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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF QUININE IN HUMAN PLASMA BY LC-MS

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Keywords:

Quinine, LC-MS, Human plasma, Bioanalytical

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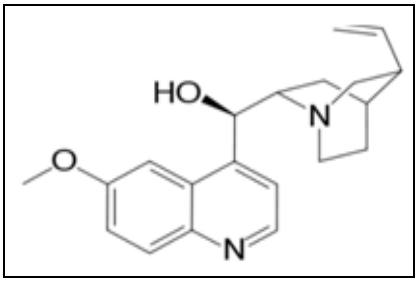
ABSTRACT: To develop a simple, specific, sensitive and robust bioanalytical method for determining Quinine in the human plasma with the help of LC-MS/MS API2000 various found parameters. Different Equipment experiment was found within the acceptance criteria, Different Column experiment was found within the acceptance criteria, and the Proposed Bioanalytical method for quantifying Quinine from K3EDTA based Human plasma was satisfactorily validated. It can be used to quantify Quinine from K3EDTA-based Human Plasma in Bioequivalence and Bioavailability Study.

INTRODUCTION: The reliability of analytical findings is important in forensic and clinical toxicology, as its off-course is a precondition for the correct construal of toxicological findings. This is especially true in the context of quality management and accreditation. Selective and sensitive analytical methods and bioanalytical methods for the quantitative and qualitative evaluation of drugs and their metabolites (analytes) are critical for the successful conduct of preclinical and/or biopharmaceutics and clinical pharmacology studies. The first studies measuring drugs in biological fluids were carried out to determine possible overdosing as part of the new science of forensic medicine/toxicology. Some techniques commonly used in bioanalytical studies include. Quinine is a natural white crystalline alkaloid having antipyretic (fever reducing), antimalarial, analgesic (painkilling), anti-inflammatory properties it is a well-known bitter antimalarial drug occurring among the alkaloids of cinchona bark and Ramijia. Cinchona bark contains about thirty alkaloids, but its antimalarial activity is mainly due to quinine, quinidine, cinchonine and cinchonidine; the theorized mechanism of action for quinine and related anti-malarial drugs is that these drugs are toxic to the malaria parasite. Specifically, the drugs interfere with the parasite's ability to break down and digest haemoglobin. Consequently, the parasite starves and/or builds up toxic

Structure:



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Method Development: Liquid Chromatography coupled with tandem quadrupole mass spectrometry operated in multiple reactions monitoring (MRM) mode has been found as a very sensitive technique for determination as well as quantitation of drugs and their metabolites in biological matrix which is very much useful in pharmacokinetic studies.

Determination of Parent Ion: The parent ion for the respective analytes had been determined by AB SCIEX triple quadrupole mass spectrometer API 2000. The instrument was equipped with software 'Analyst 1.4.2' which had been used for subsequent quantitation and other relevant calculations. For Q1 scanning of the analytes, a long mass range (100-400) was selected.

Optimization of MRM Transitions: The parent ion breaks into several fragments on application of collision energy. The optimum collision energy was selected by studying the change of pattern in intensity of ionization of parent ion *i.e.* the intensity of daughter ion increased by increasing collision energy with proportionate decrease in parent ion intensity. The other compound-dependant parameters liked clustering potential (DP), Entrance potential (EP), Focusing potential (FP), Collision cell Entrance potential (CEP), Collision cell exit potential (CXP) were optimized similarly by varying simply and studying the change of the intensities of parent and daughter ion peaks

Finalizing the MS/MS Method Development: After getting all the optimized parameters, a multiple reaction monitoring (MRM) mode was built to create MS/MS method for the determination and subsequent quantitation of Quinine in biological matrix (*i.e.* plasma) along with Quinine D3 using as internal standard.

MRM Transition for Quinine D3 (Q1):

Optimized MRM Transition: Optimized MRM transitions and compound dependent and source dependant parameters for the Quinine and Quinine D3.

