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A STUDY ON BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DORAVIRINE IN HUMAN PLASMA BY RP-HPLC

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ABSTRACT

For analytical purposes, a variety of approaches are employed to separate medicines from biological matrices. Some of the more popular ones are chromatography-based. Chromatography refers to a broad range of techniques used to separate chemically similar components from mixtures. In chromatography, the components to be separated are divided between two phases, one of which is stationary phase, and the other of which is mobile phase, which moves over the stationary phase in a certain direction. The phases are selected so that various sample elements have different affinities for every phase. There are numerous HPLC separation methods. The classification of the mode of liquid chromatography is based on how the solutes interact with the stationary phases. Size exclusion, ion-exchange, normal, and reversed phase are only a few of the several LC modes. A pump, injector, column, detector, and data system are components of HPLC apparatus. The column where separation takes place is the brain of the system. The mobile phase must be pumped through the column at high pressure because the stationary phase is made up of porous micrometer-sized particles. The solute is injected onto the top of the column to start the chromatographic process.

KEY WORDS: Bioanalytical Method, Development, Validation, Doravirine, Human Plasma**.**

INTRODUCTION

In the treatment of human immunodeficiency virus (HIV) infection, doravirine, a non-nucleoside reverse transcriptase inhibitor, is used in conjunction with other antiretroviral medications1. Although doravirine has not been linked to instances of clinically obvious acute liver injury, it is related with a low risk of transitory serum aminotransferase increases while undergoing therapy. For the treatment of HIV-1 infection in adult patients who have never had antiretroviral therapy, doravirine is appropriate in combination with other antiretroviral medications.

It is a branch of science that investigates the chemistry, physiology, and behaviour of matter. Gathering and interpreting chemical data is the goal of chemical analysis. To gather and evaluate qualitative, quantitative, and structural data on the nature of matter, a variety of approaches and methodologies are applied. Before estimating and identifying a component, it is possible to separate it. Identification of the elements, species, and/or compounds contained in a sample is known as qualitative analysis. Calculating the absolute or relative amounts of the elements, species, or compounds contained in a sample is known as quantitative analysis. Structural analysis is the process of identifying distinctive atoms and/or functional groups as well as the spatial arrangement of atoms in an element or molecule. Due to the fact that humanity is plagued by a number of potentially fatal diseases, the use of therapeutic medicines has dramatically expanded throughout time. According to the need, they are injected into the biological system via a variety of routes in order to address all health issues.

As a result, pharmaceutical products are essential to the health care system and have integrated into daily life as basic necessities. The identification, purity, safety, and efficacy of medications must be perfect in order for them to fulfil their intended function. The development of analytical procedures is based on the physico-chemical characteristics of drug substances as well as the characteristics that are measured for qualitative and quantitative evaluation. The fast development of electrical and computing technologies has led to the emergence of many complex analytical tools. Instrumental methods refer to any method that uses instruments to measure an analyte's physical characteristics. For the separation, identification, quantification, and characterization of several pharmaceutical products, biologics, contaminants, and metabolites, instrumental techniques analysis has developed into a potent tool. Drug research and discovery now depend on it inextricably.

RESEARCH METHODOLOGY

MATERIALS

DRUG TEST SAMPLES

We bought doravirine and hydrochlorothiazide from Spectrum Pharma research solutions in Hyderabad, India, to use as internal standards.

METHODOLOGY

SELECTION OF WAVELENGTH

A 10 g/mL doravirine UV spectrum in acetonitrile Water was detected using scanning in the 200–400 nm range. The medication displayed good absorption at 264 nm in the UV spectrum, hence this wavelength was chosen.

SELECTION OF CHROMATOGRAPHIC METHOD

The type of material, including its ionic/ionisable/neutral character, molecular weight, and solubility, affects the choice of chromatographic procedure. The medication chosen for the investigation had polar properties. Therefore, ion-pair or exchange chromatography, as well as reverse phase HPLC, must be used. For the initial separations, the RP-HPLC method was chosen because to its adaptability and simplicity.

