Journal of Bioequivalence & Bioavailability - Open Access

JBB/Vol.2 Issue 1

Research Article | OPEN ACCESS Freely available online | doi:10.4172/jbb.1000024

Pharmacokinetic Evaluation of Metolazone Tablets using Healthy Human Volunteers

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Abstract

A sensitive and reproducible high performance liquid chromatography (HPLC) method has been developed and validated for the quantification of metolazone in human plasma, after solid phase extraction (SPE). A Good resolution was achieved on a reverse-phase LichroCART Purospher® C_{18} column using the mobile phase acetonitrile–0.5% triethylamine (35:65) in isocratic elution with a total run time of 15 min. The analyte, metolazone, was detected by using high performance liquid chromatography with the support of photo diode array detector. Limit of detection and Lower limit of quantification was found to be 1 and 2.5 ng/mL. The present method was successfully applied in the pharmacokinetic study of metolazone in human plasma.

Keywords: Metolazone; Bioavailability studies; Pharmacokinetic evaluvation

Introduction

This work deals with the studies carried out by the writer in this laboratory for the past one year on the development of analytical method used for the estimation of Metolazone in biological fluids and Bioequivalence studies on Metolazone tablets. Metolazone is a diuretic/saluretic/antihypertensive drug of the quinazoline class. The chemical name is 7-chloro-1,2,3,4-tetrahydro-2-methyl-3-(2-methylphenyl)-4-oxo-6-quinazolinesulfonamide. Metolazone is a quinazoline diuretic, with properties generally similar to the thiazide diuretics. The actions of Metolazone result from interference with the renal tubular mechanism of electrolyte reabsorption. Metolazone acts primarily to inhibit sodium reabsorption at the cortical diluting site and to a lesser extent in the proximal convoluted tubule. Sodium and chloride ions are excreted in approximately equivalent amounts. The increased delivery of sodium to the distal tubular exchange site results in increased potassium excretion. Metolazone does not inhibit carbonic anhydrase. A proximal action of Metolazone has been shown in humans by increased excretion of phosphate and magnesium ions and by a markedly increased fractional excretion of sodium in patients with severely compromised glomerular filtration. This action has been demonstrated in animals by micropuncture studies. The antihypertensive mechanism of action of Metolazone is not fully understood but is presumed to be related to its saluretic and diuretic properties (wikipedia.com). Only limited methods have been reported for HPLC and LC-MS (Brodie et al., 1981; Farthing et al., 1990; Farthing et al., 1994; Wei et al., 2007; Jadhav et al., 2009; Guidance for Industry Bioanalytical Method Validation. U.S, 2001; Guidance for Industry, Statistical Approaches in Establishing Bioequivalence. U.S, 2001). Our aim is to develop a new and sensitive bioanalytical method.

Experimental

Reagents and materials

Working standard of Metolazone with 99.86% purity was obtained from German Remedies Ltd., Mumbai, India. Celecoxib (purity 99.38%) working standard was obtained from Cadila Health Care Ltd., Ahmedabad, India). Acetonitrile (HPLC grade), obtained from Qualigens Fine Chemicals, Mumbai and potassium dihydrogen ortho phosphate, ortho phosphoric acid, methanol, and trichloroacetic acid (all analytical grade reagent) were purchased from S.D. Fine Chem. Ltd., Mumbai. In house mill Q water was used throughout the study. Fresh frozen human plasma used in the method development was obtained from the Vijay Hospital, Ooty, and was stored at -70±2°C until required.

Instrumentation

The HPLC system consisted of a HPLC 10 AT-VP (Shimadzu Ltd., Japan), Manual injector port with 100µL loop (Rheodyne, USA) and UV detector (Shimadzu Ltd., Japan). The wavelength of the detector was set at 225 nm. Detector output was quantified on CLASS VP (Version 6.01) chromatography software. Separation was carried out on a LichroCART Purospher® C_{18}, (250 mm × 4.6mm, 5µm), Japan, using mixture of 0.5% triethyl amine (pH 3.5) and acetonitrile (65:35, v/v) as a mobile phase, at a flow rate of 1 mL/min. Total analysis time was 15 min. All analysis was performed at room temperature.

Preparation of calibration standard and quality control standards

Stock solutions of metolazone and celecoxib (I.S) (1 mg/ml) were prepared in a mixture of water and acetonitrile (1:1 v/v) and stored at 8°C. The stock solution of metolazone was further diluted with the mixture of water and acetonitrile to give series of standard solutions. Galibration standard of metolazone (2.5, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0, 250.0 and 500.0 ng/ml) were prepared by spiking appropriate amount of the standard solution in blank plasma. Lowest quality control standards (LQC), median quality control standards (MQC) & highest quality control stan-

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Received December 29, 2009; **Accepted** January 21, 2010; **Published** January 22, 2010

Citation: Babu B, Muralidharan S, Meyyanathan SN, Suresh B (2010) Pharmacokinetic Evaluation of Metolazone Tablets using Healthy Human Volunteers. J Bioequiv Availab 2: 015-017. doi:10.4172/jbb.1000024

dards (HQC) were prepared by spiking drup free plasma with metolazone to give solutions containing 6.0, 50.0, and 500.0, ng/mL, respectively. They were stored at -20±2°C till analysed.

