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ORIGINAL ARTICLE

Liquid chromatography–tandem mass spectrometry method for the estimation of adefovir in human plasma: Application to a pharmacokinetic study

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Abstract An analytical method based on solid phase extraction was developed and validated for analysis of adefovir in human plasma. Adefovir-d_4 was used as an internal standard and Synergi MAX RP80A (150 mm × 4.6 mm, 4 μm) column provided the desired chromatographic separation of compounds followed by detection with mass spectrometry. The method used simple isocratic chromatographic condition and mass spectrometric detection in the positive ionization mode. The calibration curves were linear over the range of 0.50–42.47 ng/mL with the lower limit of quantitation validated at 0.50 ng/mL. Matrix effect was assessed by post-column infusion experiment to monitor phospholipids and post-extraction addition experiment was performed. The degree of matrix effect for adefovir was determined as 7.5% and ion-enhancement in five different lots of human plasma was 7.1% and had no impact on study samples analysis with 4.5 min run time. The intra- and inter-day precision values were within 7.7% and 7.8%, respectively, for adefovir at the lower limit of quantification level. Validated bioanalytical method was successfully applied to clinical sample analysis.

1. Introduction

Adefovir, an acyclic phosphonate analog of deoxynucleoside monophosphate (IUPAC name: {[2-(6-amino-9H-purin-9-yl) ethoxy] methyl} phosphonic acid, PMEA), is a broad spectrum antiviral agent acting as a DNA polymerase inhibitor [1]. It has activity against herpes virus (Epstein–Barr) and retroviruses including the human immunodeficiency virus (HIV) [1]. Adefovir is largely used to treat chronic hepatitis B in adults, though the drug is reported for poor oral bioavailability [2]. The oral bioavailability of adefovir has been substantially improved by using the bis-pivaloyloxymethyl ester of adefovir (bis-POM PMEA, adefovir dipivoxil, Fig. 1) as a pro-drug

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with enhanced lipophilicity and achieving higher systemic adefovir levels. Adefovir dipivoxil spontaneously hydrolyzes to mono-POM-PMEA, which is rapidly converted into PMEA (adefovir) by enzyme. Adefovir is an acyclic nucleoside analog of adenosine monophosphate which is phosphorylated to the active metabolite adefovir diphosphate by cellular kinases [2].

Although several methods have been reported to quantify adefovir in human plasma [3–6] including serum [7], by employing liquid chromatography–tandem mass spectrometry (LC–MS/MS), analytical limitations could not be overcome. The published methods demonstrated LC–MS/MS method for adefovir estimation but lacked sensitivity and had lengthy run time [5,7]. Xie et al. [6] developed an LC–MS/MS method for the determination of adefovir with limit of quantitation 0.5 ng/mL but this method had matrix related issue. The reported method failed to use labeled/deuterated analog of adefovir for estimation from plasma to compensate equivalent matrix effect with that of analyte. Vela et al. [8] had developed an LC–MS/MS method using a very tedious and complex ion-pairing technique for adefovir estimation. An interesting LC–MS/MS method of adefovir had been reported with emphasis on hydrophilic interaction but failed to achieve lower limit of quantification (LOQ) below 1.00 ng/mL [9]. Moreover, Chen et al. [10] achieved sensitivity 0.25 ng/mL using protein precipitation extraction method. But the method had lengthy analysis run time (>7 min) and also the method-related issue was not addressed adequately.

Bioavailability/bioequivalence studies are frequently conducted on healthy volunteers with adefovir dipivoxil 10 or 20 mg tablet, marketed as Hespera (Gilead Sciences, Inc., Foster City, CA). Regulatory guidance [11,12] suggests that LOQ should be sufficient to characterize pharmacokinetic parameters based on expected peak plasma concentration (C_{max}). European Medicine Agency [12] suggests 5% of C_{max} should be achieved to have sufficient sensitivity to capture profile in elimination phase of a drug. A monograph on adefovir states that the following oral administration of a 10 mg single dose of Hespera in chronic hepatitis B patients, the mean C_{max} was 18.4 ng/mL with mean elimination half-life of 7.48 h [13]. But, published literature reflected high variation (14.9–24.7 ng/mL) in mean C_{max} for 10 mg adefovir tablet, though administered to healthy volunteers [9,10]. Such variation could be attributed to matrix effect or any other aspects of method limitations. Therefore, it becomes imperative to develop a precise, accurate, and high throughput method for estimation of adefovir in human plasma. For conducting the bioequivalence study on adefovir (i.e. 10 mg Hespera tablet), method sensitivity should be such that concentration profile up to 36 h (~5 half lives) could be plotted. Though 1.0 ng/mL LOQ could have sufficed [12] to characterize pharmacokinetic parameter, we further decreased method sensitivity to 0.5 ng/mL.

