Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

# New ultra-performance liquid chromatography-tandem mass spectrometry method for the determination of irbesartan in human plasma

*Tanveer A. Wani* [a,*], *Seema Zargar* [b]

[a] *Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*
[b] *Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia*

ARTICLE INFO

*Article history:*
Received 22 October 2014
Received in revised form
9 February 2015
Accepted 10 February 2015
Available online 26 March 2015

*Keywords:*
high throughput analysis
irbesartan
pharmacokinetic
tandem mass spectrometry
toxicokinetic
ultra-performance liquid chromatography

ABSTRACT

With the objective of reducing analysis time and maintaining good efficiency, there has been substantial focus on high-speed chromatographic separations and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) is a preeminent analytical tool for rapid biomedical analysis. In this study a simple, rapid, sensitive, and specific ultra-performance liquid chromatography-MS/MS method was developed and validated for quantification of the angiotensin II receptor antagonist, irbesartan (IRB), in human plasma. After a simple protein precipitation using methanol and acetonitrile, IRB and internal standard (IS) telmisartan were separated on Acquity UPLC BEH C18 column (50 mm × 2.1 mm, i.d. 1.7 μm, Waters, Milford, MA, USA) using a mobile phase consisted of acetonitrile: methanol: 10 mM ammonium acetate (70: 15: 15 v/v/v) with a flow rate of 0.4 mL/min and detected MS/MS in negative ion mode. The ion transitions recorded in multiple reaction monitoring mode were *m/z* 427.2→193.08 for IRB and *m/z* 513.2→469.3 for IS. The assay exhibited a linear dynamic range of 2–500 ng/mL for IRB in human plasma with good correlation coefficient of (0.995) and with a lower limit of quantitation of 2 ng/mL. The intra- and interassay precisions were satisfactory; the relative standard deviations did not exceed 9.91%. The proposed UPLC-MS/MS method is simple, rapid, and highly sensitive, and hence it could be reliable for pharmacokinetic and toxicokinetic study in both animals and humans.



* *Corresponding author*. Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, P.O. Box 2457, Riyadh 11451, Saudi Arabia.
E-mail address: tanykash@yahoo.co.in (T.A. Wani).

http://dx.doi.org/10.1016/j.jfda.2015.02.008

## 1. Introduction

Irbesartan, 2-butyl-3-{[2-(tetrazol-5-yl)biphenyl-4-yl]-methyl}-1,3-diazaspiro[4.4]non-1-en-4-one, is a potent and selective angiotensin II subtype 1 receptor antagonist widely used for the treatment of hypertension and heart failure in clinical patients. Angiotensin II is an octapeptide regarded as the main effector of AT1 receptor in the renin–angiotensin system. It causes vasoconstriction, tachycardia, increase of aldosterone secretion from the adrenal cortex, and retention of sodium and body fluid [1,2]. The oral absorption of irbesartan is rapid and complete with an average absolute bioavailability of 60–80%. Following an oral administration of irbesartan in therapeutic dose (75–300 mg), peak plasma concentration of irbesartan is attained at 1.5–2 hours after dosing. Irbesartan exhibits linear pharmacokinetics over the therapeutic dose range [1–4]. Irbesartan also demonstrates superior antihypertensive efficacy versus losartan and valsartan [5]. Angiotensin II receptor blockers have been the choice of drugs for diabetic nephropathy by the World Health Organization (WHO)/International Society of Hypertension (ISH) guidelines [6].

A literature review revealed that there are several analytical methods reported for the quantitative estimation of IRB in biomatrices mainly based on liquid chromatography [7–22], capillary electrophoresis (CE) [23–26], and spectrophotometry [27–30]. Liquid chromatography is the major method for measurement of irbesartan in human plasma and urine. It is combined with a UV detector [8,9], diode array detector (DAD) [10–12], fluorescence (Flu) detector [13–17], electrospray ionization mass spectrometric detection [18], and tandem quadrupole mass spectrometer [19–22]. Literature survey also revealed that there is a high variation in the limit of quantitation (1–10 μg/mL) as reported by different authors [8–12]. There are many sample preparation methods used in biological samples, such as solid-phase extraction (SPE), solid-phase microextraction (SPME), liquid–liquid extraction (LLE), and protein precipitation [7–22]. SPE is the principal way to clean up the biosamples, but it is complicated and time-consuming. SPME is a very suitable sample preparation technique for a small amount of samples, but it is not widely used in human plasma samples. LLE has a tedious sample processing process and is time consuming.

