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Development and validation of a method for quantitative determination of valsartan in human plasma by liquid chromatography-tandem mass spectrometry

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Abstract

A sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of valsartan in human plasma was developed and validated. A 0.5 ml aliquot was extracted using solid-phase extraction in an Empore® high performance extraction disk plate, universal resin 96-well format. The estimated calibration range of the method was 2–2000 ng/ml.

The method was fully validated with intra-day mean accuracy and precision of 94.8–107% and 2.19–5.40% and inter-day mean accuracy and precision of 93.5–105% and 1.87–5.67%, respectively. No significant loss of valsartan in processed samples was confirmed in processed samples for up to 24 h at 10 °C. Sample dilution up to 50-fold with blank human plasma provided acceptable analyses. No interference peaks or matrix effects were observed. No effect of QC sample location results was observed in a 96-well plate. This LC-MS/MS technique was found to improve quantitative determination of valsartan allowing its pharmacokinetic evaluation with clinically relevant doses.

Keywords: Liquid chromatography-tandem mass spectrometry; Valsartan; Human pharmacokinetics; Matrix effects; Inter-subject variability

1. Introduction

Valsartan (*N*-valeryl-*N*[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl] methyl]valine, Fig. 1) is an orally active, potent and specific competitive angiotensin II antagonist acting at the ATI receptor, which mediates all known effects of angiotensin II on the cardiovascular system. Valsartan is widely used in the treatment of hypertension [1]. The drug in unchanged form shows strong pharmacologically activity with high affinity for the ATI receptor. Valsartan is metabolized only to a small extent (ca. 10%) and even its most abundant metabolite (M1) possesses negligible affinity for the ATI receptor (1/200 that of valsartan). In order to determine the relationship between exposure and receptor response, an analytical method for unchanged valsartan with high accuracy is of great importance. The pharmacokinetics (PK) of valsartan in human shows rapid absorption with a peak plasma concentration reached about 2 h after administration and a terminal elimination phase with a half-life of about 7 h [2]. There is considerable individual variability (CV of ca. 30%) in peak plasma concentrations (C_{max}) and area under the curve (AUC) [3]. Therefore, an analytical method for the determination of valsartan in human plasma that is not affected by inter-subject sample deviation is required for appropriate PK evaluation.

Several analytical methods for the determination of valsartan in human plasma by high performance liquid chromatography (HPLC) have been reported [4–7]. These methods utilize a fluorescence detector (FP) after extraction from plasma by solid-phase extraction (SPE) [4,5] or liquid–liquid extraction [6]. They require long chromatographic run times of more than 10 min/sample. Recently, a HPLC/FP analytical method with short analysis run time (ca. 3 min/sample) has been reported [7]. This method, however, has a lower limit of quantification (LLOQ) of only 100 ng/ml. Available PK profiles were only provided for the higher-dose (160 or 320 mg) subjects. In order to eliminate these obstacles, we selected a liquid

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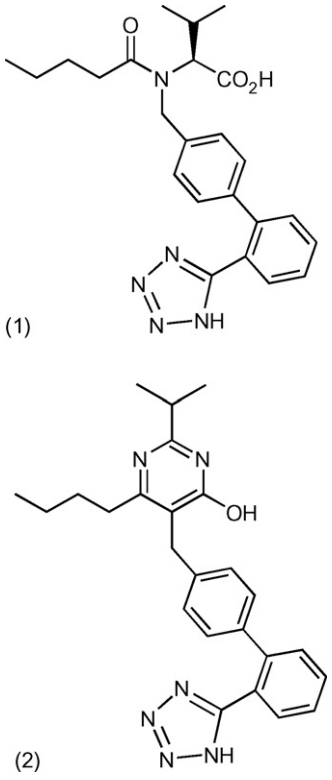

Fig. 1. Chemical structures of (1) valsartan and (2) its analytical internal standard (CGP48791).

chromatography-tandem mass spectrometry (LC-MS/MS) as an analytical instrument. The target features of the method are high sensitivity (<LLOQ of a 5 ng/ml) and short analytical run time (<10 min). For achieving high throughput before LC-MS/MS analysis, solid-phase extraction (SPE) in a 96-well format was also investigated.

