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Method development and validation of Irbesartan using LC-MS/MS: Application to pharmacokinetic studies

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ABSTRACT

A simple, accurate liquid chromatography with tandem mass spectrometry (LC/MS-MS) method has been developed and validated in human plasma. The method employed liquid-liquid extraction. Samples containing Irbesartan were chromatographed on a Hypersil gold column (C18, 5µm, 100 x 4.6 mm) at a temperature of 40°C. The isocratic mobile phase composition was a mixture of 2 mM ammonium formate (pH 4.0) / methanol (20:80 v/v), which was pumped at a flow rate of 0.5 mL / min with split ratio of 20:80.The retention time under these chromatographic conditions was found to be 2.20 minutes with run time 2.82 minute. Ethyl acetate & n-Hexane (80:20, v/v) was found to be good extracting and produced a satisfactory chromatogram. The developed LC/MS-MS method was found to be selective, simple, sensitive, accurate and linear for the analysis of Irbesartan in human plasma. The retention time and in-turn run time was very short, hence required less mobile phase for the method, making it more economical and rapid. The method was applicable for the pharmacokinetic study of Irbesartan.

Key words: Irbesartan, LC/MS-MS, Validation, Plasma.

INTRODUCTION

Irbesartan is a non-peptide compound, chemically described as 2-butyl-3-[*p* -(*o* -1 *H* -tetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one[1] used in hypertension. Literature survey reveals that few HPTLC and HPLC methods are reported for the estimation of irbesartan in biological samples such as urine and plasma [2, 3]. The present study was designed to develop and validate a new LC/MS-MS method for the analysis of Irbesartan in human plasma. Several HPLC methods have been described previously for the determination of Irbisartan in

pharmaceuticals and biological samples [4-12]. This paper described a newly developed LC–MS/MS method for the quantitation of Irbesartan in human plasma with more sensitivity compared to other methods.

EXPERIMENTAL SECTION

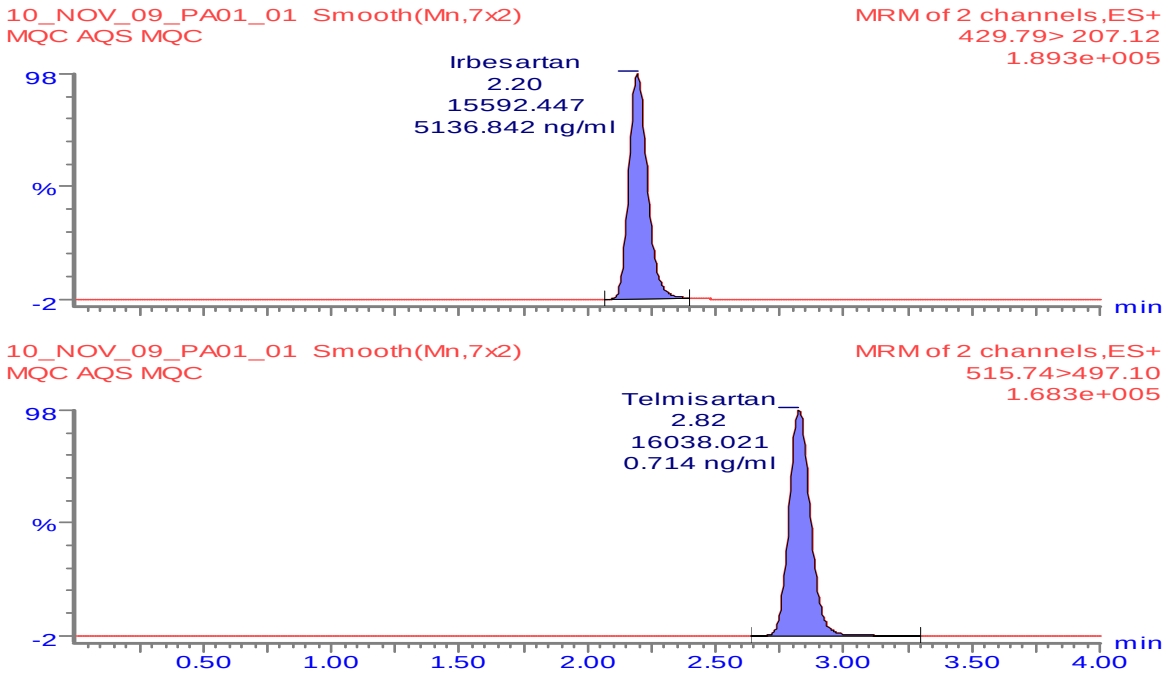
Materials

Irbesartan reference standards and Telmisartan (internal standard) was obtained from the Dr.Reddy's Laboratories Ltd, Hyderabad, India, Ethyl acetate (GR Grade), n-hexane (GR Grade), Acetonitrile (HPLC Grade), methanol (HPLC Grade), Di-Potassium hydrogen phosphate anhydrous (GR Grade) and Ammonium format (GR Grade) from Merck (India). A Milli-Q system (Millipore, Bedford, MA, USA) was used.