Optimized MRM Transitions and Parameters for Quinine and Quinine D3:

MS Transition	Quinine	Quinine D3
Ql	325 amu	328.20 amu
Q3	160 amu	163.20 amu

Compound Dependant Parameters		
Polarity	**+ve**	**+ve**
DP (Volts)	80.00	80.00
FP (Volts)	58.00	80.00
EP (Volts)	10.00	10.00
CEP (Volts)	25.00	16.72
CE (Volts)	51.00	47.00
CXP (Volts)	16.00	16.60
Dwell time (msec)	200.00	200.00

Source Dependant parameters	
CUR	20.00
IS	3000.00
Tem(‘c)	350.00
Gsl	40.00
Gs2	60.00
ihe	ON
CAD	6.00

Development of Liquid Chromatographic Method: The process of bioanalysis in a regulated environment involves the development of a highly sensitive MS/MS assay to detect an analyte in the low nanogram to pictogram level. Critical to the success of this assay is the separation of Quinine from the internal standard used as well as endogeneous materials present in plasma, such as phospholipids and other exogeneous metabolites from the analytes. Rigorous demands are placed on developing LC method to detect and quantify Quinine in a biological matrix. Criteria that the method must include: accuracy, precision, specificity, detection limit, quantitation limit,

linearity, range, robustness. A method development strategy that is systemic in its approach can greatly simplify the process and allow the development optimal conditions quickly and efficiently.

Optimization of Chromatographic Condition: C18 and C8 are columns used during LC Method development. A column that can be operated over a wide range of pH (pH 2 to pH 8) is suitable for experimentation with concentration, strength, and ratio of various types of mobile phases consisting of organic solvent and buffers while providing sufficient resolution, sensitivity and response for Quinine. Using a shorter column enabled the analyst to achieve faster separation for the analyte. Since, the drug was non-polar, C18 column was preferred for separating dug from the plasma matrix because C8 column was showing poor resolution. There was no other interfering peak around the retention time 1.90 min of Quinine.

Optimization of a Mobile Phase: LC-MS/MS system requires a mobile phase containing volatile component. Mobile phase composition was altered using buffer solution in different proportions of varying pH with mobile phase. Electro-spray ionization is more efficient with a higher organic modifier concentration in mobile phase. A mobile phase was developed using acetonitrile as an organic solvent. Acetonitrile has higher eluting power than methanol; methanol is more polar than acetonitrile in reversed phase chromatography; the objective of this study was to develop a simple reliable method that would facilitate analysis of Quinine in human body fluid in large number of samples over a relatively short period of time. In cost-effective manner the suitability of mobile phase with 90% acetonitrile as organic modifier was investigated. The presence of a buffer solution in mobile phase is essential to achieve a peak of good resolution along with better sensitivity in reverse-phase chromatography.

Optimization of Sample Extraction Procedure: Adequate sample preparation is a key aspect of quantitative bioanalysis and can often cause bottlenecks during high-throughput analysis. Achievement of cleanliness in the extracted sample *i.e.* free from endogeneous as well as exogeneous interferences, gives assurance on long-term stability of the assay involving biological matrix performance. Four main principles of sample extraction are applied in general LC: Precipitation by addition of organic solvents, inorganic acids and/or chaotropic salts, protein filtration, Liquid – Liquid extraction (LLE), and Solid phase extraction (SPE).

RESULTS AND DISCUSSION:

System Suitability: The system suitability experiment was performed before the analytical day, and % RSD of area ratio of analyte to IS for MQC samples and signal to noise ratio (S/N) ratio for LLOQ sample was found within the acceptance criteria.