The reliability of quantitative LC-MS/MS data obtained from determination of drugs in biological matrices can be adversely affected by endogenous components in biological fluids. Matrix effects are a major issue in quantitative bioanalytical examination using LC-MS/MS. Taylor reported that assessment of matrix effects from individual matrix sources was required to support quantitative LC-MS/MS analysis. Inter-subject variability within 15% at each quality control (QC) level (expressed as % relative standard deviation; %R.S.D.) has been proposed as an acceptance criterion [8]. This approach has been used and found practical in our previous study [9]. If an analytical method is compromised by matrix effects, its results may not be reliable. In the present study, matrix effects were also evaluated based on Taylor's approach.

Assessment of intra-well variability in a 96-well format has been reported [10–12]. Likewise, intra-well variability was evaluated in our validation study. The 96-well format SPE was used for simplification of sample preparation procedure.

The purpose of the present study was to develop and validate a simple, accurate, reproducible and sensitive analytical method for the determination of valsartan in human plasma using a 96-well format SPE for the sample preparation procedure and an LC-MS/MS instrumentation without individual or intra-well variability. The analytical method established was to be applied to samples obtained from a PK study in healthy volunteers who received oral dose of valsartan.

2. Experimental

2.1. Chemicals and reagents

Valsartan and CGP48791 (analytical internal standard, IS, Fig. 1) were supplied by Novartis Pharma AG (Basel, Switzerland). Methanol and acetonitrile (HPLC grade) were purchased from Kanto Chemical Co Inc. (Tokyo, Japan) and trifluoroacetic acid (HPLC grade) from PIERCE (Rockford, IL, USA). Water was deionized and purified on a Millipore water purification system (Milford, MA, USA). Human heparinized plasma was purchased from the New Drug Development Research Center (Iwamizawa, Japan).

2.2. Calibration standards (CS), quality control (QC), mock samples and dilution test samples

Stock solutions for CS and QC were prepared separately in methanol. CS samples were prepared by spiking into heparinized human plasma of respective stock solutions of valsartan to yield concentrations of 2, 5, 20, 100, 500, 1000 and 2000 ng/ml. QC samples were prepared at 2, 4, 16, 80, 400 and 1800 ng/ml in the same manner as CS samples. Mock samples were used to ascertain reproducible extraction on the entire 96-well plate. The mock samples were prepared by adding stock solution to blank human plasma aliquots to obtain a final concentration of 80 ng/ml.The results from the mock samples were not used in any other evaluations.

Dilution test samples were prepared by spiking in blank human plasma of respective methanol standard solutions. The concentrations used for the dilution test were 1800 (the highest QC sample), 5000 and 10,000 ng/ml.

All prepared plasma samples were stored at −20 °C and all prepared stock solutions were stored at 4 °C.

2.3. Sample preparation

A 500 μl aliquot of each plasma sample was placed in each well of 96-well collection plates along with 50 μl of IS solution (500 ng/ml in methanol/H_2O, 50/50, v/v) and 250 μl of 2% trifluoroacetic acid. The entire volume of the resulting sample was transferred to a 96-well solid-phase extraction plate (Empore® high performance extraction disk plate, universal resin, 3 M, NJ, USA) that had been pretreated with 100 μl of methanol and 500 μl of 1% trifluoroacetic acid. Each well was rinsed, in order, with 800 μl of 1% trifluoroacetic acid, 800 μl of 1% trifluoroacetic acid/methanol (95/5, v/v), 800 μl of 1% trifluoroacetic acid/methanol (80/20, v/v), and 800 μl of 1% trifluoroacetic acid. The sample was eluted into a 96-well collection plate with 500 μl of a methanol/water solution (90/10, v/v). The eluate was evaporated to dryness under a nitrogen stream at 50 °C. The residue was reconstituted in 100 μl of methanol/0.1% trifluoroacetic acid (50/50, v/v), transferred to a 96-well filter plate