Validated liquid chromatography/tandem mass spectrometry method for determination and Pharmacokinetic study of metolazone in rat plasma

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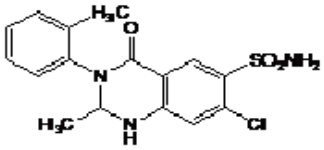
Highlights

- Simple LC-MS/MS method for the determination of metolazone (MET) in rat plasma.
- Validation of the method was carried out according to current bioanalytical guidelines.
- Highly sensitive (LLOQ 0.05 ng/mL) and cost effective (total run time was less than 2 min) method.
- Application of the method to monitor plasma concentration-time profiles in rats.

Graphical Abstract

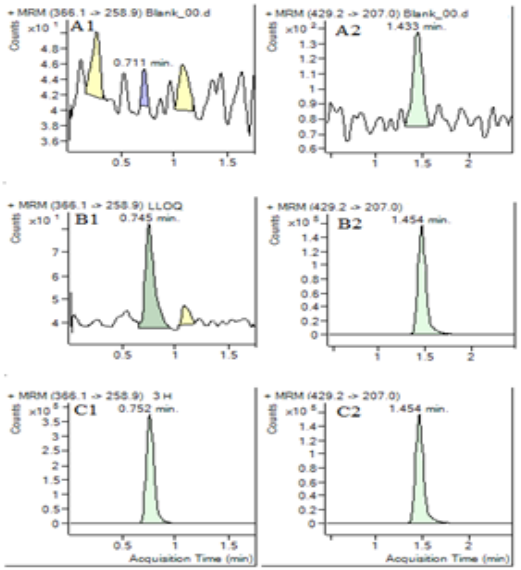


Oral dose

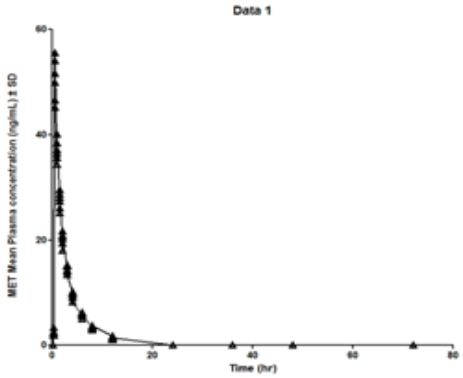
Plasma

PPT and
LC-MS/MS

Representative MRM chromatogram of
Metolazone and IS



PK Analysis



Mean plasma concentration Vs time curve of
Metolazone

Introduction

- Metolazone (MET), chemically known as (7-chloro- 2-methyl-3-(2-methylphenyl)-4-oxo-2,3-dihydro-1Hquinazoline- 6-sulfonamide is a quinazoline diuretic, It acts primarily to inhibit sodium reabsorption at the cortical diluting site and to a lesser extent in the proximal convoluted tubule.

- Quantification of antihypertensive drugs in biological matrix is very important and necessary because majority of these drugs prscribed in combination to get better result and control blood pressure at the adequate level.

Objective

- Developed and validated a simple, sensitive and high-throughput LC–MS/MS method for simultaneous quantification of MET in rat plasma and applied it in preclinical pharmacokinetic studies.

Experimental

- 1260 infinity HPLC system Agilent - 6460 Triple Quadrupole mass spectrometer
- *m*/*z* 366.1→258.9, and *m*/*z* 429.2→207.0 for MET and IS (Irbesartan), respectively.
- *Sample preparation – Simple protein precipitation*
  - 0.1 % formic acid in methanol and that in water (80:20, *v*/*v*)
  - Column Agilent Poroshell 120, EC- C18 (50 mm × 4.6 mm, i.d., 2.7 μm)
  - Total Run time – 2 min., MET (0.73 min) and IS (1.54 min)

Method Validation

Food and Drug Administration (FDA) guidelines

- Selectivity
- Extraction recovery and matrix effects
- Sensitivity and linearity
- Precision and accuracy
- Stability studies

Application to Pharmacokinetics study

- Wistar rats weighing 250 ± 50 g
- Oral administration of MET at 1 mg/Kg, it is dissolved in 0.1 % carboxy methyl cellulose.
- Non-compartmental methods.
- Phoenix® WinNonlin® software for pharmacokinetics data analysis(version 6.3; Certara USA, Inc., St. Louis, USA)

Results

Table I. Results of Recovery and Matrix Effect of MET and IS in rat plasma (*n* = 6)