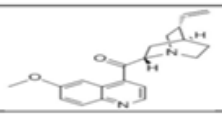
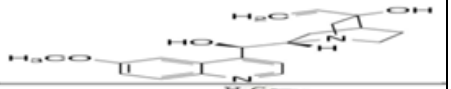
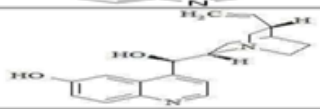
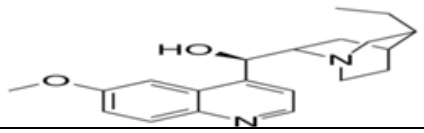
System Suitability Experiments and Signal-to-Noise (S/N) ratio for LLOQ:

Equipment ID	Batch ID	%RSD of area ratio of analyte to IS for MQC samples	Signal to Noise (S/N) ration for LLOQ sample
INS/ANA/01	SST01	1.44	627.64
INS/ANA/01	SST02	2.37	438.11
INS/ANA/01	SST03	2.37	438.11
INS/ANA/01	SST04	1.01	253.66
INS/ANA/01	SST05	0.37	442.35
INS/ANA/02	SST06	1.81	456.85
INS/ANA/01	SST07	1.03	551.25
INS/ANA/02	SST08	1.47	658.24
INS/ANA/01	SST09	1.80	653.48
INS/ANA/01	SST10	2.36	94.32
INS/ANA/01	SST11	1.23	163.55
INS/ANA/01	SST12	1.32	248.39
INS/ANA/02	SST13	1.94	99.23
INS/ANA/01	SST14	0.94	443.39

Autosampler Carryover Test: The Autosampler carryover test was performed, and no % carryover was observed in blank samples.

S. no.	Auto sampler carryover Test samples	Area observed at RT of Analyte	% Carryover of Analyte	Area observed of RT of IS	% Carryover of IS
	Aqueous (AQ)				
1	MP	0		0	
2	AQSTD H	387656	0.00	235665	
3	MP	0		0	0.00
4	AQ STD	6692		313131	
	Extracted				
5	BLK	385890	0.00	0	0.00
6	STD H	0		238666	
7	BLK	5938		0	
8	STD A			281445	

Selectivity and Specificity:

S. N	Compound Name	Molecular Weight	Structure
01	Quininone	322.40	
02	3- Hydroxyquinine	340.18	
03	o- desmethylquinine	310.17	
04	10,11-dihydroxydihydroquinine	358.19	

Information about Metabolites: Reference: Label Information QUALAQUIN, NDA no. 021799, USFDA Reference ID: 3535706, Quinine is metabolized almost exclusively via hepatic oxidation cytochrome P 450 (Cyo) pathways, resulting in four primary metabolites, 3-Hydroxyquinine, 2- Quinone, Odesmethylquinine. and 10, 11- dihydroxydihydroquinine. The molecular weight and structure of Quinine and its metabolites are given

Molecular Weight and Structure of Quinine Metabolites: From the tablet it is evident that the molecular weight of metabolites is very much different from Quinine, so none of the metabolites can have MRM of Quinine in the current method of Quantification, MRM used for Quinine was 325/160.0 amu in positive polarity mode.

Due to the specific MRM of MRM of Quinine, these metabolites cannot be detected in the current method hence it can be concluded that analytical method used for the determination of Quinine in human plasma with K3EDTA is selective.

S. no.	Sample ID	Type of Biological MatriX	Area at RT of Analyte	% Interference of Analyte	Area at RT of Is	% Interference of IS
1	BLK01		0		0	
2	LLOQ01		6165	0.00	279733	0.00
3	BLK02		0		0	
4	LLOQ02		6708	0.00	309451	0.00
5	BLK03		0		0	
6	LLOQ03	Normal Plasma	6549	0.00	307911	0.00
7	BLK04		0		0	
8	LLOQ04		7263	0.00	314786	0.00
9	BLK05		0		0	
10	LLOQ05		8494	0.00	338108	0.00

11	BLK06		0		0	
12	LLOQ06		6200	0.00	289133	0.00
13	BLK07	Haemolyzed Plasma	0		0	
14	LLOQ07		5854	0.00	271219	0.00
15	BLK08	Lipemic Plasma	0		0	
16	LLOQ08		5303	0.00	261850	0.00

Quantification of Quality Control Sample: Quantitation of Quinine was not affected due to concomitant medication in Quality control samples.