SELECTION OF INTERNAL STANDARD

Hydrochlorothiazide was chosen and chromatographed along with the standard medication based on the polarity and solubility. Hydrochlorothiazide took 2.28 minutes to elute. The hydrochlorothiazide peak was symmetric and well separated from the doravirine peak. Therefore, hydrochlorothiazide was chosen as the internal standard for the current investigation.

EXTRACTION PROCESS OF PLASMA SAMPLES

From the spiked solutions, 250 l of plasma, 500 l of internal standard, and 250 l of doravirine were transferred to a set of polypropylene tubes that had already been pre-labeled and contained 1.5 mL of acetonitrile. The tubes were vortexed for 2 minutes before being centrifuged at 3200 rpm for 5 minutes. The organic layer is collected after centrifugation, and 20 l were immediately injected into HPLC.

DATA ACQUISITION AND PROCESSING

The Empower 2 programme was used to obtain the chromatograms and process the data using the peak area ratio approach. The following equation of the regression analysis of the spiked plasma calibration graph using 1/x2 as the weighing factor was used to determine the concentration of the unknown samples.

Y= m x + c

X = Internal Standard Concentration / Analyte

Y = Internal standard area / Analyte (Peak area ratio) M is the calibration curve's slope.

C is the intercept value.

RESULTS AND DISCUSSION

Table 1 provide an overview of the method development trails' optimization.

Table-.1 Optimization of RP-HPLC method

Trail No.	Column	Mobile phase composition	Injection volume	λmax (nm)	Flow rate (mL/min)	Observation
1	Phenomenex C18 (250×4.6mm,5µ)	Acetonitrile:Na_2HPO_4 (65:35)	20µl	264nm	1.0mL/min	Peak shape was not good.
2	Phenomenex C18 (250×4.6mm,5µ)	Acetonitrile: OPA (70:30)	20µl	264nm	1.0mL/min	Low plate count was observed.
3	Agilent C18 (250×4.6mm,5µ)	Acetonitrile: OPA (60:40)	50µl	264nm	1.0mL/min	Low plate count was observed.
4	Phenomenex C18 (250×4.6mm,5µ)	Acetonitrile:KH_2PO_4 (65:35)	20µl	264nm	1.0mL/min	Good peak shape, tailing and resolution were observed.

DETERMINATION OF Λ_{MAX} FOR DORAVIRINE

Doravirine's isobestic point (max) was discovered to be at 264 nm. It suggests that the most sensitive wavelength for HPLC operations would be detection at 264 nm. For the development of an HPLC technique for doravirine, this max was used.

METHOD VALIDATION FOR BIO ANALYTICAL STUDIES OF DORAVIRINE

The technique was validated to satisfy the requirements of the industrial guidance for the validation of bioanalytical methods.

SPECIFICITY AND SELECTIVITY

Six different random blank human plasma samples at either the Doravirine or ISTD retention durations failed to reveal any interfering peaks. According to the chromatogram shown above, the overall run duration was 6 min, and the retention times for the analyte and the ISTD were 3.1 and 2.2 min, respectively. There were no interfering peaks near the peaks for Doravirine and the internal standard in the chromatogram of blank plasma. The chromatogram of blank plasma spiked with ISTD shows the same thing.

SYSTEM SUITABILITY

Doravirine and the ISTD area ratio were found to have a %CV of 0.57%. As a result, it was found to be system suitable.

Table 2.: System suitability Results of Doravirine

SampleName	AnalyteArea	AnalyteRt (min)	ISTD Area	ISTD Rt (min)	Area Ratio
MQC	33994	3.189	61813	2.285	0.5499
MQC	34079	3.199	61503	2.286	0.5541
MQC	34132	3.199	61934	2.286	0.5511

MQC	33980	3.203	61947	2.286	0.5485
MQC	33759	3.208	61734	2.291	0.5468
MQC	33663	3.208	61733	2.291	0.5453
Mean		0.321		2.288	0.54931
SD		0.0071		0.0027	0.003142
%CV		0.22		0.12	0.57

Acceptance Criteria: The retention time's%CV must be less than 2.00%. The area ratio's CV should be less than 5.00%.