Analytes	**Nominal conc. (ng/mL)**	**Recovery (%)**		**Matrix effect (%)**	
		Mean ± SD	%RSD	**Mean** ± SD	**RSD (%)**
MET	0.05	75 .25	9.80	90.20	10.20
	0.5	78.60	6.95	93.50	6.68
	80	80.26	4.60	90.95	5.50
	200	80.53	3.10	89.85	3.85
IS	200	93.60	3.15	92.90	3.06

Results

Table III. Stability of METIS after storage under indicated condition (mean % ± S.D., n=6)

Analytes	Nominal Conc. ng/mL	Bench – top stability at 24 ºC		Long term stability at -80 ºC (90 days)		Auto sampler stability at 24 ºC (24 h)		Freeze thaw stability (from – 80 ºC to 24 ºC		Stock solution stability 4 - 8 ºC (7 days)	
		Conc. found ng/mL ± SD	CV (%)	Con. Found ng/mL ± SD	CV (%)	Conc. found ng/mL ± SD	CV (%)	Conc.found ng/mL ± SD	CV (%)	Conc. found ng/mL ± SD	CV (%)
MET	0.5	0.51 ± 0.20	3.87	0.53 ± 0.10	3.55	0.49 ± 0.15	3.90	0.51± 0.25	3.81	0.50 ± 0.15	1.89
	80	79.95 ± 1.16	1.46	80.10 ± 1.16	1.46	79.99 ± 1.15	1.40	79.90 ± 1.50	1.55	80.10 ± 1.15	1.50
	200	201. 00 ± 2.09	1.04	201.00 ± 1.19	1.03	203.15 ± 2.15	1.09	200.8 ± 2.18	1.01	202.10 ± 1.48	1.60
IS	200	196.20 ± 5.10	2.89	196.13 ± 2.17	4.85	197.14 ± 3.12	2.54	195.4 ±1.89	3.33	196.98 ± 1.87	2.10

Results

Table: IV. Pharmacokinetic parameters obtained after oral administration of 1 mg/kg MET

AUC(0-48h) (hr × ng/mL)	638.31
AUCinf (hr × ng/mL)	655.20
Cmax (ng/mL)	58.77
Kel (1/hr)	0.07
MRT(0-48h) (hr)	11.16
T1/2(z) (hr)	9.39
Tmax (hr)	3.00
SE_AUC(0-48h) (hr × ng/mL)	9.48
SE_Cmax (ng/mL)	0.38

