)	*F*-value (6.39)
		Proposed method	Official method		
Acivir [a] DT tablets	200	195.64±2.62	196.18±1.21	0.45	4.69
	400	396.26±4.98	399.23±3.51	1.11	2.01
	800	789.72±10.64	792.50±8.81	0.45	1.46
Ocuvir [b] tablets	200	196.50±2.32	200.95±3.21	2.54	1.91
	400	386.57±5.82	388.09±6.02	0.41	1.07
	800	779.19±10.34	782.81±11.15	0.53	1.16
Ocuvir [b] suspension	400 mg per 5 ml	398.09±6.17	399.84±7.11	0.42	1.33

[a] Marketed by Cipla India Ltd.
[b] Marketed by FDC India Ltd.

Table 4
Results of recovery studies by standard-addition method

Tablet investigated	Amount of drug in the tablet solution (μg)	Amount of pure drug added (μg)	Total found (μg)	Recovery [a] pure drug added (%)
Acivir (200 mg)	97.82	200.00	291.87	97.03
	97.82	300.00	383.90	95.36
	97.82	400.00	491.85	98.51
Acivir (400 mg)	99.07	200.00	305.95	103.44
	99.07	300.00	393.48	98.14
	99.07	400.00	506.56	101.87
Ocuvir (800 mg)	97.40	200.00	292.05	97.33
	97.40	300.00	391.44	98.01
	97.40	400.00	496.39	100.25
Ocuvir suspension (400 mg per 5 ml)	99.52	200.00	308.76	104.62
	99.52	300.00	409.36	103.28
	99.52	400.00	510.96	102.86

[a] Mean value of three trials.

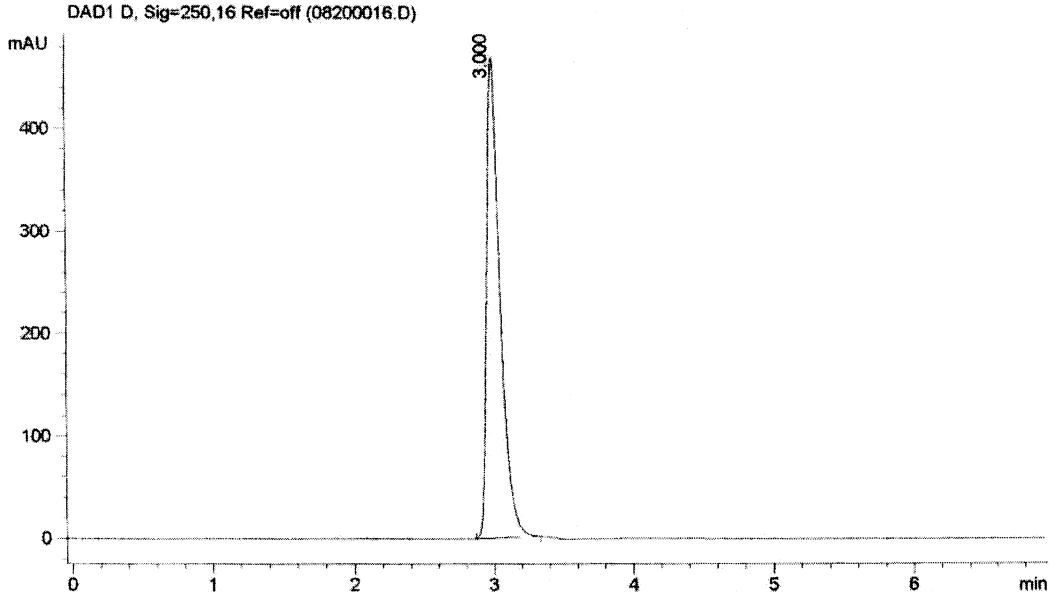

Fig. 3. Typical chromatogram of acyclovir preparation (39.6 μg ml^{-1}).

employing an inexpensive mobile phase. Moreover, the proposed assay method is more sensitive than the existing HPLC methods [25–28] for acyclovir in dosage forms. The results obtained indicate that the method described is capable of good accuracy and precision. It appears to have a considerable value as a rapid and sensitive method to be used as a routine procedure, and should help in the analysis of acyclovir in pharmaceutical preparations.

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