The Bioscan 5(1) : 117 - 121, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

ANALYSIS OF RELATED SUBSTANCES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY [HPLC] METHOD FOR VALACYCLOVIR HYDROCHLORIDE

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KEY WORDS
Valacyclovir hydrochloride
HPLC
Herpes virus
Antiviral

Received on :
17.08.2009
Accepted on :
22.12.2009

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ABSTRACT
Valacyclovir hydrochloride is a purine nucleoside analogue derived from Acyclovir and is used in the treatment of Herpes simplex and Herpes zoster infections. Valacyclovir hydrochloride is used to treat certain infections like Genital Herpes and Herpes cold sores on the face and lips. In the present study, it was aimed to perform qualitative analysis of related substances by High performance liquid chromatography [HPLC] for Valacyclovir hydrochloride, to obtain auto scaled chromatogram for the standard and impurity blends of Valacyclovir hydrochloride. The flow rate of the compound was maintained at 1.0micro/minute. The column used for Valacyclovir hydrochloride analysis is Inertsil ZODS 3V, 250X4.6mm ID, 5 micro meter particle size. Column temperature was maintained to 40°C.Different peaks were observed at different time intervals. Peak purity was checked for principle compounds and also for impurities. Valacyclovir was as effective and well tolerated in the treatment of recurrent genital herpes simplex virus infection as 5-times-daily acyclovir. Valacyclovir is well tolerated first line therapy with an established place in the treatment of immunocompetent patients with Herpes zoster.

INTRODUCTION

A purine nucleoside analogue that is derived from acyclovir and used in the treatment of *Herpes simplex* and *Herpes zoster* infections is known to be valacyclovir hydrochloride. *Varicella zoster* virus (VZV), the pathogen responsible for *Herpes zoster*, belongs to the *Herpes* virus family and is sensitive to the antiviral drug acyclovir (Furman *et al*., 1981). However, the low oral bioavailability of acyclovir has to some extent limited its efficacy in the treatment of *Herpes zoster* and has prompted the development of the more readily absorbed oral prodrug valacyclovir. In large comparative study Valacyclovir, (1000mg 3 times daily for 7 days) was at least as effective as acyclovir (800mg 5 times daily for 7 days) in controlling the symptoms of acute *herpes zoster*. Importantly, Valacyclovir alleviated zoster – associated pain and postherapeutic neuralgia significantly faster than acyclovir (Goodman and Gilmans, 1996). Valacyclovir and acyclovir demonstrated similar efficacy for the control of cutaneous lesions and ocular complications in patients with *Zoster ophthalmicus*.

Preliminary results of a large controlled trial indicate that Valacyclovir 1000mg 3 times daily and Famciclovir (prodrug of Penciclovir) 500mg 3 times daily are of similar efficacy in speeding resolution of acute H*erpes zoster* rash and shortening the duration of postherapeutic neuralgia. Valacyclovir hydrochloride should not be used by anyone with a weak immune system, such as those with HIV infection or those who have undergone a bone marrow or kidney transplant. It can cause serious side effects including death in such people (Dogan- Topal *et al*., 2007). In the present study qualitative analysis carried out by High Performance Liquid Chromatography [HPLC] to study the safety and efficacy of Valacyclovir hydrochloride in presence of related substances.

MATERIALS AND METHODS

Valacyclovir hydrochlorid was obtained as gift sample from Dr.Redd's Labs,Hyderabad,India. All other chemicals used for the study were purchased from S.D.Fine Chemicals,India.

Typical chromatographic conditions

Inertsil ODS 3V, 250×4.6 mm ID, 5 μm particle size column is required. A filtered and degassed mixture of buffer and Methanol in the ratio of 90:10 prepared and named as Mobile phase A, a filtered and degassed mixture of buffer, Methanol and Acetonitrile in the ratio of 50:30:20 and named as Mobile phase B. Buffer was prepared which includes 3.4g of potassium dihydrogen ortho phosphate (KH_2PO_4) dissolved in 1000mL of water and adjusted the pH to 6.7 with Triethylamine. Maintaining flow rate 1 mL/minute (gradient), detector wavelength 254 nm, injection volume 20 μL, column temperature at 40°C,run time for 65 minutes. Diluent-1, consists of water: Dimethyl Sulfoxide [DMSO] in the ratio of 1:1 and Diluent-2 consists of 3.4 g of potassium dihydrogen ortho phosphate (KH_2PO_4) dissolve d in 1000mL of water

(Mayur *et al*., 2007).

