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DETERMINATION OF PROPRANOLOL HYDROCHLORIDE IN HUMAN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETERY/MASS SPECTROMETERY (HPLC-MS/MS)

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ABSTRACT

Biological fluids like blood, serum, urine, tissue extracts or plasma may contains compounds like drugs and their metabolite, and Bioanalysis is the term which is commonly used for the purpose of quantitative determination of this compounds. An accurate LC-MS/MS technique to quantify Propranolol in the range 2.0500 ng/mL to 250.3060 ng/mL was developed and validated. Propranolol become extracted from 250 µL plasma by means of the usage of solid Phase extraction method turned into accomplished on Hypersil gold 50 x 4.6 mm column. The plasma (200 µL) processed using Liquid-Liquid extraction technique.

Keywords: Bio-analytical method, Propranolol, HPLC-MS/MS, Human plasma, Standard deviation

INTRODUCTION

Propranolol is a drug under Beta blockers **[1]**. Propranolol is used for the treatment of Cardio vascular diseases such as High blood pressure, Essential tremors, Capillary hemangiomas, and Performance anxiety **[1-3]**. For the structure and Profile details refer **Figure 1** and **Table 1**.

Biological fluids like blood, serum, urine, tissue extracts or plasma may contains compounds like drugs and their metabolite, and Bioanalysis is the term which is commonly used for the purpose of quantitative determination of this compounds **[4]**. One of the main techniques which is currently used for this purpose is HPLC-MS/MS **[5]**. Liquid chromatography has the power to separate various components from complex mixtures and mass spectrometery is used for the selective and specific detection of these separated compounds **[6-7]**.

HPLC has lots of advantage over normal Liquid chromatography. Normally ordinary liquid chromatography uses the gravitational force for the flow of solvent, while operational pressure of HPLC techniques are high, about 50-350 bar. The column dimension of HPLC is 2.1-4.6 mm diameter and it made up of smaller adsorbent particles with an average particle size of 2-50µm.These all features enhance the resolving power of HPLC techniques and thereby make it one of the most popular technique. The information of HPLC has no shortage **[8-14]**.

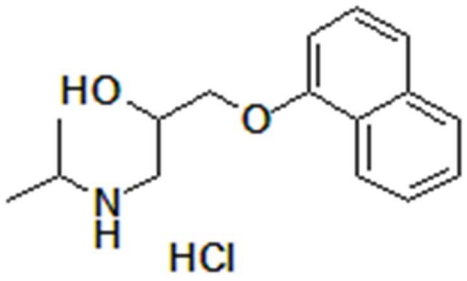


Figure 1: Chemical structure of Propranolol Hydrochloride

Table 1: Profile of Propranolol HCL and IS

Profile	Propranolol	Propranolol D7
Drug Name	Propranolol hydrochloride	Propranolol D7 HCl
IUPAC	1-naphthalen-1-yloxy-3-(propan-2-ylamino)propan-2-ol;hydrochloride.	1-(1,1,1,2,3,3,3-heptadeuteriopropan-2-ylamino)-3-naphthalen-1-yloxypropan-2-ol;hydrochloride.
Chemical Formula	$C_{16}H_{22}CLNO_2$	$C_{16}H_{14}D_7NO_2.HCL$
Molecular Weight	295.80g/mol	302.85g/mol
Solubility	DMSO	DMSO
Physical Properties	Off-white to light yellow solid	Off-white,solid
Half Life	4-5 Hours.	4-5 Hours

MATERIALS AND METHOD

Materials

The Propranolol (Working standard), Propranolol D7 (Internal standard) are collected from MTS Lab, Chennai. The authentication of samples is done by melting and solubility studies. The HPLC-MS/MS containing liquid chromatography with software 1.6.2 distributed from Applied Biosystems MDS as the software. The following solvents are used in the process. The Acetone-M, Water, Acetonitrile, TBME and Ethyl acetate are used in HPLC grade. The ammonium Formate and Ammonium Acetate are used in Analytical research grade.

Preparation of Buffer-1 (20 mM Ammonium formate solution)

Weighed about 1.2612 g of Ammonium formate and add 1000 milliliter of water to dissolve.

