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Bioanalytical Method Validation for The Determination of Bisoprolol in Human Serum By Lc/Ms/Ms Detection.

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ABSTRACT

Bisoprolol was extracted from an aliquot of human serum using Solid phase extraction method and then injected into a liquid chromatograph, equipped with Mass Spectrometry Detector. Internal standard method was used for quantitation of Bisoprolol. Linear regression with 1/X2 weighting was performed to determine the concentration of the drug from serum samples. All regressions and figures presented in this validation report were generated by analyst 1.4.1 software. The method was validated over a linear range of 0.50 ng/ml to 200.00 ng/ml and the limit of quantification were 0.50 ng/ml. Recoveries was observed above 90% for the analyte. The storage stability of Quality control samples was investigated under various conditions. Quantification of serum concentrations was achieved by the internal standard method using peak area ratio. Alprazolam was used as internal standard.

KEYWORDS | Bisoprolol, LC-MS/MS, Pharmacokinetic studies

Introduction:

Bisoprolol is been used individually and in combination with other antihypertensive agents for the treatment of hypertension. The incidence of hypokalemia with the bisoprolol and hydrochlorothiazide combination is significantly lower than with hydrochlorothiazide alone. BISO, 1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl) phenoxy]propan-2-ol , is a highly selective β1 receptor blocking agent used for the treatment of hypertension and angina pectoris. Some procedures have been described for the determination of either Hydrochlorothiazide or Bisoprolol in biological fluids, such as gas chromatography–mass spectrometry and capillary electrophoresis with UV-absorbance detection and HPLC with UV-absorbance detection Liquid chromatography coupled to tandem mass spectrometry has become a method of the choice for the determination of small molecules in biological matrices, including bisoprolol and other antihypertensive agents, because of its superior LLOQ,sensitivity, and improved selectivity. An attempt has been made to extract Bisoprolol from human serum instead of plasma, We herein describe a simple, sensitive and high throughput method based on liquid–liquid extraction and LC–MS/MS for routine measurement of Bisoprolol in human serum in support of pharmacokinetic study.

Materials and methods

Bisoprolol, and Alprazolam were purchased from Synfine Research, Canada or Sigma Aldrich, USA. Methanol, Acetonitrile, Ammonium acetate, and Trifluoroacetic acid were obtained from Qualigens (Worli, Mumbai) India. De-ionized water was prepared on Milliq Laboratory Plant (Millipore, Bedford. USA). Organic solvents and reagents used were of analytical grade. Serum was prepared from the blood obtained from suraktam blood bank, Vadodara.

Equipments and chromatographic conditions

The chromatographic system consist of LC-2010HT (Shimadzu,Japan) equipped with SIL-HTc autosampler. Mass Spectrometric analysis were conducted using API 3200 Q-trap Triple quadrapole instrument (Applied Biosystem, Sciex, Concord, Canada), equipped with a pneumatically assisted APCI(heated nebulizer) and ESI (electrospray) ionization source, which was operated in negative mode. The whole system was controlled using Analyst software version 1.4.1(Applied-Biosystem-Sciex, Concord, Canada).

Chromatographic separation was achieved on Phenomenex C18 , 5µm, 50*4.6 mm column in atmospheric pressure electrospray ionization. The mobile phase composition was a isocratic mixture of Buffer : Acetonitrile (25:75 %v/v),(Buffer: 30 mg Ammonium Acetate & 60 µl Trifluoroacetic acid in Water). Flow rate was maintained at 0.5 ml/min.

MS tuning

Tuning of mass spectrometer involves optimizing voltages, currents, flow rates and optimization of ion source parameters to achieve the maximum mass spectral sensitivity and proper resolution. Mobile phase was introduced in to the mass spectrometer via the ESI source operating in the negative ion mode under multiple reaction monitoring conditions (MRM). Quantitation was performed using selective ion Monitoring (SIM) mode at m/z 326.25, and 308.99 for Bisoprolol and Alprazolam respectively.