Instrumentation

The system (Waters, Milford, USA) is equipped with an Acquity SM sample manager, Acquity BSM binary solvent manager and thermo stated column compartment. The chromatography was performed on a Hypersil gold column (C18, 5µm, 100 x 4.6 mm) at a temperature of 40°C. The isocratic mobile phase composition was a mixture of 2 mM ammonium formate (pH 4.0) / methanol (20:80 v/v), which was pumped at a flow rate of 0.5 mL / min with split ratio of 20:80. Mass spectrometric detection was performed on a Quattro premier XE triple quadrapole instrument (Waters, Milford, USA) using multiple reaction monitoring (MRM). A turbo electrospray interface in positive ionization mode was used. Data processing was performed using Masslynx 4.1 software.

Figure 1 Representative Chromatogram of an Aqueous Standard Solution for Irbesartan



Preparation of standard solution

A stock solution was prepared by dissolving accurately weighed quantity of irbesartan in methanol to yield a final concentration of 1mg /mL, sonicated for 5 minutes, allowed to equilibrate to room temperature and suitably diluted with methanol. The stock solution was

further diluted by suitable dilution with methanol. The standard chromatogram is presented in Figure 1.

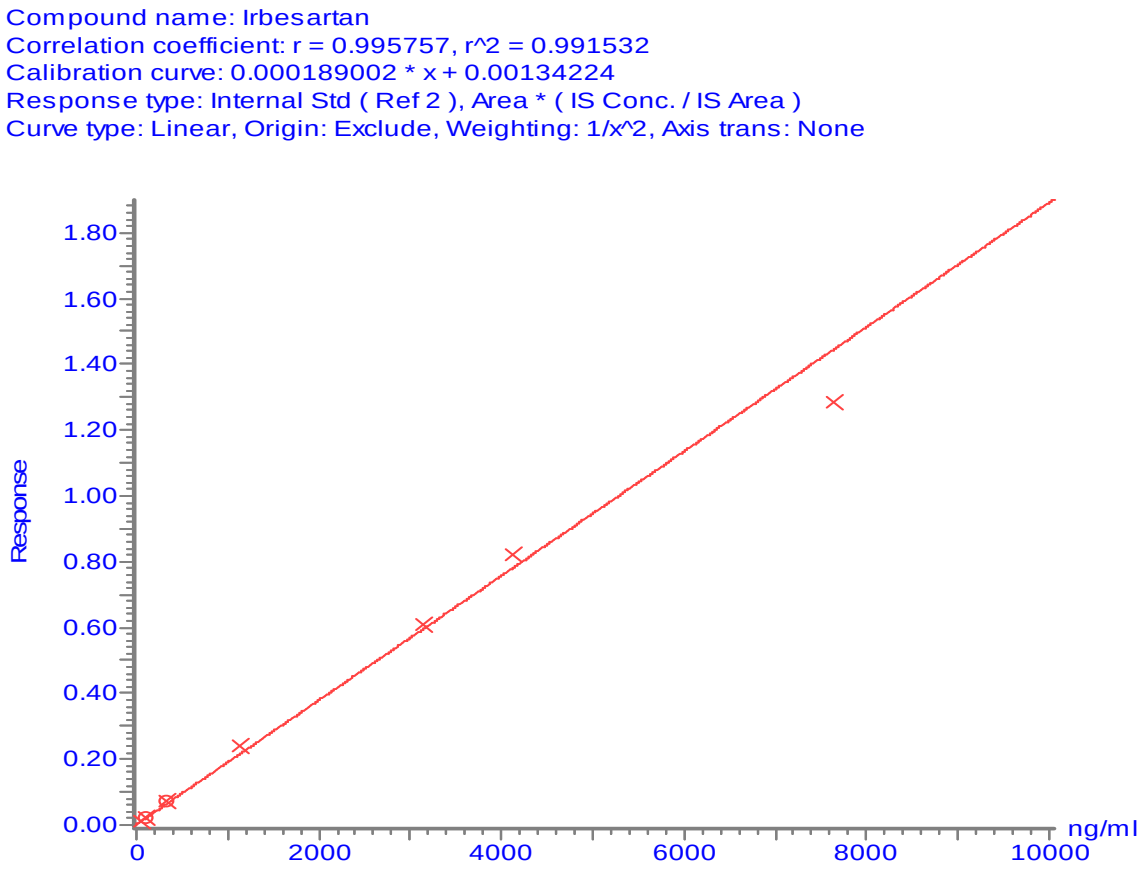
Extraction of Irbesartan from plasma

A 100 µL volume of plasma was transferred to a 4mL ria vial, and then 50 µL of IS working solution (5.0 µg/mL) was spiked. After vortexing for 30 sec, add 100 µL of 1.0 M Di-Potassium hydrogen phosphate anhydrous solution. Then 2.5 mL aliquot of the extraction solvent ethyl acetate: n-Hexane (80:20, v/v) was added. The sample was vortex-mixed for 10 min and then centrifuged at 1891 $\pm$ 100 for 5 minutes at 10°C. The organic layer (2.0 mL) was quantitatively transferred to a 4 mL glass tube and evaporated to dryness at 40°C under a stream of nitrogen. Then, the dried extract was reconstituted in 500 µL of Mobile phase and a 5 µL aliquot was injected into the chromatographic system.

Preparation of calibration curve and Quality control samples

Standard stock solution of Irbesartan (2 mg/mL) and Telmisartan ISTD (1 mg/mL) were separately prepared in methanol. Spiking solutions for calibration curve and quality controls were prepared by appropriate dilution in methanol:water (50:50). The IS working solution (5 µg/mL) was prepared by diluting its stock solution with methanol:water (50:50). Spiking solutions (0.2 mL) were added to drug-free human plasma (9.8 mL) as a bulk, to obtain Irbesartan spiking concentration levels of 45.8280 to 10052.5460 ng/mL. The calibration curve is presented in Figure 2.

Figure 2 Representative Regression Analysis of a Calibration Curve for Irbesartan



