Stability Test of Acyclovir at Severe Conditions

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In this paper, the influence of stability of acyclovir in severe conditions was tested (acid and alkaline environment) and the acyclovir impurities were evaluated using ion-pairing HPLC method and mobile phase H_2O : ACN 5 : 95% (v/v) counter-ion $C_8H_{17}SO_4$ Na 0.002 M, pH 2.5, KH_2PO_4 0.02 M, flow rate 1 mL/min, UV detection 253 nm. The method was precise and linear (R > 0.99985, n = 11) in the range of 1–100 µg/mL and reproducible (RSD = 2.65%).

Key Words: HPLC, Ion-pair, Acyclovir, Guanine, Stability.

INTRODUCTION

Stability indicating assay can be described as a method of analysis by which the parent constituent(s) of the pharmaceutical product can be selectively analyzed. It can be developed either to determine only the active or only the degradation product, or the method can be used to separate and determine the parent compound in presence of its impurities and degradation products. The choice of the method depends upon the chemical nature of the drug, the number of impurities, the flexibility of the dosage form and, of course, on practical considerations relating to the availability of resources and instruments.

Currently, chromatographic methods are the methods of first choice for controlling, conformity, purity, stability and strength of the active ingredient. A logical and systematic approach to the development of a stability indicating assay can be carried out initially through a review of internal information present within the laboratory or company or from extern information, from literature sources. In addition, biological, toxicological, pharmacological information, physicochemical properties including solubility, partition coefficient, dissociation constant and spectrophotometric properties should also be reviewed.

One method of checking impurities in the active ingredient is to subject it to conditions of stress, such as heat, light and moisture in order to predict probable routes of degradation. Short and long term stability studies of the active ingredient and storage conditions should also be examined. On completing development of

the method it must be evaluated and therefore validated according to the criteria of specificity, linearity, precision, accuracy, sensitivity and reproducibility. There is no report yet on the development of stability indicating assay method for the drug acyclovir.

This study relates to acyclovir [9-(2-hydroxyethoxymethyl)guanine] which is a modified nucleoside that shows strong activity against viruses of the herpes group. It derives from guanine, which is a constituent of purine. In the previous work, a reversed phase ion pair HPLC method was developed to separate acyclovir from its major degradation product, guanine[1]. The objective of the current study was to develop a validated stability indicating assay method for acyclovir after subjecting the drug to stress conditions.

EXPERIMENTAL

The chromatographic system consisted of a Shimadzu LC 5A pump (Touzart, Matignon, France) connected to a Rheoyden 7520 syringe loading sample injector valve fitted with a 20 µL sample loop, a variable wavelength UV detector (UV SPD 10 Å) and a Shimadzu chart recorder. The column was a 150×4.6 mm i.d. packed with Hypersil ODS C18, 3 µm particle size (Touzart, Matignon, France). Acyclovir (Acv) and guanine (Gu) were obtained from Sigma-Aldrich Chemical Company. Acyclovir (zovirax) tablets were obtained from Welcome Laboratories.

RESULTS AND DISCUSSION

1. Effect of temperature: Tablets of acyclovir extracted in mobile phase were subjected to temperature in the range 35–60°C. The centrifuge tubes containing analytes were kept in a thermostat bath at different temperature. For each tube 20 µL of a particular solution were injected on to the column. A full chromatogram was then obtained and showed no effect and the drug was stable to temperature in the range 35–60°C. Fig. 1 shows a chromatogram of acyclovir at standard temperature (20°C) and Fig. 2 shows retention of both acyclovir and guanine.

2. Effect of light: In order to test photodegradation, the same tablets were subjected to the effect of UV light and the chromatogram obtained was compared to a reference chromatogram. A full chromatogram was then obtained and showed no effect and the drug was stable.

3. Extraction with 0.5 M and 1 M of NaOH solution: In assessing the accelerated degradation profile both bulk acyclovir and pharmaceutical tablet (zovirax) formulation were extracted in sodium hydroxide (0.5 M and 1 M respectively). Full chromatograms were recorded and compared with that extracted in mobile phase composition. As from Fig. 3 (a, b) there are some impurities or degradation products related to the drug (more than 0.3%).