Preparation of calibration standard

Stock solution of Bisoprolol, was prepared by accurately weighing and dissolving respective reference standards in methanol to give the final concentration of 1000 µg/ml of each. Stock solution of internal standard i.e Alprazolam was obtained in methanol at a concentration of 5 µg/ml and was used directly for serum sample preparation. Stock solution of Bisoprolol was further diluted with methanol to give serial concentrations of , 10.00, 20.00, 40.00, 100.00, 200.00, 400.00, 1000.00, 2000.00, 4000.00 ng/ml to form working solution of calibration standards. Quality control standard solutions of Bisoprolol was prepared in methanol at concentration of 30.00, 600.00 and 3000.00 ng/ml. Working solution of analytes as well as internal standard were stored at 5°C .

Sample Preparation

A common procedure for the isolation of Bisoprolol from serum samples prior to LC/MS/MS was developed. For analysis of analyte, 25 µl of Alprazolam, 5 µg/ml and 500µl of 10% aqueous ortho phosphoric acid solution were added to 250µl human serum. The mixture was vortexed for several seconds. Conditioned the HLB cartridge with 1 ml methanol followed

by equilibration with 1 ml water. 1 ml of serum sample was loaded, washed with 1 ml of 2% methanol and 1 ml water. Eluted with 525µl of Elution solvent, i.e., Buffer : Acetonitrile (25:75 %v/v),(Buffer: 30 mg Ammonium Acetate & 60 µl Trifluoroacetic acid in Water).

Method validation
The method was validated for specificity, linearity, precision, accuracy, recovery and stability.

Results:

Limits of Quantitation
The lower limit of quantitation i.e., lowest standard level with a coefficient of variation less than 20 % was 0.50 ng/ml, between batch % coefficients of variation for Bisoprolol was ranged between 3.80 % to 16.50 % and accuracy was ranged between 92.08 % to 104.53 %. The results are within ± 15 %, The Upper limit of Quantitation for Bisoprolol was 200 ng/ml The within batch % coefficient of variation for Bisoprolol was ranged between 3.60 % to 17.69 % and % accuracy was ranged between 92.29 % to 105.39%

Linearity and Sensitivity
Good linearity was achieved over the concentrations in the range of 0.50 to 200 ng/ml for Bisoprolol. The data of linearity are listed in Table 1. The limit of quantification (LOQ) was 0.50 ng/ml using 500 µL of serum for Bisoprolol, with accuracy, precision ≤ 20%. Back calculations were made from the calibration curves to determine Bisoprolol, concentration of each calibration standard. For Bisoprolol The co-efficient of correlation were found to be better than 0.9968.

Table No 1: Intermediate precision ,accuracy and linear regression parameters of Bisoprolol determination in human serum by LC-MS-MS detection.

Added Concentration (ng /ml)	Mean measured Concentration (n = 5)(ng/ml)	Precision (RSD,%)	Accuracy (%)[a]
Bisoprolol			
0.50	0.53	0.01	105.00
1.00	0.91	2.01	90.67
2.00	1.87	2.98	93.35
5.00	5.44	2.23	108.70
10.00	10.78	0.79	107.76
20.00	20.79	0.23	103.97
50.00	50.23	2.68	100.46
100.00	98.18	0.86	98.18
200.00	183.92	0.52	91.96
Calibration curve			
Slope		0.0145	
Intercept : 0.0005		Correlation Coefficient : 0.9987	

Specificity
Presence of any interference from endogenous substances was estimated by analyzing human serum from six different lots of analyte (s) free human serum including hemolised and lipemic serum used for analysis .No significant interference was observed at the retention times of both analyte (s) and internal standard.

Precision , Accuracy and Recovery of Method
A good precision and accuracy was observed in this method. The intra and inter-day precision and accuracies are summarized in Table 2. The intra-day CV (%) were less than 6.60% and inter-day CV (%) were less than 7.43 % for Bisoprolol. The **Intra- day** accuracies was found between 92.29 to 105.39% and the inter-day accuracies was between 92.08 to 104.53% for Bisoprolol. The recovery of the method was found between 91.35 to 97.42% for Bisoprolol. These datas were found satisfactory for pharmacokinetic studies.