SENSITIVITY

Doravirine's CV was determined to be 3.06%, while its mean accuracy was determined to be 100.08%. It so passed the sensitivity test. Acceptance Criteria: Four out of six samples, or at least 67%, must fall within the range of 80.00 to 120.00. The recommended range for mean accuracy is 80.00–120.00%. CV must be less than 20.00%.

THE MATRIX EFFECT

By contrasting the responses of post-extracted plasma standard QC samples (n=6) with the responses of the analyte from neat samples at comparable concentrations, the matrix of plasma constituents over the ionisation of the analyte was identified. Human plasma that had been chromatographically filtered was used to evaluate the matrix effect. At HQC and LQC, Doravirine has a precision (%CV) of 1.38% and 2.00%, respectively. Acceptance Criteria: Samples at each level must be within the range of 85.00-115.00% in at least 67% (2 out of 3 cases). At least 80% (5 of 6) of the matrix lot must meet the requirements for acceptance. LQC and HQC samples made from various biological matrix lots should have a mean accuracy of back estimated concentration within the range of 85.00-115.00%.

LINEARITY

A weighted (1/x2 where X is concentration) least square regression analysis of the standard plots connected to the six point standard curve of doravirine was used to assess the method's linearity. Over the Doravirine concentration range of 50.00-2000.00 ng/mL, the standard curve was linear. It was discovered that the correlation coefficient was 0.998. The ratio of the analyte peak area to the ISTD peak area was used to quantify samples. Peak area ratios and plasma concentrations were plotted.

PRECISION AND ACCURACY

Six replicates of samples containing doravirine were examined at six different QC levels in order to assess the intra-assay precision and accuracy. The analysis of the four levels of QC samples from four independent runs was used to calculate the inter-assay precision. Except for LLOQ QC, where it should be within 80-120% for accuracy and 20.00% of RSD, the criteria for acceptable data include accuracy within 85-115% from the actual values and a precision of within 15% relative standard deviation.

ANALYTE RECOVERY

At low, medium, and high quality control concentration levels, the recovery of the medication and ISTD were assessed. By comparing its reaction in replicate samples with that of neat standard solution responses, recovery was estimated. The extraction efficiency of an analyte from a sample matrix is measured by comparing the analytical response to the amount of analyte added to the value obtained from the sample matrix. Doravirine has fundamental qualities, hence Acetonitrile solvent was used for extraction. In experiments using spiked chemicals, analyte recoveries ranged from 97.0% to 99.80%, and ISTD recoveries were 97.40%.

Table 3: Recovery of analyte of Doravirine

plicate No.	HQC		MQC		LQC	
	Unextracted Response	Extracted Response	Unextracted Response	Extracted Response	Unextracted Response	Extracted Response
1	56162	54731	34143	33994	5655	5516
2	55831	54259	34026	34079	5662	5523
3	56165	54611	34189	34132	5686	5508
4	55806	54156	34112	33980	5628	5556
5	55910	54355	34210	33759	5640	5538

6	56059	54250	34313	33663	5700	5514
n	6	6	6	6	6	6
Mean	55989	54394	34166	33935	5662	5526
SD	161.55	227.06	97.07	184.40	27.24	18.00
%CV	0.29	0.42	0.28	0.54	0.48	0.33
%Mean Recovery	97.15		99.32		97.60	
verall % Mean Recovery	98.024					
verallSD	1.1475					
Overall %RSD	1.17					

The %RSD of recovery at each QC level and for ISTD must be less than 15.005. For all QC levels, the overall mean recovery and RSD should be less than 20.00%.

