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Research Article

LC/MS/MS METHOD FOR THE SIMULTANEOUS ESTIMATION OF LOSARTAN POTASSIUM AND IRBESARTAN IN RAT PLASMA

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ABSTRACT

A rapid and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method has been developed and validated for simultaneous quantification of Losartan potassium (LOS) and Irbesartan (IRB) in rat plasma using Phenomenex polar RP 80 4µ.The mass transition ion-pair has been followed as *m/z* 423.4→207.2 for LOS, *m/z* 429.3→195.1 for IRB.The method involves precipitation extraction from plasma, with gradient elution chromatographic conditions and mass spectrometric detection using an API 3000 instrument that enables detection at nanogram levels. Ketoconazole was used as the internal standard. The proposed method has been validated with a linear range of 5.01–1000.8 ng/ml for LOS and for IRB.

Keywords: Losartan, Irbesartan, API 3000 LC/MS/MS

INTRODUCTION

Irbesartan chemically (2-butyl-3-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one) and Losartan (2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol monopotassium salt) belongs to Angiotensinogen antagonists class of antihypertensive drugs .IRB is itself an active molecule but LOS converts to 5-carboxylic acid metabolite in the body. Ketoconazole an anti-fungal drug was used as an internal standard. The mass transition ion-pair for Ketoconazole (KET) *m/z* 531.2 → 82.0.

Different methods have been reported in the literature for monitoring plasma levels of LOS[2,3] and IRB individually[4,5] and in dosage forms also[1,6]. Some other techniques used in individual analysis of LOS from plasma include HPLC with Mass Spectroscopy, UV but these are not sensitive. Since there is no specific method available in literature for the quantification of Losartan and Irbesartan in rat plasma using LC/MS/MS system, the aim of the study was the development and validation of simple, sensitive, rapid and specific method.

EXPERIMENTAL

Chemicals used

Dimethylacetamide (Sigma), Ethanol (Hayman), Propylene glycol (Merck) Water for injection were used in formulation and Acetonitile (Merck), Methanol (Merck), Millipore water (in house) were used in method development.

Instrumentation

API 3000 LC/MS/MS was used.

MASS PARAMETERS

The mass spectrometer was operated in the positive ion mode. The developed mass and LC parameters for the estimation of LOS and IRB with Ketoconazole (KET) as internal standard are given below.

The compound dependent parameters for Losartan Declustering potential was 50eV, Focussing potential was 170eV, Entrance potential10eV, Collision energy CE 35 psi.

For Irbesartan Declustering potential was 53eV, Focusing potential was 185 eV, Entrance potential 8 eV, Collision energy CE 31 V.

For Ketoconazole Declustering potential was 40eV, Focusing potential was 200 eV, Entrance potential 10 eV, Collision energy CE 95 V.

Source dependent parameters for the method are CUR -15 psi, TEMP -550°c, ISV-5500 V, CAD-18psi.

MULTI REACTION MONITORING (MRM)

The mass transition ion-pair has been followed as *m/z* 423.4→207.2 for LOS, *m/z* 429.3→195.1 and *m/z* 531.2→82.0 for KET.