Table No 2 : Intra- day and Interday precision and accuracy for Bisoprolol of QC.

	Intra- day precision and accuracy for Bisoprolol of QC (n = 5)			
Added Concentration (ng/ml)	Mean measured Concentration (ng/ml)	standard deviation	CV (%)	Accuracy (Mean relative error)
1.50	1.38	0.09	6.60	92.29
30.00	31.62	1.14	3.60	105.39
150.00	148.38	6.48	4.37	98.92
	Inter- day precision and accuracy for Bisoprolol of QC (n = 30)			
Nominal Concentration (ng/ml)	Mean found Concentration (ng/ml)	standard deviation	CV (%)	Accuracy (Mean relative error)	% Recovery
1.50	1.37	0.10	7.43	92.08	97.42
30.00	31.36	1.19	3.80	104.53	94.14
150.00	145.16	5.79	3.99	96.77	91.35

Samples Stability
Bisoprolol, showed a good stability under the conditions used for storage and processing. Bisoprolol was highly stable under the influence of autosampler stability, short term stability as well as three freeze/thaw cycles.

Discussion
A convenient method for the determination of Bisoprolol in human serum has been developed. The analytical method was validated as per the well defined standard operation procedure of Bioanalytical laboratory. The calibration curve for the standard was linear over the range from 0.50 to 200.0 ng/ml .

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References
1 K Ding L, Zhou X, Guo X, Song Q, He J, Xu G. LC-ESI-MS method for the determination of bisoprolol in humanplasma. J Pharm Biomed Anal, 2007; 28:44(2): 520-525.
2 Bhatt J, Subbaiah G, Kambli S, Shah B, Patel M, Saxena A, Baliga A, Nigam S, Parekh H, Yadav G,A high throughput and sensitive liquid chromatographytandem mass spectrometry (LC-MS/MS) method for the estimation of bisoprolol in human plasma using multiplexing technique. J Chromatogr B Analyt Technol Biomed Life Sci , 2007; 852(1-2): 374-381
3 Liu M, Zhang D, Sun Y, Wang Y, Liu Z, Gu J, Liquid chromatographic-electrospray tandem mass
spectrometric determination of bisoprolol in human plasma Biomed Chromatogr,2007;21: 508-513
4 Tutunji LF, Tutunji MF, Alzoubi MI, Khabbas MH, Arida AI. Simultaneous determination of irbesartan and hydrochlorothiazide in human plasma using HPLC coupled with tandem mass spectrometry: Application to bioequivalence studies. J Pharm Biomed Anal. 2010, 4: 985-990
5 Tutunji MF, Ibrahim HM, Khabbas MH, Tutunji LF. Simultaneous determination of bisoprolol and hydrochlorothiazide in human plasma by HPLC coupledwith tandem mass spectrometry. Chromatogr B Analyt Technol Biomed Life Sci., 2009;.877(16-17): 1689-1697.
6 Myriam C. Salvadori, Roberto F. Moreira Bruno C. Borges Maristela H. Andraus Cristina P. Azevedo, Ronilson A. Moreno Ney C. Borges .Simultaneous Determination of Losartan and Hydrochlorothiazide in Human Plasma by LC/MS/MS with Electrospray Ionization and Its Application to Pharmacokinetics,Clinical and Experimental Hypertension, 2009;31: 415-427.
7 Maha F. Tutunji, Husam M. Ibrahim, Manal H. Khabbas and Lara F. Tutunji Simultaneous determination of bisoprolol and hydrochlorothiazide in human plasma by HPLC coupled with tandem mass spectrometry Journal of Chromatography B, 2009; 877: 1689-1697.
8. US Department of Health and Human Services, Food and Drug Administration, 2001, Guidance for Industry, Bioanalytical Method Validation. FDA/CDER/CVM, 2001.[Cited 2009 May 2]. Available from: URL: http://www.fda.gov/cder/guidance/4252fnl.pdf.