Results

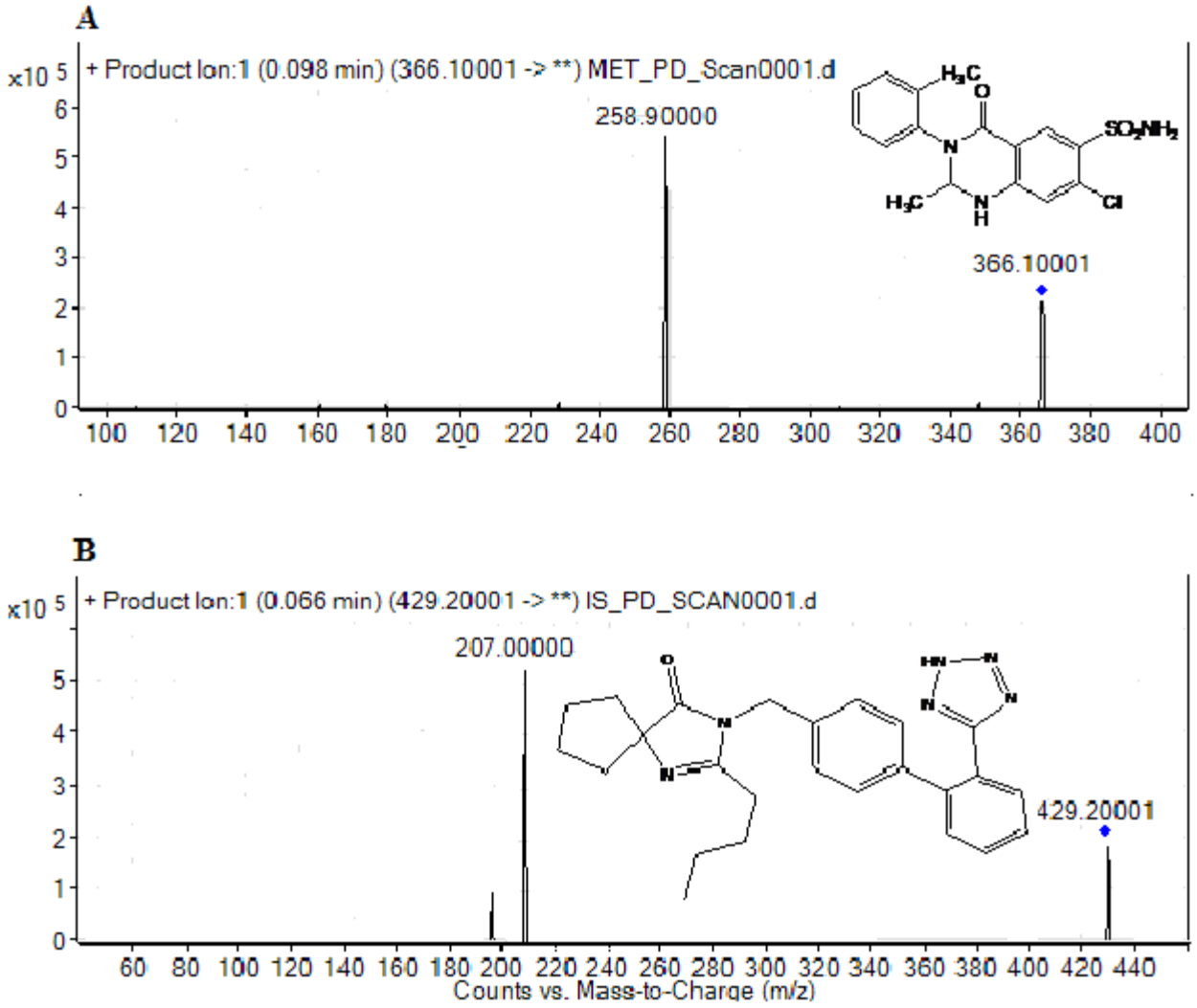


Fig. 1 Chemical structure and product ion mass spectra of [Metolazone (MET), (A)] and [Irbesartan (IS), (B)].