Ultra-performance liquid chromatography (UPLC) is a new category of separation science that builds upon well-established principles of liquid chromatography, using sub-2μm porous particles. These particles operate at elevated mobile phase linear velocities to produce significant reductions in separation time and solvent consumption. Literature indicates that a UPLC system allows approximately ninefold decreases in analysis time as compared to the conventional high-performance (HP) LC system using 5-μm particle size analytical columns, and approximately threefold decrease in analysis time in comparison with 3-μm particle size analytical columns without compromise on overall separation [31–35]. Acquity UPLC columns contain hybrid X-Terra sorbent, which utilizes bridged ethyl siloxane/silica hybrid (BEH) structure, ensures the column stability under the high pressure and wide pH range (1–12) [35]. In all documented references, no UPLC-MS/MS method has been used to determinate IRB presence and concentration in human plasma until now.

The current study describes the development and validation of a UPLC method coupled with tandem mass spectrometry (UPLC-MS/MS) for the determination of IRB in human plasma. The proposed method used is a relatively simple extraction procedure using methanol and acetonitrile to directly precipitate protein in combination with UPLC-MS/MS detection.

## 2. Materials and methods

Irbesartan and telmisartan were obtained from AK Scientific Inc. (California, USA). Human plasma was obtained from normal healthy volunteers at King Khalid University Hospital (Riyadh, Saudi Arabia), and they were kept frozen at −20°C until analysis. HPLC-grade acetonitrile, methanol, and ammonium acetate were obtained from WinLab, London, UK. All other reagents were of analytical grade unless stated otherwise. All aqueous solutions was prepared using water that was purified using Milli-QR Gradient A10R (Millipore, Moscheim Cedex, France) having a pore size 0.22 μm.

### 2.1. Liquid chromatography

The UPLC system included quaternary solvent manager, a binary pump, degasser, autosampler with an injection loop of 10 μL and a column heater-cooler. The separation was performed on Acquity UPLC BEH $C_{18}$ column (50 mm × 2.1 mm, i.d., 1.7 μm, Waters, Milford, MA, USA) maintained at 25°C. The mobile phase was composed of acetonitrile: methanol: 10 mM ammonium acetate acid (70:15:15 v/v/v) pumped at a flow rate of 0.4 mL/min. The injection volume was 5 μL in partial loop mode and the temperature of the autosampler was kept at 4°C.

### 2.2. Mass spectrometric conditions

Waters Acquity liquid chromatography system coupled with a Waters TQD triple quadrupole mass spectrometer was used. Mass spectrometric detection was carried out using an electrospray interface (ESI) operated in the negative ionization mode with multiple reaction monitoring (MRM) for both IRB and IS. Nitrogen was used as a desolvating gas at a flow rate of 500 L/h. The desolvating temperature was set at 400°C and the source temperature was set at 150°C. The collision gas (argon) flow was set at 0.1 mL/min. The capillary voltage was set at 3.2 kV. The MS analyzer parameters were as follows: LM1 and HM1 resolution 10.0 and 8.0; ion energy 1, 1 V; LM2 and HM2 resolution 15.0 and 10.0, respectively; ion energy 2, 0.1 V, dwell time, 0.146 seconds. The cone voltage and collision energy were optimized in case of each analyte so as to maximize the signal corresponding to the major transition observed in the MS/MS spectra, following the fragmentation of the $[M+H]^+$ ions corresponding to the selected compounds. The Mass Lynx software (Version 4.1, SCN 805, Waters, Milford, MA, USA) was used to control the UPLC-MS/MS system as well as for data acquisition and processing.