4. Extraction with 0.5 M and 1 M of HClO₄: The same procedure as with NaOH was carried out with $HClO_4$ and Fig. 4 (a, b) shows degradation product of acyclovir (guanine).
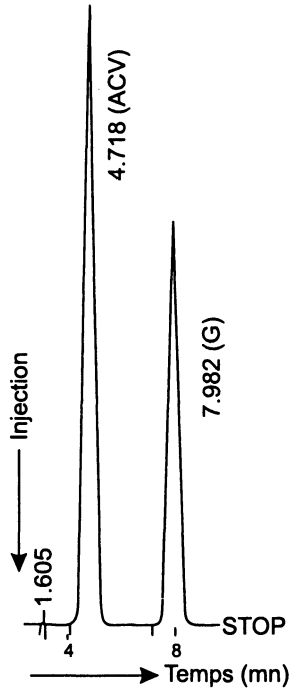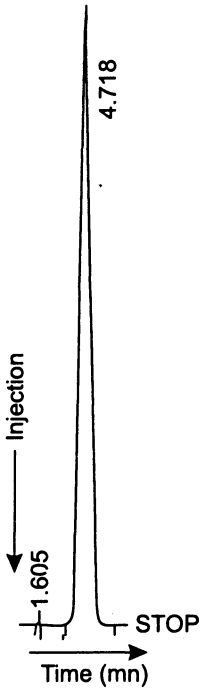
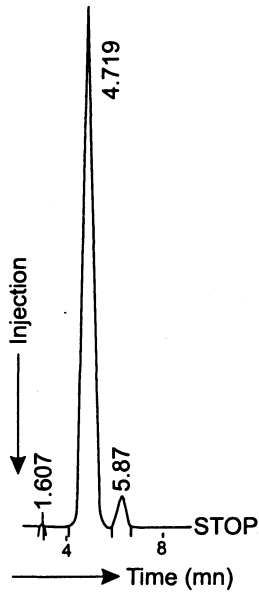Fig. 1. Chromatogram of acyclovir at standard   Fig. 2. Retention of acyclovir and guanine
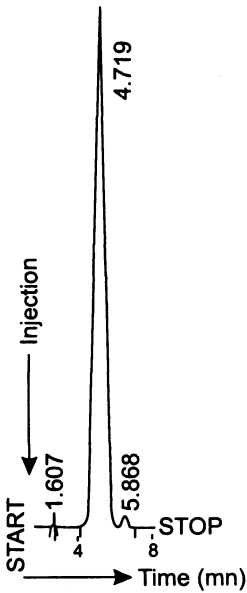   temperature

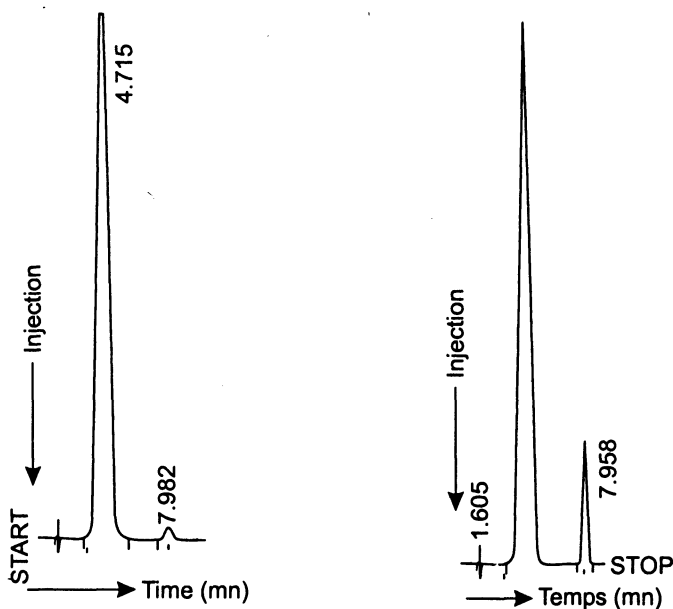Fig. 3 (a, b).   Extraction with 1 M and 5 M NaOH solution, respectively