Fig. 1: Parent ion scan of Irbesartan

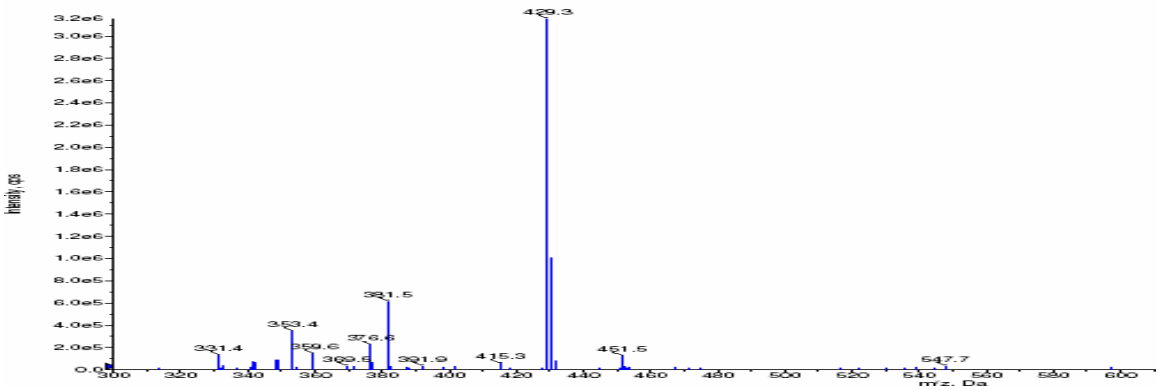


Fig. 2: Product ion scan of Irbesartan

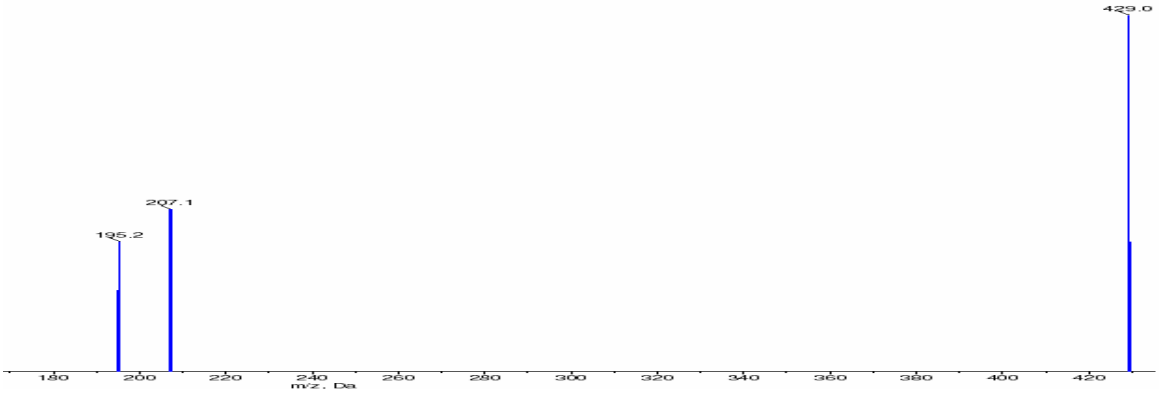
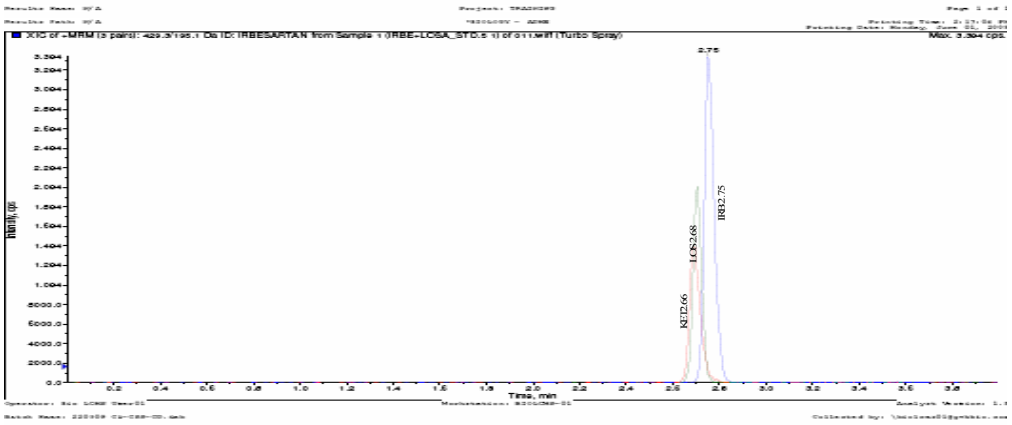


Fig. 3: Chromatogram representing Losartan, Irbesartan and Ketoconazole



From the chromatogram above the retention time (Rt) for Ketoconazole was found to be 2.66 min, Losartan 2.68 min and Irbesartan 2.75 min.

