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ORIGINAL ARTICLE

Validated spectrometric determination of penciclovir and entecavir in bulk and in pharmaceutical preparations

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Abstract Simple, rapid and sensitive spectroscopic methods were developed and validated for the determination of antiviral agents, namely, penciclovir (PCV) and entecavir (ETV). The first method is based on measuring the native fluorescence of each of the cited drugs at its optimum excitation and emission wavelengths. The fluorescence intensity was measured for PCV & ETV at 363 nm and 370 nm upon excitation at 260 nm and 254 nm, respectively. The calibration curves were linear over the concentration range 0.1–0.8, 0.025–0.4 μg ml^{-1} for PCV and ETV, respectively. The second method is based on measuring the amplitude of ETV in the fourth derivative with $\Delta\lambda = 8$ and scaling factor = 100 at 256.4 nm at which its acidic degradation showed zero reading over the concentration range 5–60 μg ml^{-1}. The proposed methods were applied for the determination of the cited drugs in bulk and pharmaceutical preparations. ICH guidelines were used for method validation.

1. Introduction

Penciclovir (PCV), [2-amino-9-[4-hydroxy-3-(hydroxymethyl) butyl]-6,9-dihydro-3*H*-purin-6-one] and entecavir (ETV), [2-Amino-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-methylidenecyclopentyl]-6,9-dihydro-3*H*-purin-6-one] are antiviral drugs, Fig. 1.[1–3] PCV and ETV are nucleoside analogs, more specifically guanosine analogs, that inhibit reverse transcription, DNA replication and transcription in the viral replication process by inhibiting DNA polymerase.[4,5]

A detailed survey of analytical literature of PCV revealed several methods based on various techniques, HPLC using UV[6,7] fluorescence[8–11] and mass spectrometry[12,13] as detectors and capillary zone electrophoresis.[14] Besides, different analytical methods for the determination of ETV have been reported, including UV-spectrophotometry,[15,16] HPLC using UV[17–19] and mass spectrometry[20–24] as detectors, and capillary zone electrophoresis.[25]

Accordingly, the literature revealed that there is no reported spectrofluorimetric method for the determination of

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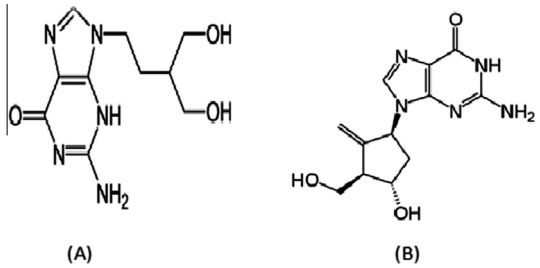

Figure 1 Chemical structures of (a) penciclovir, (b) entecavir.

PCV or ETV although they have native fluorescence which represents a direct method for the determination of the two drugs. Moreover, no spectrophotometric method has been reported for the determination of ETV in the presence of its acidic degradation. Thus, the development of these spectroscopic methods for the determination of the cited drugs in bulk and pharmaceutical preparations were of interest. Moreover, the device used for the determination is available in each QC laboratory.

2. Experimental

2.1. Instrumentation

A Shimadzu RF-1501 spectrofluorimetric (Kyoto-Japan). A Crest ultrasonic processor model 575DAE (USA). Electronic balance model HR200 (USA). UV Shimadzu UV/Visible recording spectrophotometer 1601 (Japan) connected to an IBM compatible computer and supported with UV probe software version 2.2.1.

2.2. Reagents and reference samples

Pharmaceutical grade PCV (certified to contain 99.00%) and penciclovir® cream, nominally containing 10 mg of PCV per g (batch No. 12042) were supplied by Sabaa Company, Egypt. ETV (certified to contain 99.90%) and Hepaclude® tablets, nominally containing 1 mg of ETV per tablet (batch No. 120953A) were supplied by Chemipharm Company, Egypt. Sulfuric acid analytical grade was supplied by Sigma–Aldrich Laborchemikalien GmbH, Germany and distilled water was supplied by ADWIC B.N.13100025, Egypt.