The quality control pools were divided into aliquots and stored in the freezer at −70°C until analysis. Each validation run consisted of a double quality control, system suitability sample, blank samples (a plasma sample processed without an IS), a zero sample (a plasma processed with IS), calibration curve consisting of eight non-zero samples covering the total range (45.8280 to 10052.5460 ng/mL) and QC samples at four concentrations (n = 6, at each concentration). Such validation runs were generated on 4 consecutive days. Calibration samples were analyzed from low to high at the beginning of each validation run and other samples were distributed randomly through the run. Linearity was assessed by a weighted ($1/x^2$) least squares regression analysis and the calibration curve had a correlation coefficient (r^2) of 0.99. The acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value except. LLOQ.

System suitability tests

Throughout the study, the suitability of the chromatographic system was monitored by calculating the trailing/asymmetry factor, theoretical plates and relative standard deviation.

Intraday accuracy and precision

Within-batch and between-batch accuracy and precision evaluations were performed by repeated analysis of Irbesartan in human plasma. The run consisted of a calibration curve with six replicates of each LLOQ, low, medium and high quality control samples. During routine analysis, each analytical run included a set of calibration samples, a set of QC samples in duplicate and plasma samples to be determined. The overall precision of the method expressed as relative standard deviation and accuracy of the method.

RESULTS AND DISCUSSION

The mobile phase selected achieved a good resolution and symmetric peak shapes for the analyte and IS with a short run time. The high proportion of organic solvent eluted the Irbesartan and Telmisartan (IS) at retention times of 2.54 and 3.15 min, respectively. A flow rate of 0.5 mL/min produced good peak shapes and permitted a run time of 4.0 min. Liquid-liquid extraction (LLE) was used for the sample preparation in this work. A mixture of ethyl acetate & n-Hexane (80:20, v/v) was found to be optimal, which can produce a clean chromatogram for a blank plasma sample. The average recoveries of Irbesartan from spiked plasma samples at low, medium and high level are 70.76%, 54.62% and 64.66% respectively and for Telmisartan (ISTD) is 90%. Recoveries of the analytes and IS were good and it was consistent, precise and reproducible. Therefore, the method has proved to be robust in high-throughput bioanalysis. The percentage CV of matrix factor for Irbesartan and internal standard were found to be 3.44 and 4.02 respectively. The matrix effect percentage of Irbesartan and Internal Standard were found to be 89.59 and 94.99 respectively.