Concomitant Medication Experiment– Quantification of Quality Control Samples:

Sample ID	**LQC**	**HQC**
Nominal concentration (ng/mL)	**202.10**	**4021.89**
Acceptable Limit(ng/mL)	**171.79 to 232.42**	**3418.61 to 4625.17**
S. no.	**Back calculated concentration**	**(ng/mL)**
1	195.23	3989.96
2	191.91	4092.46
3	187.39	4066.34
4	188.21	4064.57
5	197.33	4215.27
6	191.06	3953.28
Mean	191.86	4063.65
SD	3.8788	91.0368
%RSD	2.02	2.24
% Nominal	94.93	101.04

SUMMARY AND CONCLUSION:

Parameters	**Criteria**	**Limit**	**Results**
System Suitability	% RSD of area ratio of Analyte to IS for six consecutive injections for MQC samples	NMT 5.00%	0.37 to 2.37
Autosampler Carryover Test	Suppose any peak is present at the retention time of Analyte in Mobile phase injection or Blank sample. In that case, its area response should be < 20.00 % of area response of an Aqueous STD A (LLOQ) or Extracted STD A (LLOQ) respectively. If any peak is present at the retention time of an IS in Mobile phase injection or Blank sample, its area response should be < 5.00% of area response of an Aqueous STD A (LLOQ) or Extracted STD A (LLOQ) respectively	1. If any peak is present at the retention time of Analyte in Mobile phase injection or Blank sample, its area response should be < 20.00 % of area response of an Aqueous STD A (LLOQ) or Extracted STD A (LLOQ) respectively. 2. If any peak is present at the retention time of an IS in Mobile phase injection or Blank sample, its area response should be < 5.00% of area response of an Aqueous STD A (LLOQ) or Extracted STD A (LLOQ) respectively	Complies
Selectivity	Response of an interfering peak at the retention time of Analyte should be < 20.00% of respective LLOQ and response of interfering peak in blank at the retention time of IS should be 5.00 % of the response of IS in LLOQ	Response of an interfering peak at the retention time of Analyte should be < 20.00% of respective LLOQ and response of interfering peak in blank at the retention time of IS should be< 5.00 % of the response of IS in LLOQ	Complies
Haemolysis Effect	% RSD		
	LQC	NMT 15.00%	3.44
	HQC		1.56
	% Nominal		
	LQC	85.00% to 115.00%	92.86
	HQC		95.02
	% RSD		
Lipemic Effect	LQC	NMT 15.00%	2.44
	HQC		1.28

	% Nominal		
	LQC	NMT 15.00%	90.43
	HQC		93.89
Matrix Factor	%RSD		
	LQC	NMT 15.00%	3.18
	HQC		1.67
Concomitant Medication Experiment	Response of an interfering peak at the retention time of Analyte should be < 20.00% of respective LLOQ and response of interfering peak at the retention time of IS should be< 5.00% of the response of IS.	Response of an interfering peak at the retention time of Analyte should be < 20.00% of respective LI,OQ and response of interfering peak at the retention time of IS should be < 5.00% of the response of IS	Complies

	% Nominal		
	LQC		94.93
	HQC	85.00% to 115.00%	101.04
	%RSD		
Concomitant Medication Experiment (Quantification of Quality Control samples	LQC	NMT15.00%	2.02
	HQC		2.2
	Correlation Coefficient	>0.99	0.9988 to 0.9999
Calibration Curve	% Nominal at LLOQ level	80.00% to 120.00%	98.55
	% Nominal at levels other than LLOQ level	85.00% to 115.00%	98.38 to 103.31

ACKNOWLEDGMENT: Nil

CONFLICT OF INTEREST: Nil

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