Gradient time programme

Preparation of system suitability solution

Reference solution-A Stock: Weighing accurately 5mg of Guanine into 50 mL volumetric flask dissolve and dilute to volume 0.1N sodium hydroxide solution.

Reference solution – B Stock: Weighing accurately 5 mg of Acyclovir, 5mg of Alanine impurity of Valacyclovir, 5mg of Isoleucine impurity of Valacyclovir, 5 mg of O-Acetyl acyclovir, 5 mg of N- formyl valacyclovir and 5 mg of valacyclovir hydro chloride into 50mL volumetric flask dissolve and dilute to volume with diluents.

Reference solution (0.15%): Transfer 150μL of Reference solution-A Stock and Reference solution – B Stock into 10 mL volumetric flask containing diluents 2.

Valacyclovir hydrochloride standard solution preparation: Weighing accurately 50mg of Valacyclovir hydrochloride standard sample in 50 mL volumetric flask, dissolve in 5 mL of diluents-1 solution and dilute to volume with diluents-2.

Acceptance criteria: The resolution between N- formyl Valacyclovir and Isoleucine impurity of Valacyclovir obtained from reference standard solution should not be less than 1.5. The column efficiency determined from the Valacyclovir peak in Valacyclovir hydrochloride standard solution which is injected before test sample is not less than 3000 theoretical plates, tailing factor for the Valacyclovir peak is not more than 2.0.

Impurity content

Standard solution: Weighing about 50mg of Valacyclovir hydrochloride working standard into a 50 mL volumetric flask, dissolved in 5 mL of diluents-1 and dilute to volume-2 (1.0mg/mL).

Sample solution

Weighing about 50mg of Valacyclovir hydrochloride sample into a 50 mL volumetric flask. Dissolving 5 mL of diluents-1 and dilute to volume with diluents-2(1mg/ml) preparing the sample solution for six times from the same. Ensure the system meets required system suitability by injecting the reference standard solution. Inject 20 μL of blank as prepared for the sample, standard solution and sample solution each once (Mayur *et al*., 2007).

RESULTS AND DISCUSSION

This study shows that Valacyclovir is equivalent in efficacy and safety to the recommended dosage in patient –initiated episodic treatment of recurrent genital herpes. Thus, results reflect the usual use of Valacyclovir in recurrent genital herpes,

Table 1: Gradient time programme.

Time	A%	B%
0	100	0
2.5	100	0
15	85	15
30	55	45
40	10	90
55	10	90

Table 2: Peak results

Sl. no.	RT	Area	Height (μV)	% Area	Total area
1	41.184	2466	154	0.629	392175
2	43.662	823	100	0.210	392175
3	44.833	24521	571	6.253	392175
4	46.249	364364	22649	92.908	392175

Sample name: blank, sample type: unknown, Vial: 97, Injection: 1, Injection vol: 20μL, run time: 65 minutes.

Table 3: Peak results

S. no.	RT	Area	Height (μV)	%Area	Name	Total area	USP Resolution
1	5.014	107448	12768	18.431	guanine	582980	
2	7.159	104793	9848	17.975	Acyclovir	582980	8.40+000
3	8.929	71824	5838	12.320	Alanine imp of Valacyclovir	582980	5.78e+0000
4	17.654	74088	5539	12.708	O-acetyl acyclovir	582980	2.54e+001
5	22.119	55644	3983	9.545	VOV	582980	1.21e+001.
6	26.969	58280	4332	9.997	N-formyl vvalacyclobir	582980	1.31e+001
7	28.578	55787	3829	9.569	Isoleucine	582980	4.28e+000
8	51.424	55118	2923	9.454		582980	5.05e+001

Sample type: sst, Sample type: unknown, vial: 102, Injection: 1, Injection volume: 20μL, runtime: 65 minutes.