2.3. Standard and test solutions

2.3.1. Preparation of standard solutions

2.3.1.1. For spectrofluorimetric method. Standard stock solutions of 2.5 μg ml^{-1} of PCV and 1 μg $ml^{-1}$of ETV were prepared in 0.1 M H_2SO_4.

2.3.1.2. For derivative method. Standard stock solution of 200 μg ml^{-1} of ETV was prepared in distilled water.

2.3.2. Preparation of test solutions

2.3.2.1. For spectrofluorimetric method.

2.3.2.1.1. For penciclovir cream®. Penciclovir cream (0.50 g) was weighed and quantitatively transferred to a 100 ml volumetric flask, then dissolved and completed to volume with 0.1 M H_2SO_4. The solution was sonicated for 5 min then filtered through a filter paper. First 5 ml was rejected then an aliquot of the filtrate was diluted using 0.1 M H_2SO_4 to obtain a test solution of concentration of 2.5 μg ml^{-1}.

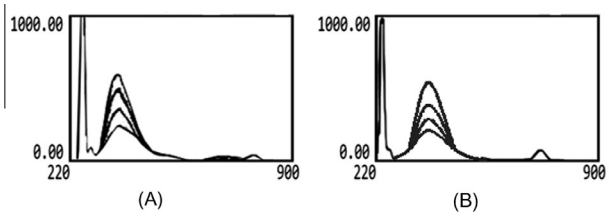

Figure 2 The fluorescence emission spectra of (A) Penciclovir at concentrations of 0.1, 0.15, 0.4, and 0.5 μg ml^{-1} and (B) Entecavir at concentrations of 0.025, 0.05, 0.1, and 0.2 μg ml^{-1}.

2.3.2.1.2. For Hepaclude tablets®. Ten tablets were accurately weighed and a quantity equivalent to 2 mg ETV was transferred to a 100 ml volumetric flask. Twenty ml of 0.1 M H_2SO_4 was added and the solution was sonicated for 15 min then completed to volume using the same solvent. The solution was filtered through filter paper to obtain a test solution of concentration 20 μg ml^{-1}. Further dilution was done using 0.1 M H_2SO_4 to obtain a final concentration of 1 μg ml^{-1}.

2.3.2.2. For derivative method. Ten Hepaclude tablets were accurately weighed and a quantity equivalent to 5 mg entecavir was transferred to a 25 ml volumetric flask. Ten ml distilled water was added and the solution was sonicated for 15 min then completed to volume using the same solvent and the solution was filtered to obtain a test solution of concentration 200 μg ml^{-1}.

2.3.3. Preparation of acidic ETV degradation stock solution

Entecavir (100 mg) was refluxed in 25 ml 5 N HCl for 72 h at 70 °C. An aliquot (1 ml) of this solution was neutralized using 5 N NaOH then completed to 100 ml in a volumetric flask with distilled water to prepare acidic ETV degradation stock solution containing degradation equivalent to 40 μg ml^{-1} of ETV.

2.4. General procedures and calibrations

2.4.1. For spectrofluorimetric method

Accurately measured aliquots of working standard solutions equivalent to 0.1–0.8 and 0.025–0.4 μg ml^{-1} of PCV and ETV respectively were separately transferred into two sets of 10 ml volumetric flasks; then each flask was completed to volume with 0.1 M H_2SO_4. The native fluorescence intensity (F) was measured and used to plot a curve against concentration (C), and the regression parameters were computed for each drug.

2.4.2. For derivative method

Accurately measured aliquots of working standard solutions equivalent to 5–60 μg ml^{-1} ETV were separately transferred into 10 ml volumetric flasks; then each flask was completed to volume with distilled water. The amplitude after the derivatization (A) with $\Delta\lambda = 8$ and scaling factor = 100 at 256.4 nm at which its acidic degradation showed zero reading, was measured and used to plot a curve against concentration (C), and the regression parameters were computed.

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