Preparation of Mobile Phase: [Acetonitrile: Buffer-1 (90:10 % v/v)]

Measure 900 milliliter of Acetonitrile, to this add 100 mL Buffer-1 solution and mix well in an ultrasonic bath for few minutes.

Preparation of Buffer-1 (10 mM Ammonium Acetate solution)

Weigh about 770.8 mg of Ammonium acetate and add 1000 milliliter of water to dissolve and mix and Sonicate it for few minutes.

Propranolol HCl Stock Solution for CC (1.0 mg/mL)

Weigh accurately about 2 mg of Propranolol HCl (Working Standard) and add 1 milliter of DMSO. Calculate the final concentration of Propranolol HCl in µg/mL using the following equation.

$$\frac{\text{Weight of Propranolol HCl (mg)}}{\text{2 mL}} \times \frac{\text{Potency (as is basis)}}{100} \times \frac{M_1}{M_2} \times 1000$$

M_1: Molecular weight of Propranolol Hcl (free) ,M_2 : Molecular weight of Propranolol Hcl (salt)

Preparation of IS Stock Solution: (1.0 mg/mL)

Take accurately about 2 mg of Propranolol D7 HCl and add 1 milliliter of Acetone-M to dissolve and makeup. Calculate the final concentration of Propranolol HCl D3 in µg/mL using the following formula.

$$\frac{\text{Weight of Propranolol (mg)}}{\text{2 mL}} \times \frac{\text{Potency (as is basis)}}{100} \times \frac{M_1}{M_2} \times 1000$$

M_1: Molecular weight of Propranolol HCl (free), M_2 : Molecular weight of Propranolol Hcl (salt)

Spiked Calibration Curves for Standards

Transfer 0.020 mL of the stock aliquot of corresponding concentrations of the above mentioned stock dilutions of Propranolol HCl into 1 mL volumetric flask and add pooled screened K_2EDTA plasma to make up the volume, concentrations mentioned in **Table 3**.

Table: 2 Preparation Of IS Stock Solution: (1.0mg/Ml)

STOCK CONC. (µg/mL)	STOCK ALIQUOT (mL)	DILUENT (mL)	FINAL VOLUME (mL)	FINAL CONCENTRATION (µg/mL)
1000.0000	0.100	9.900	10.000	10.0000
100.0000	0.100	9.900	10.000	0.100

Table: 3 Preparation Of Propranolol Spiked Calibration Curve Standards

STOCK CCID	STOCK CONCENTRATION (µg/mL)	STOCK ALIQUOT (mL)	PLASMA (milliliter)	FINAL VOLUME (milliliter)	FINAL CONCENTRATION (ng/mL)	SPIKED CCID
STD SS H	12.5153	0.020	0.980	1.000	250.3060	STD H
STD SS G	9.3865	0.020	0.980	1.000	187.7300	STD G
STD SS F	4.6933	0.020	0.980	1.000	93.8660	STD F
STD SS E	1.8773	0.020	0.980	1.000	37.5460	STD E
STD SS D	0.9387	0.020	0.980	1.000	18.7740	STD D
STD SS C	0.3755	0.020	0.980	1.000	7.5100	STD C
STD SS B	0.2629	0.020	0.980	1.000	5.2580	STD B
STD SS A	0.1025	0.020	0.980	1.000	2.0500	STD A

Pipette 0.250mL aliquot of each spiked calibration curve standard into polypropylene-capped tubes and store at -70°C ± 20°C in ultra low temperature freezer.

Preparation of Propranolol HCL stock solution for QC

Weigh accurately about 2 mg of Propranolol HCl (Working Standard) and add 1 milliliter of DMSO. Propranolol HCl in µg/mL calculated as follows in **Table 4**:

$$\frac{\text{Weight of Propranolol Hcl (mg)}}{2\ \text{mL}} \times \frac{\text{Potency (as is basis)}}{100} \times \frac{M_1}{M_2} \times 1000$$

M_1: Molecular weight of Propranolol Hcl (free) M_2: Molecular weight of Propranolol HCl (salt)

Table 4: Preparation of Stock Solution of Propranolol For QC

STOCK CONCENTRATION (µg /milliliter)	STOCK ALIQUOT (milliliter)	DILUENTS (milliliter)	FINAL VOLUME (milliliter)	FINAL CONCENTRATION (µg/ milliliter)	STOCK QCID
44.5384	0.420	1.580	2.000	9.3531	SS HQC
9.3531	1.000	1.000	2.000	4.6766	SS MQC
4.6766	0.405	0.000	2.000	0.9470	SS INTQC
0.947	0.580	1.420	2.000	0.2746	SS LQC
0.2746	0.760	1.240	2.000	0.1043	SS LOQQC