LC parameters

Column : Synergi 4µ POLAR- RP 80 A, 50*2mm

Mobile phase : A= 0.1% Formic acid
B= 100% Methanol
Injection volume : 10µL
Column oven temperature : 40°c

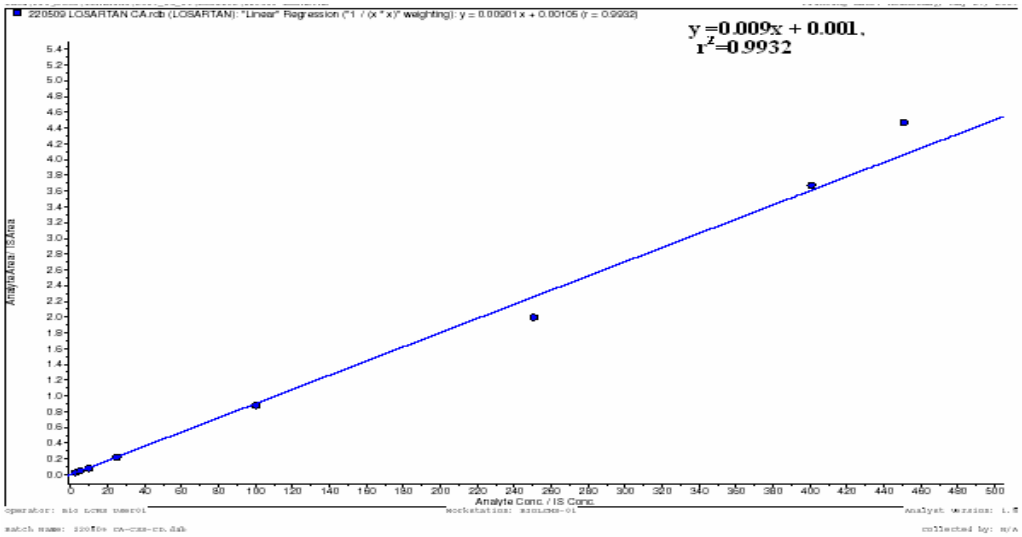
GRADIENT SYSTEM

Time (min)	Flow Rate (µL/min)	A%	B%
0	1000	95	5
2	1000	5	95
3	1000	95	5
4	1000	95	5

Preparation of calibration curve standards and quality controls for simultaneous estimation of Losartan and Irbesartan

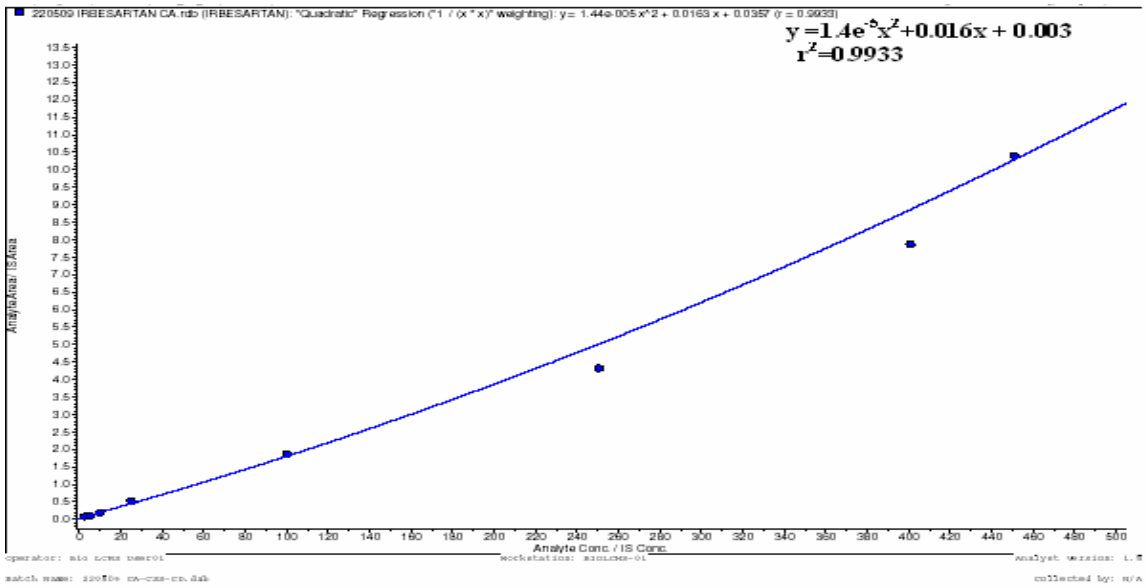
To 96µl of plasma 4µL of standard solution (SS) of Losartan was added and vortexed. Similarly 4µL of standard solution of Irbesartan was added to 96µL plasma and vortexed. These two spiked plasma samples were are pooled and 100µL was taken from it. To it acetonitrile containing IS (Ketoconazole 200 ng/ml) was added, vortexed and kept for centrifugation which rotates at 13000 rpm at 5°c. The supernatant was taken and transferred to plates for analysis. In similar way remaining calibration standards and quality controls were prepared to get calibration curve standards with arrange of 5.01-1000.8ng/ml.