RUGGEDNESS

An experiment on toughness was conducted by a separate column and analyst. Batch was processed against calibration curve standards and examined by a different analyst using a different column in order to assess ruggedness, precision, and accuracy.

Table 4: Ruggedness Linearity of Doravirine

Conc. (ng/mL)	**50**	**100**	**150**	**400**	**1000**	**1200**	**1600**	**2000**
Different column	**48.259**	**97.539**	**147.758**	**393.180**	**998.130**	**1198.120**	**1604.1**	**1998.51**
Different Analyst	**50.221**	**101.442**	**150.675**	**401.980**	**1002.59**	**1215.850**	**1597.5**	**2081.82**

Table 5: Ruggedness Precision and Accuracy of Doravirine

Batch ID	QC ID	HQC	MQC	LQC	LLOQQC
Nominal Conc. (ng/mL)		**1600**	**1000**	**150**	**50**
Differentcolumn	1	1592.790	978.210	142.751	44.263
	2	1594.380	975.520	140.730	47.220
	3	1597.980	985.320	144.680	55.260
	4	1595.750	984.580	148.699	48.221
	5	1606.890	978.900	155.780	52.251
	6	1603.390	984.260	150.810	49.289
	Mean	1598.5300	981.1317	147.2417	49.4173
	SD	5.50836	4.10431	5.60161	3.87323
	%CV	0.34	0.42	3.80	7.84
	% Mean accuracy	99.91	98.11	98.16	98.83
DifferentAnalyst	1	1594.340	985.160	140.790	48.281
	2	1592.260	988.216	142.765	46.210
	3	1596.120	975.020	144.720	48.255
	4	1598.020	974.550	147.680	52.260
	5	1603.940	982.650	156.640	50.243
	6	1601.800	980.540	150.821	49.230
	Mean	1597.7467	981.0227	147.2360	49.0798
	SD	4.45458	5.47120	5.81989	2.05151
	%CV	0.28	0.56	3.95	4.18
	% Mean accuracy	99.86	98.10	98.16	98.16

Acceptance Criteria: For LQC, MQC, and HQC samples, the within- and between-batch precision should be 15.00%, and for LLOQ QC, it should be 20.00%. With the exception of LLOQ QC, at least 67% (16 out of 24) of all QC samples and 50% (3 out of 6) at each level should fall within the range of 85.00-115%. LLOQ QC

ought to fall between 80.00 to 120.00%. The range of the mean accuracy for the LQC, MQC, and HQC samples should be between 85 and 115%, while the range for the LLOQ QC sample should be between 80 and 120%.

CONSISTENCY

A subset of the freshly prepared stock solutions (stability samples) were stored at room temperature. Stability on day zero: Stock solutions of doravirine and hydrochlorothiazide (ISTD) were made at 0.1 mg/mL in acetonitrile. By comparing the mean of the responses from six duplicates of the stability samples, the stability of the stock solution of doravirine and hydrochlorothiazide in acetonitrile was evaluated. It was discovered that the%CV and% mean accuracy for doravirine were respectively 0.35%, 1.98% and 99.94%, 100.38%. As a result, it passed the Stability test on day one.

Table 6: Stability on day Zero of Doravirine

Replicate No.	**HQC**	**LQC**
	Nominal Concentration (ng/mL)	
	1600.000	**150.000**
	Nominal concentration Range (ng/mL)	
	(1360.000-1840.000)	**(127.500-172.500)**
	Calculated Concentration (ng/mL)	
1	1592.160	152.790
2	1594.580	150.751
3	1598.340	154.720
4	1597.920	147.690
5	1607.550	150.640
6	1603.210	146.820
N	6	6
Mean	1598.9600	150.5685
SD	5.63455	2.98481
%RSD	0.35	1.98
%Mean Accuracy	99.94	100.38

ACCEPTANCE CRITERIA:

At each level, 50% (3 out of 6) and at least 67% (8 out of 12) of the total QC samples should fall within the range of 85.00-115.00%. The range for the mean LQC and HQC accuracy should be between 85.00 and 115.00%. LQC and HQC samples should have a CV of less than 15.0 %. Permanent stability Extract wet: -280C was applied to the 0.1 mg/mL stock solution for 37 days. 1.09%, 0.37%, 3.97%, 4.11% and 98.53%, 99.92%, 98.18%, 98.94%, respectively, were found to be the RSD and Mean accuracy for doravirine.