Table 4: Peak results

S. no.	RT	Area	Height (μV)	%Area	Name	Totalarea
1	5.009	2034	258	0.006	guanine	34767620
2	7.136	1040545	98599	2.993	Acyclovir	34767620
3	8.929	3567	332	0.010	Alanine imp ofValacyclovir	34767620
4	14.155	3891	342	0.011		34767620
5	17.575	5806	414	0.017	O-acetyl acyclovir	34767620
6	21.382	18318	1445	0.053		34767620
7	22.144	33359267	2056818	95.949	VOV	34767620
8	26.340	29476	2245	0.085		34767620
9	26.923	214519	16165	0.617	N-formyl valacyclobir	34767620
10	28.633	6972	345	0.020	isoleucine	34767620

Sample type: VCH-00307 LOT-1, Sample type: unknown, vial: 98, Injection:1,Injection volume: 20μL, run time: 65 minutes.

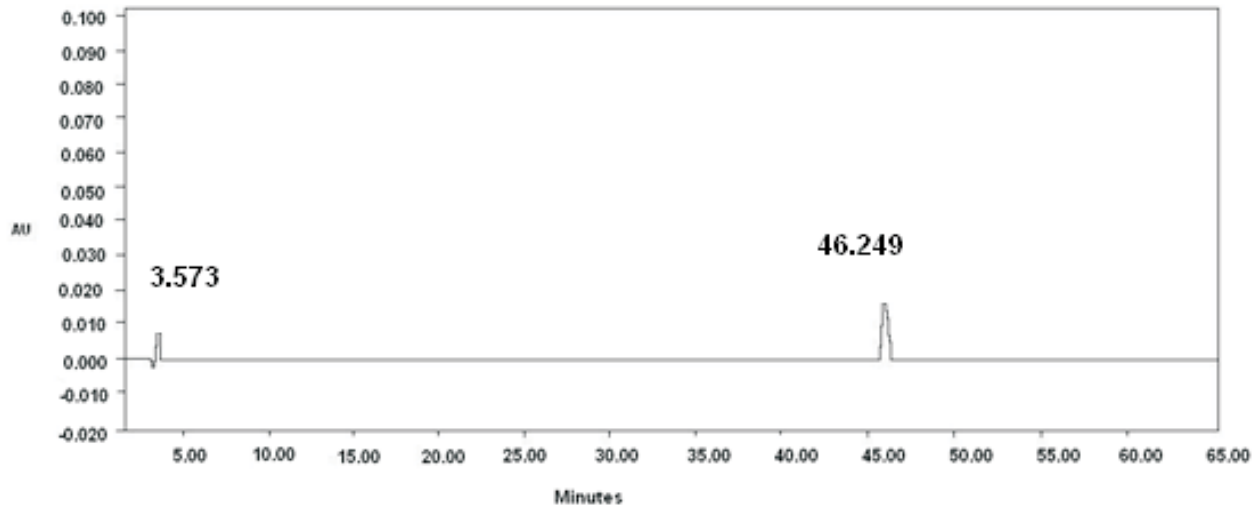

Figure: 1.a Blank chromatogram

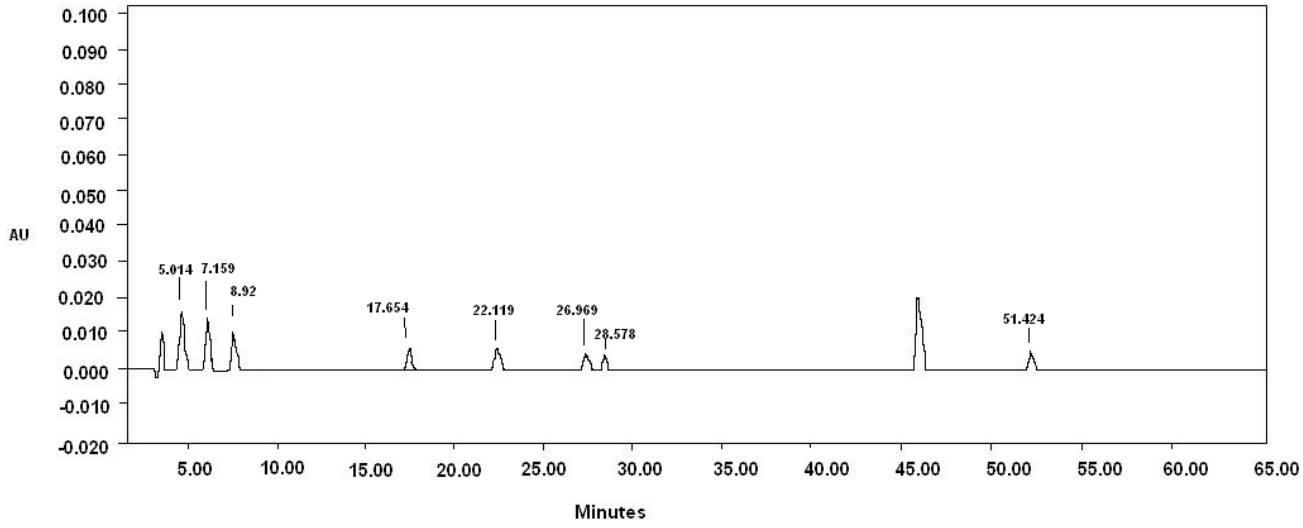

Figure 1b: Impurityblendchromatogram

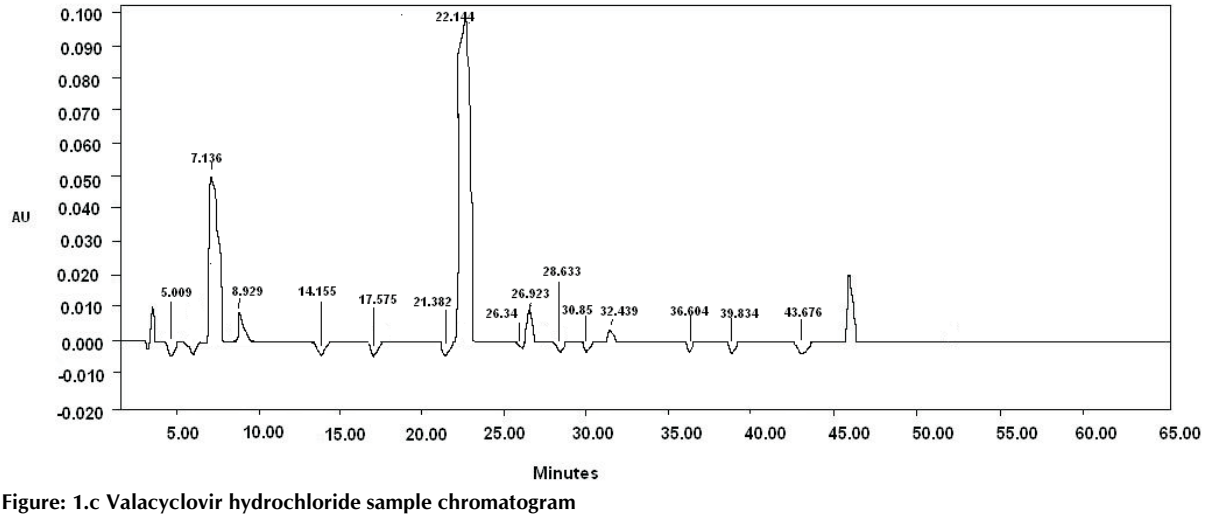

Figure: 1.c Valacyclovir hydrochloride sample chromatogram

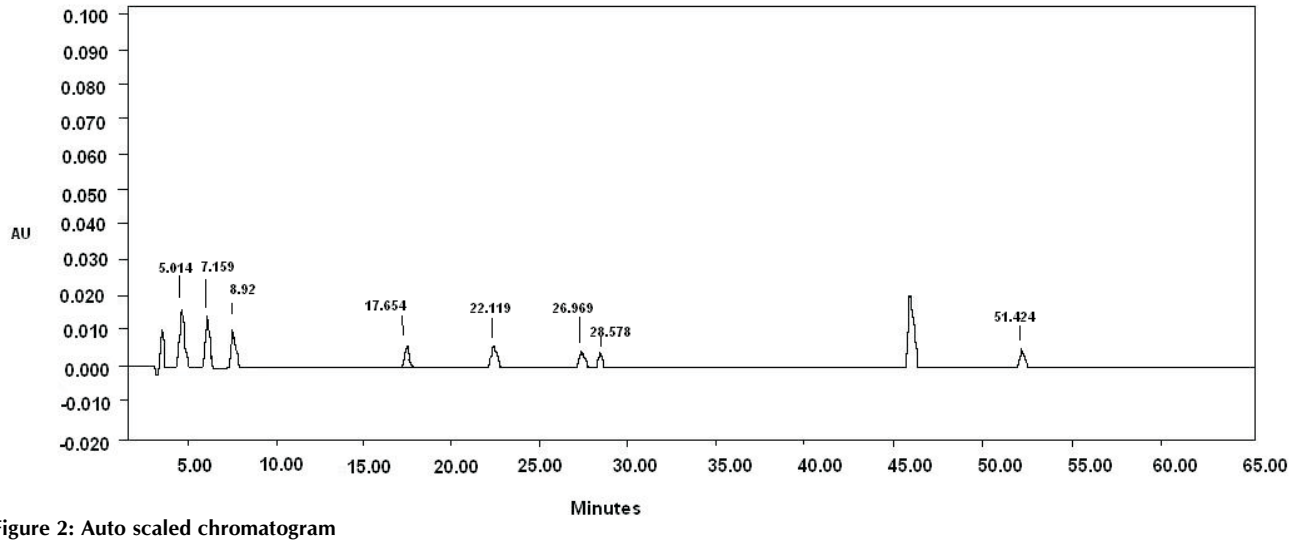

Figure 2: Auto scaled chromatogram

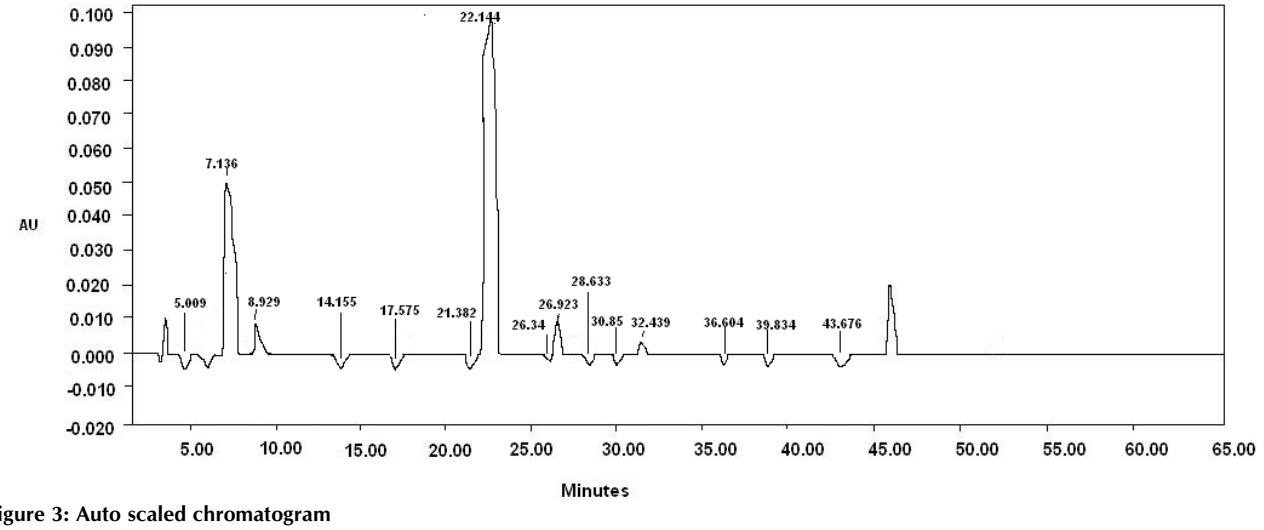

Figure 3: Auto scaled chromatogram

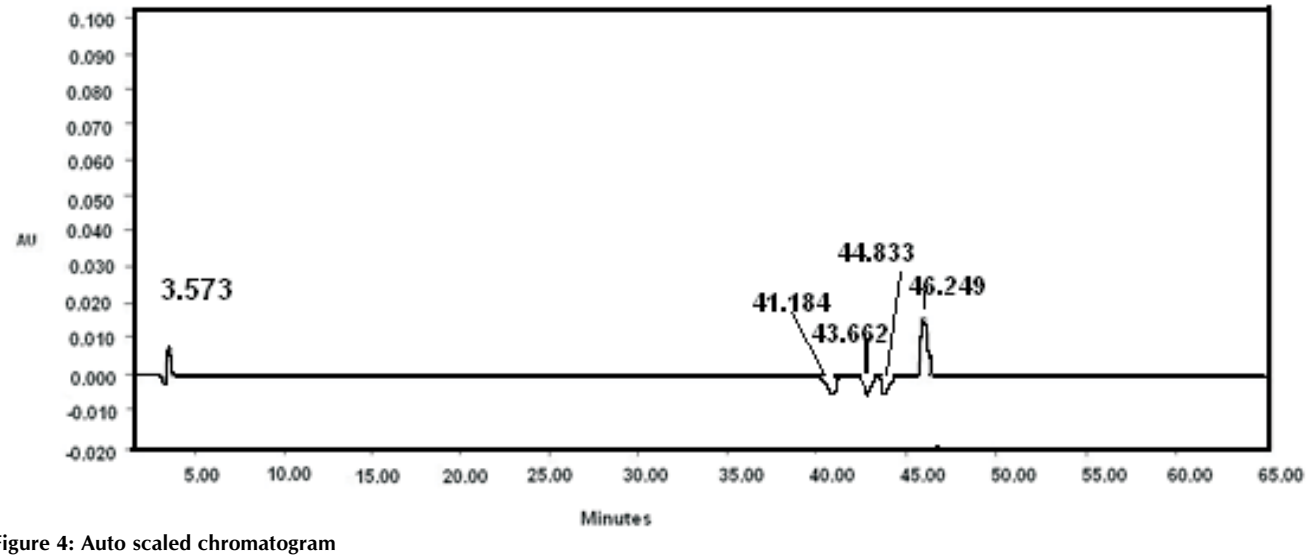

Figure 4: Auto scaled chromatogram

as compared with the artificial conditions in hospital-based phase III studies, in which viral cultures are performed daily. Moreover, the high diagnostic accuracy of genital herpes by investigators can be demonstrated by the very high frequency of patients without abortive episode exhibiting clinical; signs suggestive of herpes. The results were finalized with the help of chromatograms which were found on the recorder. Maintaining flow rate 1 mL/minute (gradient), detector wavelength 254nm, injection volume 20μL, column temperature at 40°C, run time for 65 minutes. The blank chromatogram (Fig. 1a) was obtained for reference to the following. The chromatograms were obtained for the impurity blends (Fig. 1b) which are present in the sample. The peaks were read and allowed to run the sample. At last the Valacyclovir hydrochloride samples chromatogram was read and the peaks were noted. The peaks which are present in the chromatograms show (Fig. 2 and Table 2) the related substances which are present with the sample. The peaks mainly show the related substances like amino acids (guanine, isoleucine and alanine). The main alternative product which was found in the sample analysis is Acyclovir. The final chromatogram is the auto scaled chromatograms by which the results are known. (Fig. 3, 4 and Table 3, 4).

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