In the present study, a systematic evaluation of matrix interference was investigated by using protein precipitation extraction (PPE) followed by solid phase extraction (SPE) combination technique to bring down matrix effect below 10% level effectively. The unique method highlights adefovir stability as well as selectivity in blank (untreated) plasma, hemolyzed and lipemic plasma samples. The method had been successfully applied to clinical sample analysis.

2. Experimental

2.1. Chemicals and reagents

Working standards of adefovir (purity −99.35%) and adefovir-d_4 (deuterium labeled adefovir; purity −98.0%) were procured from Ranbaxy Research Laboratories Limited, India and Toronto Research Chemicals, Canada, respectively. Ammonium acetate, formic acid, liquor ammonia and methanol were purchased from Qualigens Fine Chemicals (GSK Ltd., Mumbai, India). Oasis® MAX (30 mg/1 cc) solid phase cartridges were purchased from Waters Corporation (Milford Massachusetts, USA). Water was purified using a Milli-Q device (Millipore, Bangalore, India). Drug-free (blank) human plasma containing K_3EDTA (ethylenediaminetetraacetic acid tripotassium salt), as anticoagulant, was obtained from Yash Laboratories, New Delhi, India.

2.2. Preparation of calibration standards and quality control samples

Adefovir, a water insoluble, polar drug [13], was found to be better solubilized in acidified water (pH ~1.2). Stock solutions of adefovir and internal standard (ISTD) were prepared separately by dissolving the accurately weighed compounds in acidified water to obtain a final concentration of approximately 1 mg/mL. Stock solutions were stored at refrigerated temperature (1–10 °C). Two separate stock solutions of adefovir were prepared for bulk spiking of calibration standards (CS) and quality control (QC) samples. Primary dilutions and working standard solutions were prepared from stock solutions using methanol:water (50:50, v/v). These working (standard) solutions were used to prepare the CS and QC samples. Blank human K_3EDTA plasma was screened prior to spiking to ensure that it was free from endogenous interference at retention times of adefovir and ISTD. Eight-point calibration standards (CS) and QC samples were prepared by spiking the

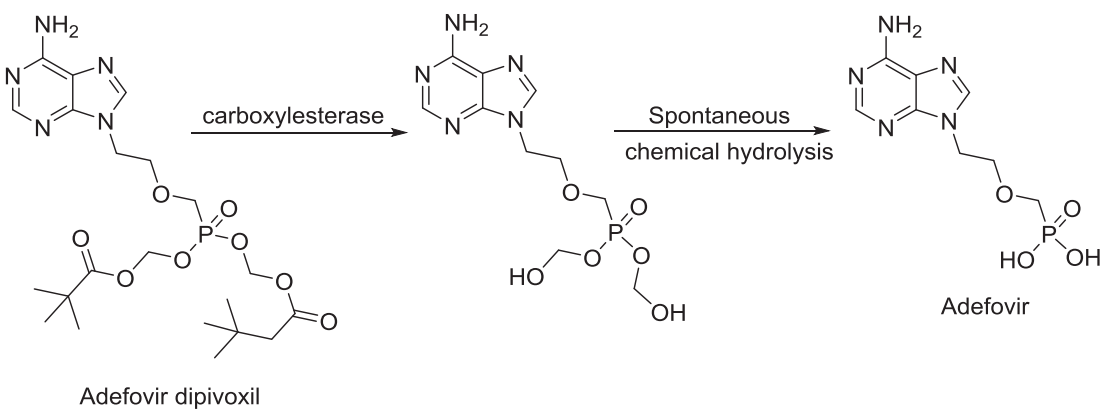

Fig. 1 In-vivo hydrolysis of adefovir dipivoxil to adefovir.