Table 7: Wet Extract of Doravirine

Replicate No.	**HQC**		**LQC**	
	Nominal Concentration (ng/mL)			
	1600.000		**150.000**	
	Nominal concentration Range (ng/mL)			
	(1360.000-1840.000)		**(127.500-172.500)**	
	Calculated Concentration (ng/mL)			
	ɔmparisonsamples	**Stabilitysamples**	**ɔmparisonsamples**	**Stabilitysamples**
1	1563.540	1594.120	142.755	143.779
2	1561.320	1592.210	140.790	141.718
3	1567.960	1598.030	144.715	144.789
4	1573.840	1596.940	147.810	152.859
5	1585.590	1607.580	150.820	149.689
6	1606.800	1603.890	156.691	157.668
N	6	6	6	6
Mean	1576.5083	1598.7950	147.2635	148.4170
SD	17.19953	5.87162	5.84042	6.10705
%RSD	1.09	0.37	3.97	4.11
%Mean Accuracy	98.53	99.92	98.18	98.94
%Mean Stability	101.41		100.78	

Over time Analyte stability in the Doravirine matrix has been demonstrated at -280°C for 37 days. Acceptance Standards: The range for stability and comparison samples should be between 85 and 115.00% for at least 67% (8 out of 12 total QC samples) and 50% (3 out of 6) for each level. The range of 85.00 to 115.00% should apply to the mean accuracy of back computed concentrations for LQC and HQC samples. LQC and HQC samples should have a CV of less than 15.0 %. LQC and HQC samples' mean stability should range from 85.00 to 115.00%.

Freeze thaw at -80^{0}C:

The 0.1 mg/mL stock solution was frozen at -800C for 37 days. 0.44%, 0.26%, 2.90%, 4.27% and 99.97%, 99.93%, 98.95%, 101.39%, respectively, were found to be the RSD and Mean accuracy for doravirine. So, at -800C, it passed the Freeze-Thaw test. Acceptance Criteria: The range of the comparison samples should be between 85 and 115.00%, and the range of the total QC samples should be at least 67% (8 out of 12) and 50% (3 out of 6) at each level. The range for the mean accuracy of the LQC and HQC samples' backcalculated concentrations should be 85.00–115.00%. LQC and HQC samples should have a CV of less than 15.0 %. LQC and HQC samples' mean stability should range from 85.00 to 115.00%.

CONCLUSION

Based on the findings of this study, it can be said that the current validated method can be used to determine doravirine in human plasma using HPLC with UV detection and that it has successfully met the acceptance criteria for selectivity, precision, accuracy, linearity, recovery, and stability over a theoretical concentration range of 0.05 g/mL to 2 g/mL for doravirine. In order to determine the presence of doravirine in human plasma and other biological fluids, the approach was shown to be linear with an acceptable correlation coefficient. Insignificant degradation of Doravirine during the prescribed storage durations and circumstances was shown by stability assessments in EDTA human plasma, stock solutions, and stock dilutions that passed the acceptance criteria. The findings were compiled in table 16 as a summary. The simplified, accurate, precise, and repeatable HPLC-UV technique. The process uses a mobile phase that is simple to prepare and is linear over a broad range. This approach is appropriate for processing several samples in a short amount of time due to its straightforward sample preparation process and reasonably quick chromatographic run time. The approach created conforms with the US-FDA guidelines' validation criteria. As a result, the devised approach can be used for human therapeutic medication monitoring and pharmacokinetic investigations.

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