Fig. 4: Calibration curve of losartan in simultaneous estimation of Losartan and Irbesartan



The calibration curve was calculated by weight $1/x^2$ least –square linear regression analysis of the peak area ratio of analyte to the internal standard versus the concentration.

Fig. 5: Calibration curve of Irbesartan in simultaneous estimation of Losartan and Irbesartan



The calibration curve was calculated by weight $1/x^2$ least – square quadratic regression analysis of the peak area ratio of analyte to the internal standard versus the concentration.

Sysytem suitability

Six replicates of Extracted standard 9 (ULOQ) sample was injected

Results are presented in table 1A, 1B.

Method validation

Objective

The objective of the work is to validate specific LC/MS/MS method for the determination of LOS and IRB in rat plasma for the study.

Injector carries over effect for analyte and is Carry over test was performed in the following sequence.

MP → STD_9 → MP → MP→ STD_1 → plasma blank .No significant injector carries over is observed for IRB and LOS and internal standard.

Results are presented in table 2A, 2B.

Blank matrix screening (Specificity)

During validation, blank plasma samples from 4 different lots were processed according to the extraction procedure and evaluate the interference at the retention times of analytes and internal standard. The 3 free interference lots were selected from the 4 lots. Presented in table 3A.

Extraction recovery

The percentage recovery of IRB and LOS and KET was determined by comparing the mean peak area of IRB and LOS in extracted LQC, MQC, HQC samples with freshly prepared un extracted LQC, MQC, HQC samples. The percentage recoveries were found to be above 75%.

Note

Since our lab is non GLP lab all other validation parameters were not performed.

Table 1A : System suitability-1

S.no	Area of IRB	Area of LOS	Area of IS (KET)	Rt of IRB (min)	Rt of LOS (min)	Rt of IS (min)
1	1805393	523107	74901	2.75	2.68	2.66
2	1743620	513891	73237	2.75	2.67	2.65
3	1834507	498756	72953	2.74	2.70	2.66
4	1799021	506431	74102	2.74	2.68	2.64
5	1815705	494678	72890	2.75	2.67	2.67
6	1825794	517230	71896	2.74	2.66	2.66
Mean	1810913	517650	73863	2.75	2.68	2.65
S.D	22031	10701	1264	0.01	0.03	0.02
%CV	1.2	2.06	1.71	0.36	1.12	0.67

Result: System suitability was passed

Acceptance criteria

%CV values of area are should be less than 5 and Rt % CV should be less than 2.

Table 1B : System suitability - 2(2nd day)

S.no	Area of IRB	Area of LOS	Area of IS	Rt of IRB (min)	Rt of LOS (min)	Rt of IS (min)
1	1804392	513108	73904	2.74	2.68	2.65
2	1713625	517891	74231	2.75	2.67	2.66
3	1814506	488756	71953	2.75	2.69	2.65
4	1789022	516432	73108	2.74	2.67	2.64
5	1814701	484671	71899	2.75	2.66	2.65
6	1795794	516230	72897	2.76	2.68	2.66
Mean	1830915	507651	71867	2.75	2.67	2.64
S.D	21031	9704	1256	0.01	0.03	0.02
%CV	1.2	2.06	1.71	0.45	1.09	0.53

Result: System suitability was passed

Acceptance criteria

%CV values of area are should be less than 4 and Rt % CV should be less than 2.