Results

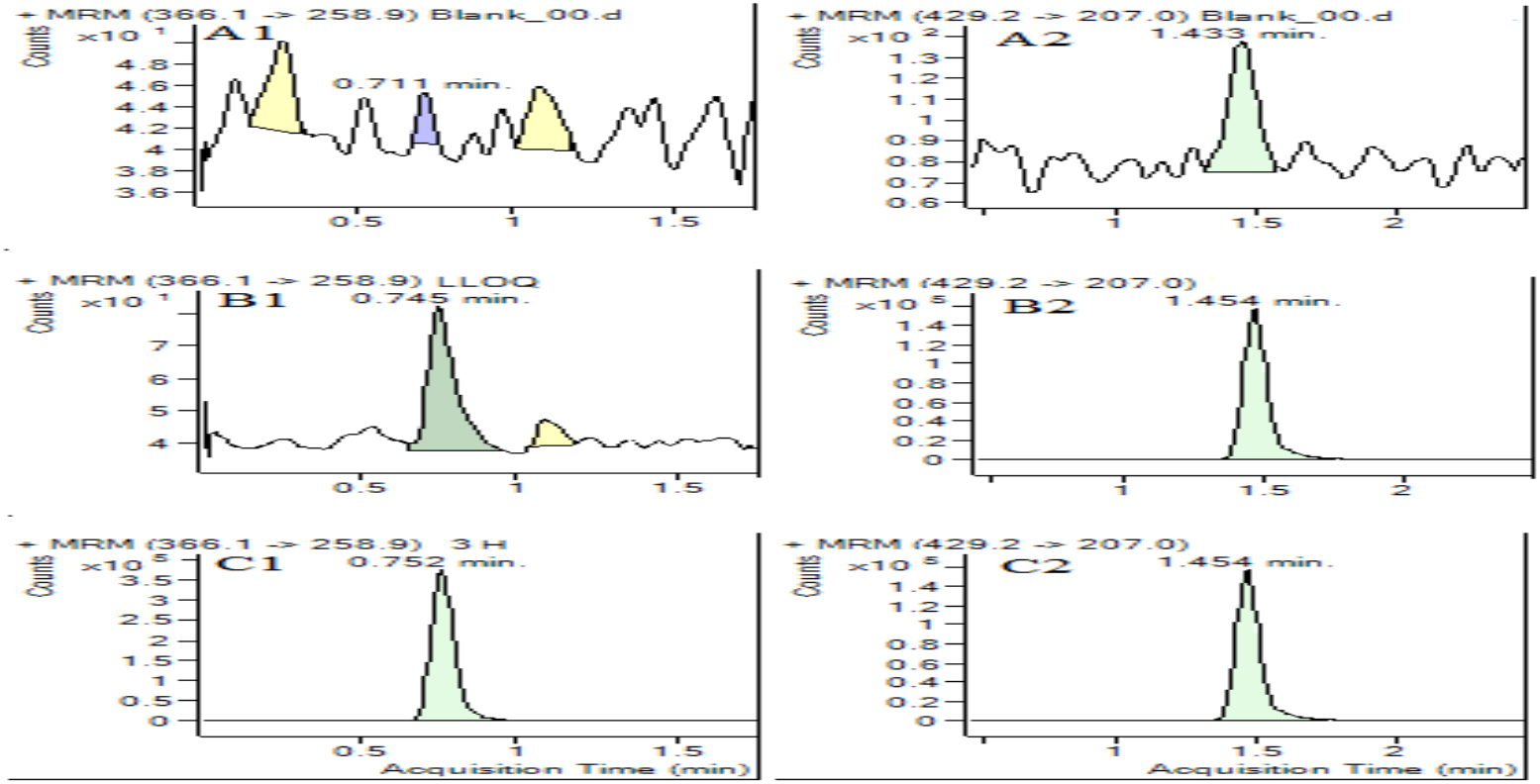


Fig. 2 Representative MRM chromatogramof blank plasma samples: MET (A1) and IS (A2). Representative MRM chromatogram of a plasma sample of MET at LLOQ level (B1) and IS at 100 ng/mL (B2). Representative MRM chromatogram of a plasma sample of MET (C1) obtained from a rat at 3 h after an oral administration of MET and IS at 100 ng/mL (C2).

Results

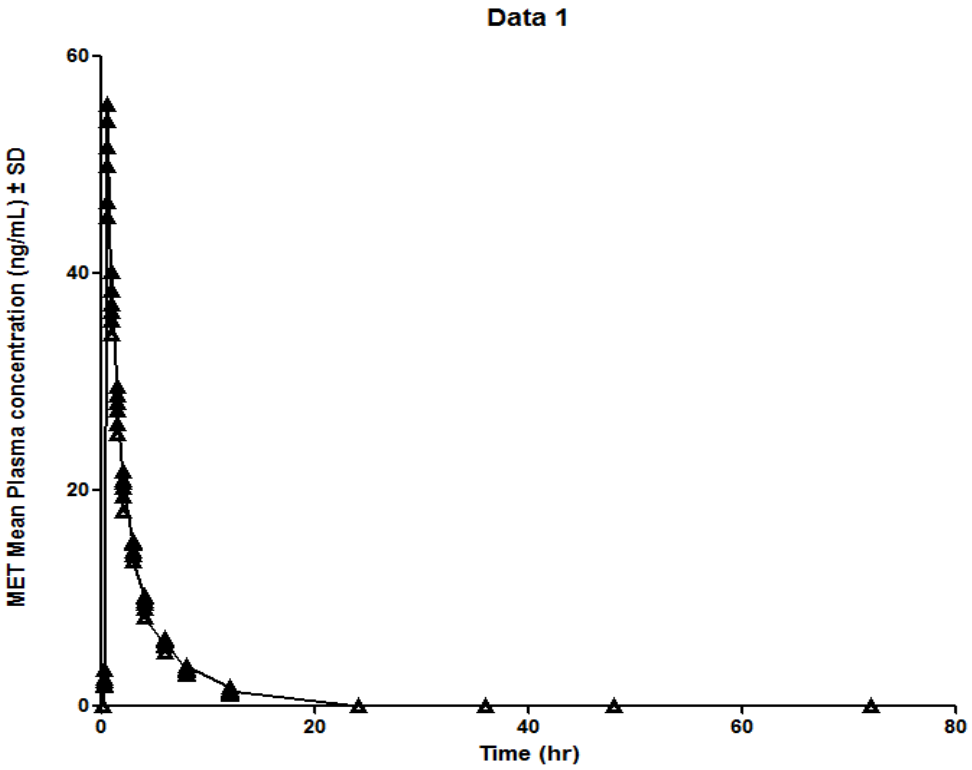


Fig. 3 Mean plasma concentration Vs time curve of MET.