As all data fall within the FDA guidelines, we conclude that the degree of matrix effect was sufficiently low to produce acceptable data and the method can be considered as valid. There were no interfering peaks present in the specificity study. The accuracy values for between and within-batch studies at the LLOQ and at low, medium and high concentrations of Irbesartan in plasma were within acceptable limits (n=6) (Table 1 & 2).

Table 1 Intra-batch Precision and Accuracy of Irbesartan

QC ID	LOQQC	LQC	MQC	HQC
Actual Concentration (ng/mL)	**46.3920**	**122.0860**	**4181.0180**	**7466.1040**
TRIALP&A - 01	56.1368	109.1036	3982.7360	7564.9464
	59.5375	109.8563	4180.0470	7806.0204
	44.9463	115.4619	4154.7798	7857.2726
	49.7561	138.6521	4395.9020	7618.1380
	42.1524	106.5795	4260.5413	7261.3645
	42.0944	119.7933	4303.3897	7752.8609
Mean	**49.10392**	**116.57445**	**4212.89930**	**7643.43380**
SD	**7.395798**	**11.831040**	**142.394210**	**217.675366**
%CV	**15.06**	**10.15**	**3.38**	**2.85**
%Nominal	**105.85**	**95.49**	**100.76**	**102.38**
P&A - 01	42.6291	120.0145	3513.6415	5912.6907
	34.2259	106.5976	4105.3525	6861.6116
	44.8543	117.7216	4099.8027	6706.1650
	48.5606	113.5732	4177.4517	7108.4657
	53.7434	128.1663	3718.9795	7180.9537
	48.6064	130.2663	3963.6062	7973.3084
Mean	**45.43662**	**119.38992**	**3929.80568**	**6957.19918**
SD	**6.673992**	**8.899119**	**260.701746**	**673.584249**
%CV	**14.69**	**7.45**	**6.63**	**9.68**
%Nominal	**97.94**	**97.79**	**93.99**	**93.18**
P&A - 02	46.5957	130.2384	4493.6161	8880.4926
	46.3241	142.7414	4217.2103	8333.6467
	44.5531	138.9865	4367.3197	8123.7939
	47.2754	105.0805	4298.6353	7397.8854
	49.8179	119.3640	6575.6248*	6317.2861
	48.5787	122.3038	4693.9951	6509.9986
Mean	**47.19082**	**126.45243**	**4414.15530**	**7593.85055**
SD	**1.838361**	**13.863502**	**186.380061**	**1032.118234**
%CV	**3.90**	**10.96**	**4.22**	**13.59**
%Nominal	**101.72**	**103.58**	**105.58**	**101.71**

Table 2 Inter-batch or Total Precision and Accuracy of Irbesartan

QC ID	LOQQC	LQC	MQC	HQC
Actual Concentration (ng/mL)	**46.3920**	**122.0860**	**4181.0180**	**7466.1040**
TRIALP&A -01	56.1368	109.1036	3982.7360	7564.9464
	59.5375	109.8563	4180.0470	7806.0204
	44.9463	115.4619	4154.7798	7857.2726
	49.7561	138.6521	4395.9020	7618.1380

	42.1524	106.5795	4260.5413	7261.3645
	42.0944	119.7933	4303.3897	7752.8609
P&A -01	42.6291	120.0145	3513.6415	5912.6907
	34.2259	106.5976	4105.3525	6861.6116
	44.8543	117.7216	4099.8027	6706.1650
	48.5606	113.5732	4177.4517	7108.4657
	53.7434	128.1663	3718.9795	7180.9537
	48.6064	130.2663	3963.6062	7973.3084
P&A -02	46.5957	130.2384	4493.6161	8880.4926
	46.3241	142.7414	4217.2103	8333.6467
	44.5531	138.9865	4367.3197	8123.7939
	47.2754	105.0805	4298.6353	7397.8854
	49.8179	119.3640	6575.6248	6317.2861
	48.5787	122.3038	4693.9951	6509.9986
Mean	**47.24378**	**120.80560**	**4305.70173**	**7398.16118**
SD	**5.705881**	**11.801255**	**627.365175**	**751.047684**
%CV	**12.08**	**9.77**	**14.57**	**10.15**
%Nominal	**101.84**	**98.95**	**102.98**	**99.09**

CONCLUSION

A simple, sensitive, and reliable LC/MS-MS method has been developed and validated for the determination of Irbesartan in human plasma. The method is accurate, reproducible, and specific. The retention time and in-turn run time was very short, hence required less mobile phase for the method, making it more economical and rapid. The method may be applicable for pharmacokinetic studies of Irbesartan in human plasma.

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