Table 2A: Assessment of Injector carry over effect for Analyte IRB and IS (KET)

Sample Identification	Analyte Response	Internal standard Response	Carry Over observed with Analyte	Carry Over observed with IS
MP	0	0	Nil	Nil
S_9	571903	878644	Nil	Nil
MP	0	0	Nil	Nil
MP	0	0	Nil	Nil
S_1	13551	865241	Nil	Nil
Plasma Blank	0	0	Nil	Nil

Result: Injector carry over effect for Analyte (IRB) and IS was passed

Acceptance criteria

The carry over must be less than 20% response of analyte (S_1) and less than 5% response of internal standard.

Table 2B: Assessments of Injector carry over effect for Analyte (LOS) and IS (KET)

Sample Identification	Analyte Response	Internal standard Response	Carry Over observed with Analyte	Carry Over observed with IS
MP	0	0	Nil	Nil
S_9	468327	848693	Nil	Nil
MP	0	0	Nil	Nil
MP	0	0	Nil	Nil
S_1	9584	855242	Nil	Nil
Plasma Blank	0	0	Nil	Nil

Result: Injector carry over effect for Analyte (IRB) and IS (KET) was passed.

Acceptance criteria

The carry over must be less than 20% response of analyte (S_1) and less than 5% response of internal standard.

Table 3: Screening of Different batches of blank matrix (RAT HEPARIN PLASMA) Screening (SPECIFICITY)

Matrix	Blank Heparin Plasma Area at		
	Analyte (IRB) RT	Analyte (LOS)RT	Internal standard RT
SD-RAT 1	0	0	0
SD-RAT 2	0	0	0
SD-RAT 3	0	0	0
SD-RAT 4	0	0	0
EX.LLOQ	9584	13551	836574

Results: Specificity test was passed.

Acceptance criteria:

The area of rat blank plasma at retention times of IRB and LOS should be less than 20% of analyte, 5% of internal standard.

Analysis of subject samples sample preparation (Extraction procedure)

A 100µL of subject plasma sample was mixed with 300µL of internal standard working solution (200ng/mL of KET) vortexed for two minutes and kept in centrifuge for 5minutes at 5°c which rotates at a speed of 13000 rpm. After that the supernatant layer was collected and analyzed in LC/MS/MS system. The chromatograms of mobile phase , Blank plasma, Internal standard, Calibration standards ,subject samples and table representing time points vs plasma drug concentrations are shown in illustrations.

RESULTS AND DISCUSSION

The goal of this work is to develop and validate a simple, rapid, sensitive method for simultaneous extraction and quantification of LOS and IRB suitable for pharmacokinetic studies. To achieve the goal different options was evaluated to optimize sample extraction, detection parameters and chromatography. Different columns (X bridge C_{18},C8, Phenomenex C_{18},C_8 but polar RP column was found to be better with minimum tailing and good retention .Coming to the mobile phase the combination of methanol and 0.1% formic acid has good eluting capacity and is cheaper. When acetonitrile is used the analytes are not at all retaining .A flow rate of 1ml/min was optimized for good retention and the column oven temperature was kept at 40°c. The LOD, LOQ values are also low (5.01ng/mL) indicating that method is very sensitive. The injection carry over, specificity, system suitability were in limits. While coming to extraction procedure precipitation was found to be better than Liquid-liquid extraction with good recoveries.