Sample preparation with SPE

To 0.5ml plasma sample containing metolazone (calibration standard), 0.5ml of internal standard (1.0µg/ml) was added and vortexed. Phenomenex Strata C18-E SPE cartridge was conditioned with acetonitrile and water sequently. The above conditioned cartridge 1 mL of sample solution was added.The cartridge was washed with water. The drug and internal standard was eluted from the cartridge using 1.0 mL of mobile phase. None of the drug free plasma samples studied in this assay yield endogenous interference at these retention times (Figure 1). We have performed with other methods such as Protien precipitation and Liquid Liquid extraction methods among these methods SPE extraction was found to be more precise (98.28% recovery) results.

Results and Discussion

Selectivity/sensitivity

Selectivity of the method described was investigated by screening six different batches of human blank plasma. Under the proposed assay condition internal standard and metolazone had a retention time of 7.97 and 10.36 min, respectively, rest of the peaks were due to the plasma components. Metolazone and internal standard were very well resolved under the proposed chromatographic conditions. None of the drug free plasma samples studied in this assay yield endogenous interference at these retention times (Figure 2).

Accuracy and precision

The mean percent accuracy of the proposed method was found to be 98.37%. Intra day precision for metolazone was 5.75±0.38, 49.19±0.17, 498.35±0.87 for the spiked concentration at 6.0, 50.0 and 500.0 ng/mL respectively. Inter day precision for metolazone was 6.1±0.21, 49.39± and 499.97±0.32 for the spiked concentration at 6.0, 50.0 and 500.0 ng/mL, respectively (Table 1).

Linearity

The linearity study was carried out on seven different concentration of metolazone were analysed (2.5, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0, 250.0 and 500 ng/mL). The peak area response was linear over the concentration range studied. Each experiment at all concentration was repeated three times on three separate days to obtain the calibration data. The coefficient of correlation '*r*2' was found to be 0.9978 (Table 2 and Figure 3).

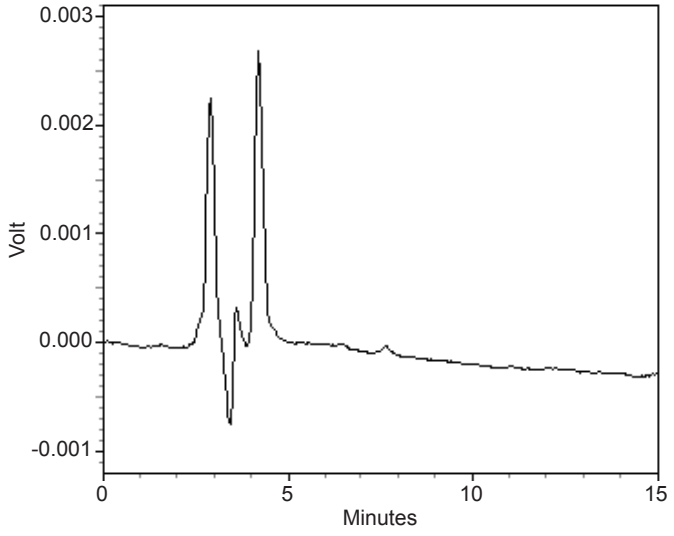

Figure 1: Blank sample chromatogram.

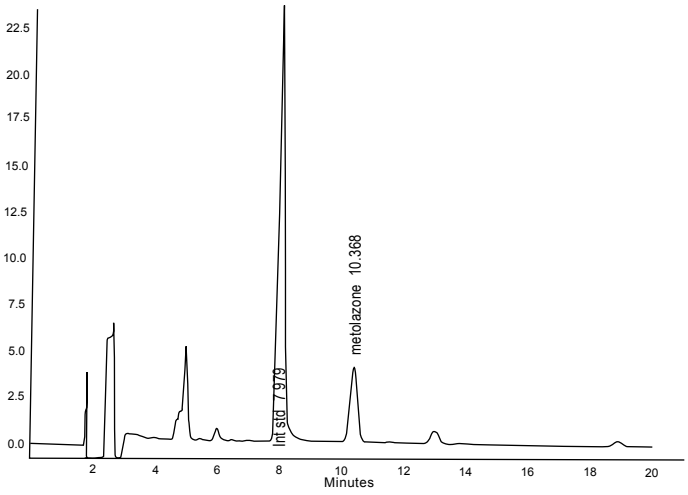

Figure 2: Sample Chromatogram.

Sample concentration (ng/mL) n=5)	Concentration found (mean±S.D.) (ng/mL)	%CV	%Accuracy
6.0	5.82±0.43	7.45	97.13
50.0	49.85±1.48	2.98	99.71
500.0	491.35±13.16	2.67	98.27

Table 1: Accuracy and Precision.