Fig. 4.(a, b). Extraction with 0.5 M and 1 M of HClO₄, respectively

5. Effect of hydrolysis: Solutions of acyclovir were prepared and left on the laboratory window sill for 1, 3 and 6 months while the experimental controls were kept in a dark cupboard and observations were recorded. As can be seen from Fig. 5 (a, b), acyclovir was stable during this period of test; however there
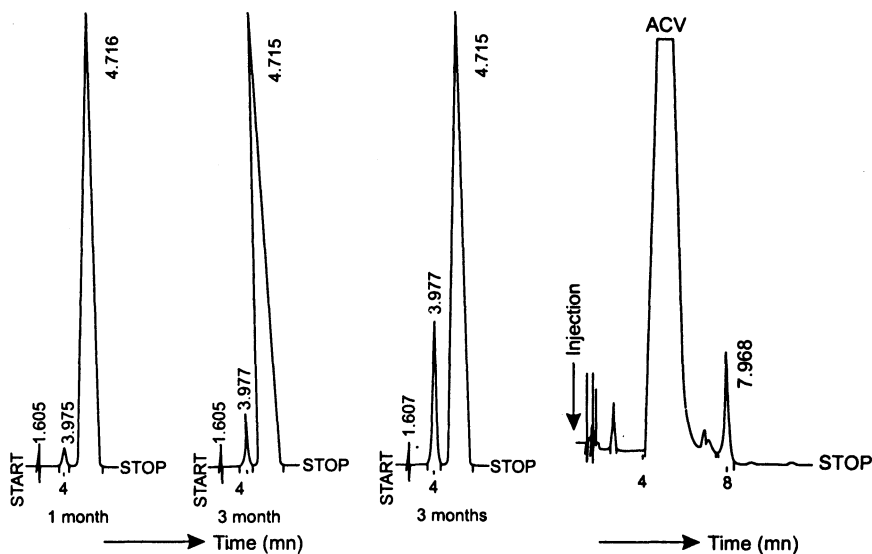
Fig. 5 (a, b). Effect of hydrolysis of acyclovir, hydrolysis of Zovirax tablet, respectively

was peak in front of acyclovir at 3.977 min, but it was well separated from acyclovir.

Validation of method

Repeatability: Ten 20 µL injections from a standard solution were injected on to the analytical column and the peak height data obtained were used in assessing system suitability and quality of analysis by calculating the relative standard deviation (RSD = 0.56%, n = 10) at 30 µg/mL.

Linearity: A stock solution of acyclovir (200 µg/mL) was prepared by dissolving the powder in the mobile phase; from this the linearity over the range of 1–100 µg/mL was examined and 20 µL of each was injected on to the analytical column. A plot peak height against analyte concentration was obtained to examine the response of the method and the results showed that the method was linear in accordance with Beer's law over this range and the linearity equation was $Y = 2.8232X + 0.01523$ and regression coefficient was R = 0.99985 (n = 11). The absolute limit of detection defined as signal to noise of 2 was examined for acyclovir and was found to be 3 ng/mL and the limit of quantitation (LOQ) was 0.1 µg/mL (20 µL was injected). Reproducibility, which expresses the precision under different conditions, such as different laboratories with different analysts, using separate instrumentation, was also examined and Table-1 shows the results obtained in two different laboratories and the RSD was between 2 and 4% .

TABLE-1

Laboratory	N	Cr (µg/mL)	Cm (µg/mL)	SD	RSD (%)
LSA (Lyon-France)	10	10	9.81	0.26	2.65
SAIDAL (Algiers)	10	10	10.26	0.38	3.70

Accuracy: Accuracy expresses the closeness of agreement between the value, which is accepted either as conventional true value or an accepted reference value (International Standard, *e.g.*, pharmacopoeal standard) and the value found (mean value) obtained by applying the test procedure a number of times. The results obtained are shown in Table-2. From these results, we can conclude that the method was accurate and precise (RF is less than 3%).

TABLE-2

N	Cr (µg/mL)	The same day				20 days later			
		Cm (µg/mL)	SD	RSD (%)	RE (%)	Cm (µg/mL)	SD	RSD (%)	RE (%)
10	10	10.02	0.076	0.76	0.20	9.93	0.17	1.71	–0.7
10	20	19.89	0.195	0.98	–0.50	20.12	0.26	1.30	0.6

Recovery: The recovery of extracted acyclovir was estimated either by comparing peak heights obtained from extracted aqueous standard and peak height obtained from spiked standard, or more conveniently by comparing the slopes of the equations. In this study, the recovery of acyclovir was 95% ($Y_{st} = 2.8232X + 0.1523$ and $Y' = 2.68411X' - 0.10304$).

Conclusion

A reversed phase ion-pair chromatography has been developed for the separation of acyclovir and its degradation product guanine. The method was used to test the degradation behaviour of acyclovir both as a bulk drug and formulation (Zovirax tablets) after subjecting the drug to severe conditions of stress. The drug was stable in acidic, alkaline and thermal conditions. The only degradation product formed after drug hydrolysis was guanine, which was identified and the method was found to apply well. Specificity of the method for the drug degradation product and impurities was proved through the nice separation of acyclovir from guanine and some impurities. The method was successful in the analysis of the drug in marketed tablets (Zovirax tablets). The method is simple, specific and precise.

REFERENCE

1. A. Hamdi, R. Fegas, B. Berka and B.J. Clarck, *Asian J. Chem.*, **17**, 1264 (2005).

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