Fig. 6: Mobile Phase Chromatogram

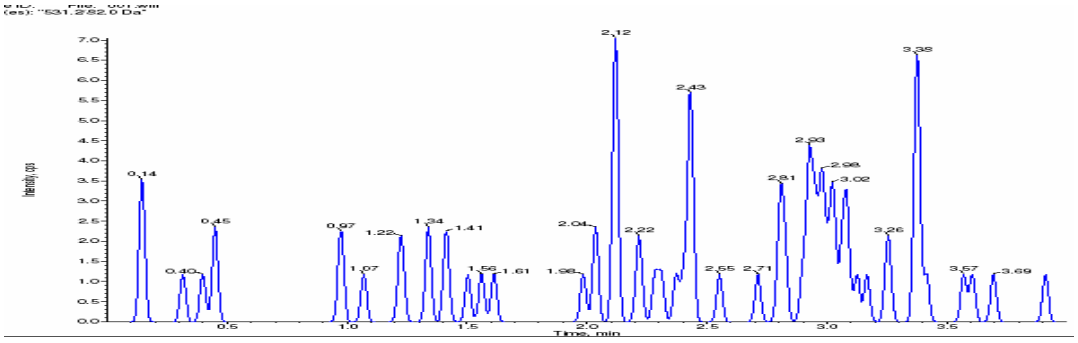


Fig. 7: Blank plasma chromatogram of losartan calibration CURVE

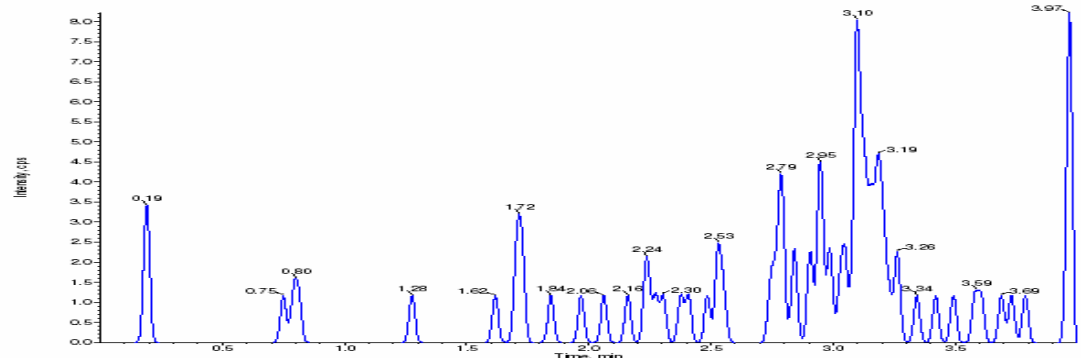


Fig. 8: Blank plasma+internal standard (ket) chromatogram of losartan calibration curve

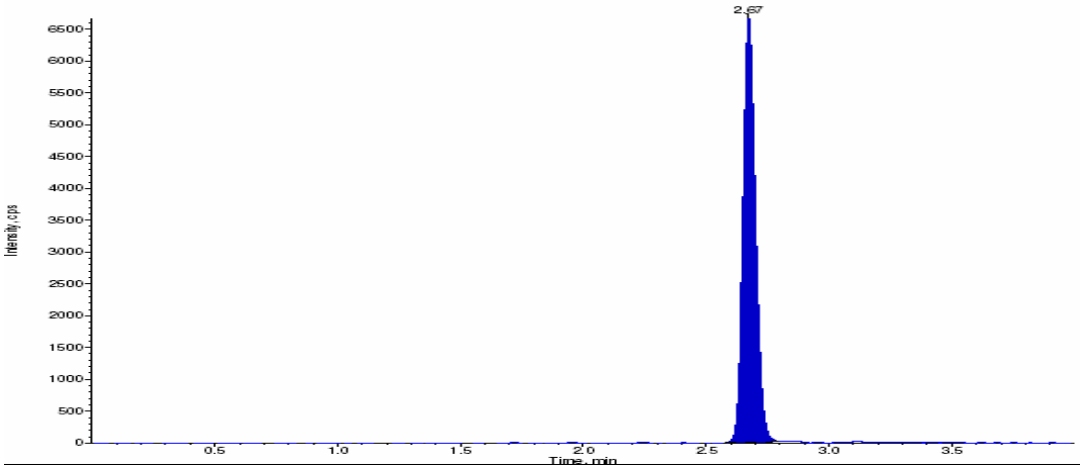


Fig. 9: Chromatogram of standard 1 (LLOQ) of losarta calibration curve in simultaneous estimation