Conclusion

- In summary, for the first time a simple, sensitive and selective LC-MS/MS method using a simple protein precipitation sample preparation procedure has been developed and validated for the determination of MET in rat plasma.
- This method can be used for the purpose of its application to measure concentration-time profiles for bioavailability, pharmacokinetic, bioequivalence and drug – drug interaction studies of MET for routine therapeutic drug monitoring.

References

[1] K. S. Channer, K. A. McLean, P. Lawson-Matthew and M. Richardson. Combination diuretic treatment in severe heart failure: A randomised controlled trial. *Br. Heart. J.* 71: 146–150 (1994).

[2] D. A. Sica and T. W. Gehr. *Clin. Pharmacokinet.* Diuretic combinations in refractory oedema states: Pharmacokinetic-pharmacodynamic relationships. 30: 229-249 (1996).

[3] R. R. Brodie, L. F. Chasseaud and L. M. Walmsley. Determination of diuretic agent metolazone in by high- performance liquid chromatography. *J. Chromatogr. B* 226: 526-532 (1981).

[4] D. Farthing, I. Fakhry, T. W. Gehr, D. A. Sica. Novel high-performance liquid chromatographic method using solid-phase on-line elution for determination of metolazone in plasma and whole blood. *J. Chromatogr. B* 534: 228-232 (1994).

[5] B. Basvan, M. Selvadurai, N. M. Subramaniya and S. Bhojraj. Pharmacokinetic evaluation of metolazone tablets using healthy human volunteers. *J Bioequiv Availab* 2: 015-017 (2010).

[6] M. I. Walash, N. El-Enany, M. I. Eid and M. E. Fathy. Simultaneous determination of metolazone and spironolactone in raw materials, combined tablets and human urine by high performance liquid chromatography. Anal. Methods. 5: 5644-5656 (2013.)

[7] W. Guangli, X. Shuhua and L. Changxiao. Determination of metolazone in human blood by liquid chromatography with electrospray ionization tandem mass spectrometry. *J. Chromatogr. B.* 845: 169-173 (2007).

[8] S. M. N. Roy, K. V. Mangaonkar, S. M. Yetal and S. S. Joshi. LC-MS-MS Method for Determination of Metolazone in Human Plasma. *J. Chem.* 5: 634-640 (2008).

[9] J. Y. Jia, M. Q. Zhang, Y. M. Liu, Y. Liu, G. Y. Liu, S. J. Li, C. Lu, L. P. Weng, Y. Qi and C. Yu. Pharmacokinetics and Bioequivalence Evaluation of Two Losartan Potassium 50-mg Tablets: A Single-Dose, Randomized-Sequence, Open-Label, Two-Way Crossover Study in Healthy Chinese Male Volunteers. *Clin. Ther.* 32: 1387-1395 (2010).

[10] Y. Chen and K. Hsu. Development of a LC-MS/MS-based method for determining metolazone concentrations in human plasma: Application to a pharmacokinetic study. *J. Food Drug Anal.* 21: 154-159 (2013).

[11] G. J. Murray and J. P. Danaceau. Simultaneous extraction and screening of diuretics, beta-blockers, selected stimulants and steroids in human urine by HPLC-MS/MS and UPLC-MS/MS. *J. Chromatogr. B.* 877: 3857-3864 (2009).

[12] A. J. Girona, K. Deventer, K. Roels and P. V. Eenoob. Development and validation of an open screening method for diuretics, stimulants and selected compounds in human urine by UHPLC–HRMS for doping control *Anal. Chim. Acta.* 721: 137-146 (2012).

[13] D. Farthing, D. A. Sica, I. Fakhry and T.W.B. Gehr. Novel high-performance liquid chromatographic method using solid-phase on-line elution for determination of metolazone in plasma and whole blood. *J. Chromatogr. B. 653:*171-176 (1994).

[14] J. S. Kang (2012). Principles and Applications of LC-MS/MS for the Quantitative Bioanalysis of Analytes in Various Biological Samples, Tandem Mass Spectrometry - Applications and Principles, Dr Jeevan Prasain (Ed.), ISBN: 978-953-51-0141-3, InTech.

[15] Guidance for Industry: Bioanalytical Method Validation; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), BP (2001).