Method validation: Sample preparation

Vortex the thawed sample. Add fifty microliter of IS (100 ng/ milliliter of Propranolol D7 HCl) to all RIA vials excluding blank. Measure 200 microliter of plasma. Add 100 µL of Buffer-2 and vortex. Add 2 mL of Extraction solvent and vortex. Load the samples into Centrifuge at 3000rpm for 5mins. Collect 1ml of supernatant into respectively labeled RIA vialsEvaporate the samples in LV. Reconstitute the sample using 1000 microliter of solvent and placed in LC-MS/MS.

Chromatographic Conditions

Column	**: Hypersil Gold, (50 Mm X 4.6 Mm, 5µm)**
Mobile Phase	**: Acetonitrile: Buffer-1 (90:10 % V/V)**
Column Oven Temperature	**: 40◦C**
Auto Sampler Temperature	**: 10◦C**
Injection Volume	**: 10 µl**
Flow Rate : 0.750 Ml/Minutes	
Run Time	**: 4.0 Minutes**
Expected Retention Time	
Propranolol HCl	**: At about 1.80 ±0.5 minutes**
Propranolol D7 HCl	**: At about 1.80 ±0.5 minutes**

LC-MS/MS Condition

The HPLC-MS/MS condition describe in the **Table 5**, the mode of ionization is positive.

Table 5: LC-MS/MS conditions (API-4000 with HPLC)

Molecule	Propranolol Hcl	Propranolol D7 Hcl
Q1 Mass (amu)	260.200	267.200
Q3 Mass (amu)	116.100	116.100
DP	15.00	15.00
EP	5.00	5.00
CE	24.000	22.000
CXP	5.000	5.000

RESULT AND DISCUSSION

Propranolol in the range of 2.0500 ng/mL to 250.3060 ng/mL was developed and validated using LC-MS/MS. Blank K_2EDTA human plasma lots were used for screening. Calibration curve of Propranolol is represented in **Figure 2** to **Figure 10**. For evaluating the selectivity 9 lots of Human plasma were analyzed and No significant interferences were observed, it found to be 12.06% and within acceptance criteria. Signal-to-Noise ratios ranged from 59.721 to 115.769 across the matrix lots evaluated so it demonstrating acceptable S/N intensity. No significance Carry over observed for both the drug and the IS. The calibration curve of Propranolol is linear (**Refer Figure10**), for the establishment of Linearity 3 accepted

precision and accuracy batches were analysed in the range of 2.0500 ng/mL to 250.3060 ng/mL of Propranolol by using IS. This values show the adequate repeatability and reproducibility. Summary of all the experimental parameters and the results are shown in **Table 6**.

Table 6: Summary of Experimental Parameters & Results

S. No.	EXPERMENTAL PARAMETERS	ACCEPTABILE RANGE/CRITERIA (IN %)	RESULTS (IN %)
1	Specificity and Selectivity % of passing lots (%CV of Area Ratio)	> 80 ≤ 20	100% 12.06%
2	Matrix Factor		
	At LQC Level Matrix Factor of IS Normalized (%CV)	0.85 – 1.15 ≤15	0.95 to 1.04 3.19%
	At HQC Level Matrix Factor of IS Normalized (%CV)	0.85 – 1.15 ≤15	0.97 to 1.05 2.53%
3	Carry Over Test	Analyte < 20 IS < 5	0.00 0.35
4	InterBatch Accuracy in LOQQC (% Nominal)	80 – 120	99.76%
	InterBatch Accuracy in LQC, INTQC, MQC & HQC (% Nominal)	85 –115	94.96% to 100.44%
	InterBatch Precision in LOQQC (% CV)	≤ 20	9.11%
	InterBatch Precision in LQC, INTQC, MQC & HQC (% CV)	≤ 15	1.92% to 3.25%
5	Intra Batch Accuracy in LOQQC (% Nominal)	80 – 120	91.57%
	IntraBatch Accuracy in LQC, INTQC, MQC & HQC (% Nominal)	85 –115	93.34% to 101.89%
	Intra Batch Precision in LOQQC (% CV)	≤ 20	8.38%
	IntraBatch Precision in LQC, INTQC, MQC & HQC (% CV)	≤ 15	1.07% to 3.59%
Analyte: Propranolol Matrix: K_2EDTA Human Plasma Validated CC range (ng/mL): 2.0500-250.3060			