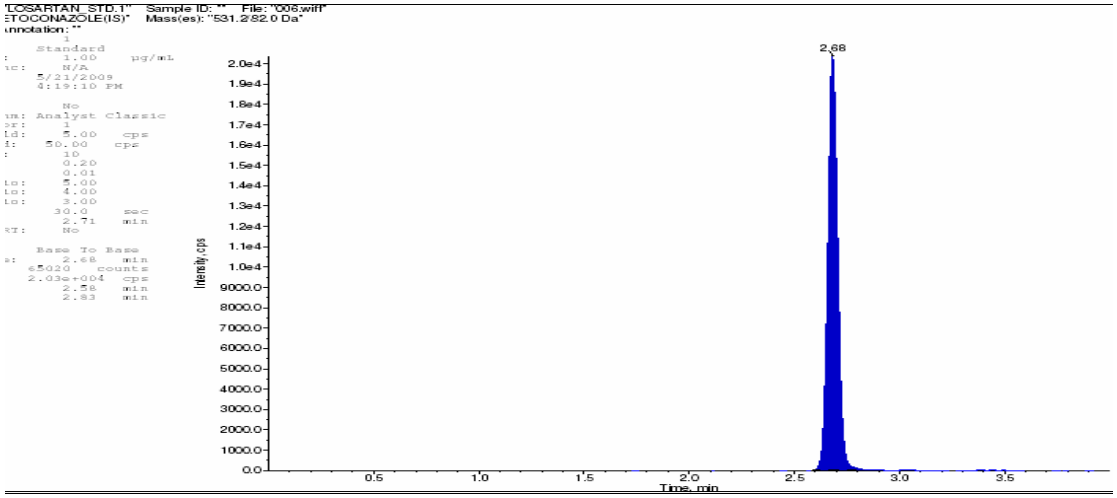


Fig. 10: Standard 9 (ULOQ) of irbesartan calibration curve in simultaneous estimation