Concentration (ng/mL)	Response Factor
2.5	0.03
5.0	0.06
10.0	0.10
25.0	0.28
50.0	0.49
75.0	0.72
100.0	1.06
250.0	2.78

Table 2: Calibration curve of metolazone.

Recovery

The mean extraction recoveries of metolazone determined over the concentration of 6.0, 50.0 and 500.0 ng/ml were 98.87%, 99.03% and 99.94%.

Stability study

Short-term and long-term stock solution stability studies evaluated, which proved no significant deviation from normal value when stored at 4°C. The stability of metolazone in plasma was determined by measuring concentration change in quality control samples over time. Stability was tested by subjecting the quality controls to three freeze-thaw cycles and compared with freshly prepared quality control samples. The mean concentration of metolazone in quality control samples did not change

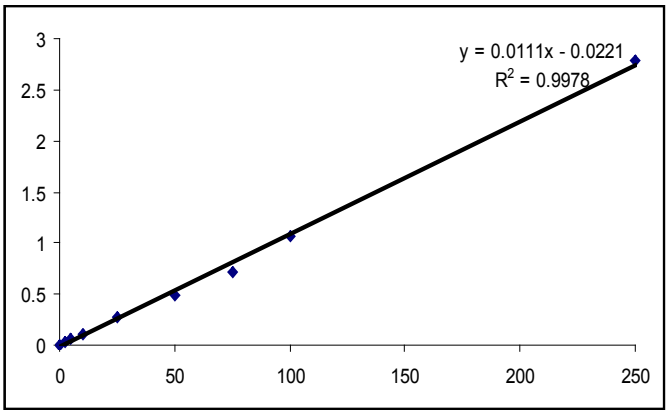

Figure 3: Calibration Curve of Metolazone.

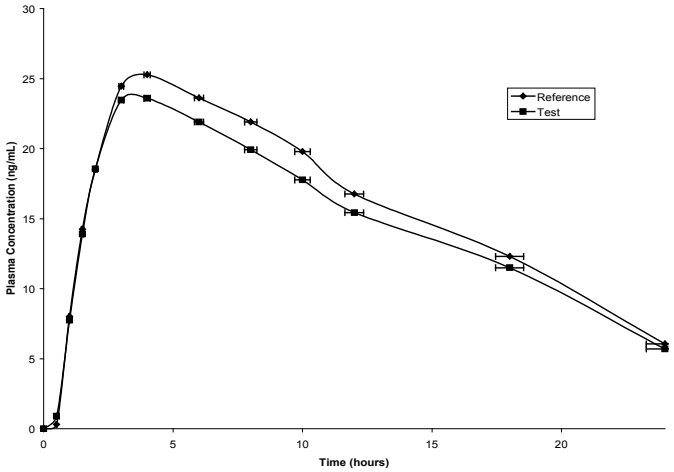

Figure 4: Mean plasma concentration.

Sample concentration (ng/mL) (n = 5)	Concentration found (mean±S.D.) (ng/mL)	%CV
Stock solution stability (6 h,15,30days)		
6.0	5.96±0.07	1.18
50.0	50.31±0.62	1.24
500.0	499.69±0.95	0.19
Short-term stability (1,2,3hrs)		
6.0	5.98±0.11	1.84
50.0	50.16±0.20	0.40
500.0	499.01±1.91	0.38
Long term Stability (4.weeks)		
6.0	6.16± 0.64	10.32
50.0	49.41±0.98	1.99
500.0	498.85±0.63	0.13
Freeze thaw Stability (3 Cycle)		
6.0	5.89± 0.53	8.96
50.0	48.82±0.41	0.85
500.0	495.31± 2.56	0.52

Table 3: Stability study report of Metolazone.

Parameters	Reference	Test
C_{max} (ng.h/ml)	25.83	24.83
T_{max} (hrs)	3.50	2.92
$T_{½}$ (hrs)	10.89	10.83
K_{eli} (hrs^{-1})	0.07	0.06
AUC_{0-24} (ng.h/ml)	377.34	351.12
AUC_{0-inf} (ng.h/ml)	478.60	446.32

Table 4:

significantly within the time period under the indicated storage conditions. Long-term stability studies results conclude that metolazone is stable in plasma matrix at least for 30 days when stored at -20±2°C (Table 3).

Pharmacokinetic evaluation

The Pharmacokinetic evaluation of *C*max, Tmax, Half Life, K elimination, $AUC_{0-t,}$ and $AUC_{0-\infty}$ these parameters were calculated. The Reference and Test formulation was compared and this result is presented in Table 4.

Conclusion

SPE of metolazone from plasma was found to be more precise than the other extraction methods. The current method guarantees a high precision, accuracy, recovery and a relatively short analysis time and will be a useful tool in the pharmacokinetic study of metolazone in humans.

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