Fig 2: AQS Std Solution For Propranolol

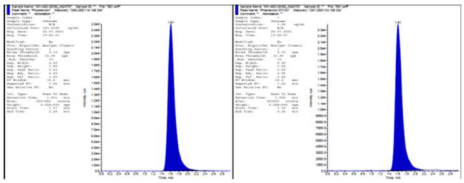


Fig 3: Standard Blank For Propranolol

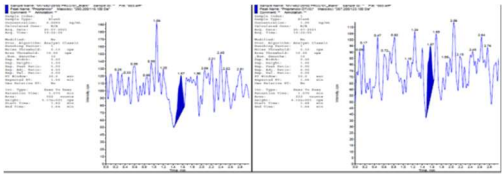


Fig 4 :Std Zero For Propranolol

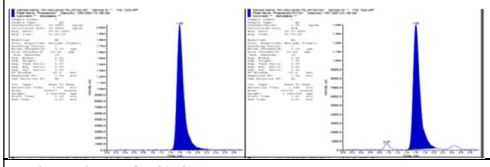


Fig 5 LLQQC Sample For Propranolol

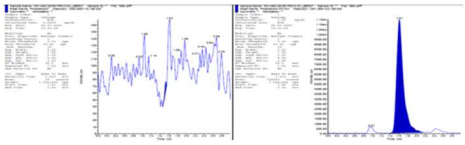


Fig 6: LQC Sample For Propranolol

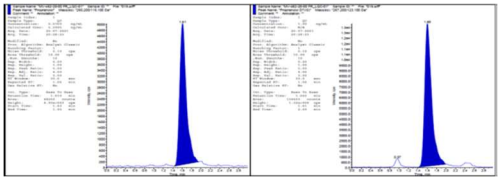


Fig 7: INTQC Sample For Propranolol

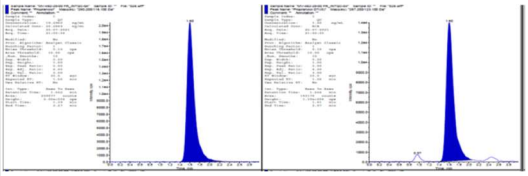


Fig 8: MQC Sample For Propranolol

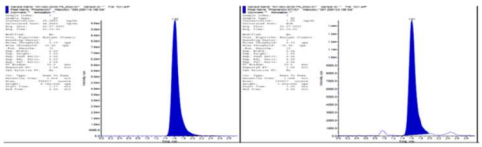


Fig 9: HQC Sample For Propranolol

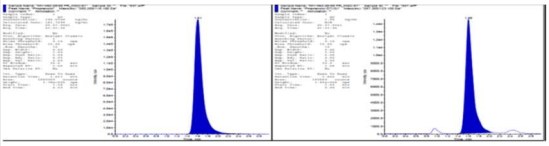
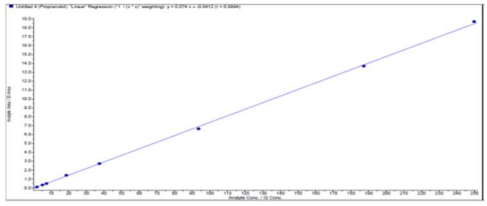


Fig 10: Representative Regression Analysis of a CC for Propranolol



CONCLUSION

The bio-analytical method for Propranolol over the range of 2.0500- 250.3060 ng / mL was successfully validated partially. LC-MS/MS which is used to separate Compounds chromatographically and then detect the compounds by mass spectrometer. Propranolol become extracted from 250 µL plasma by means of the usage of solid Phase extraction method turned into accomplished on Hypersil gold 50 x 4.6 mm column. The plasma (200 µL) processed using Liquid-Liquid extraction technique. This method is suitable for sample analysis to support bio-equivalence/ bioavailability and/or pharmacokinetic studies involving formulations of Propranolol.

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