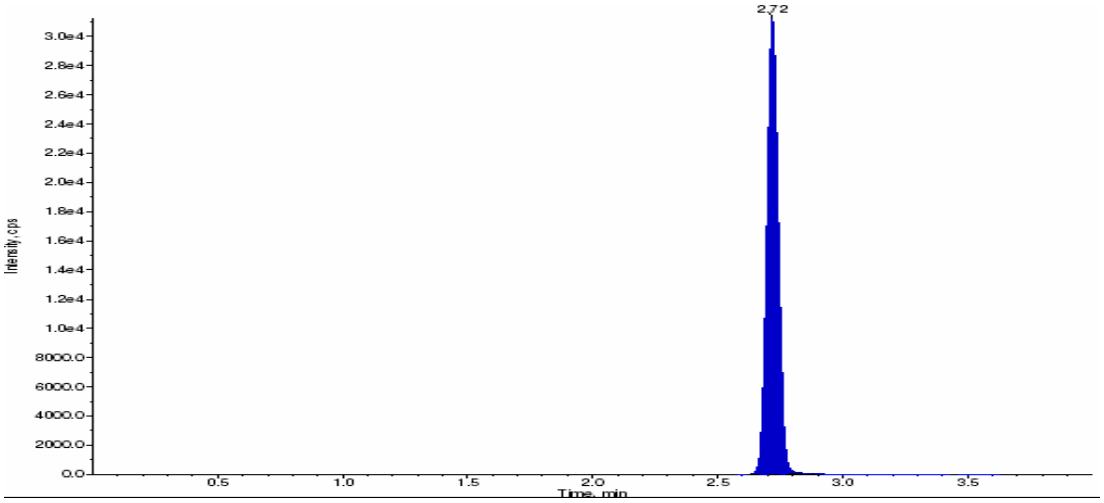


Fig. 11: Chromatogram of losartan subject sample at 0.08hr

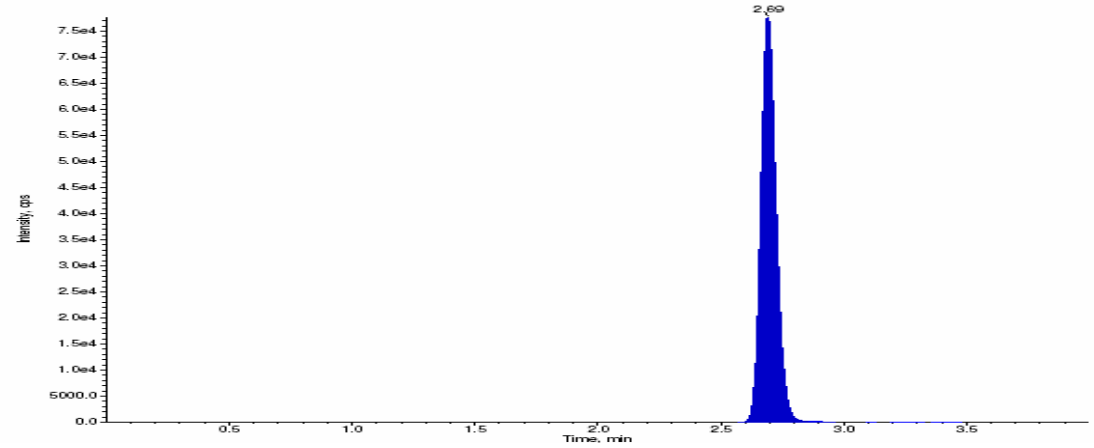


Fig. 12: Chromatogram of irbesartan subject sample at 4hr

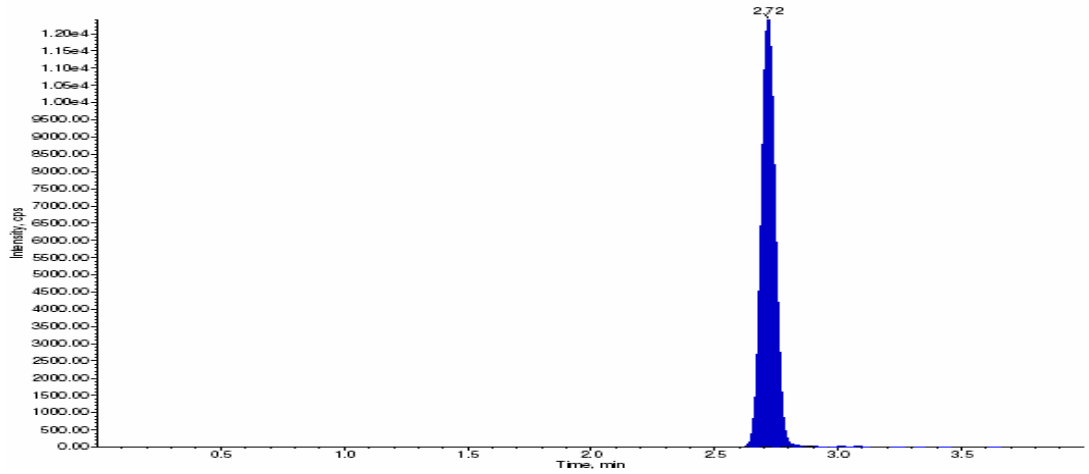


Table 4: Table indicating time points vs concentration of Losartan in simultaneous estimation of Losartan and Irbesartan

Time points (hr)	Conc. of.LOS in male SD rat 1 (ng/mL)	Conc. of.LOS in Male SD rat 2 (ng/mL)	Conc. of.LOS in Male SD rat 3 (ng/mL)
0.08	846.649	826.392	854.958
0.25	310.368	301.544	312.369
0.5	209.594	225.487	238.768
1	152.71	200.466	36.174
2	105.118	143.626	103.086
4	61.377	84.911	60.116
8	39.908	64.044	72.177
24	37.275	62.24	72.158

Table 5: Table indicating time points vs concentration of Irbesartan in simultaneous estimation of Losartan and Irbesartan

Time points (hr)	Conc. of.IRB in male SD rat1 (ng/mL)	Conc. of.IRB in male SD rat2 (ng/mL)	Conc. of.IRB in male SD rat3 (ng/mL)
0.08	2910.748	2866.942	2983.438
0.25	1765.31	1981.007	1509.763
0.5	1433.699	1752.415	1559.26
1	1466.536	2010.85	1379.263
2	1253.193	1662.359	1227.268
4	1012.077	1419.418	1061.304
8	772.783	1218.931	1404.769
24	756.044	1182.152	1346.405

CONCLUSION

A simple, specific, rapid and sensitive analytical method for the determination of LOS and IRB in rat plasma has been developed. Most of the analytical methods reported, for quantitation of LOS and IRB individually or simultaneously from human plasma, require laborious extraction procedure like liquid–liquid extraction, long run time and high quantification limit. The presented method provided excellent specificity and linearity. The other major advantage of this method is the short run time of 4 min which allows the quantitation of over 300 plasma samples per day. The recoveries in the method were also good.

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