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Research Article

VALIDATION OF PREGABALIN IN HUMAN PLASMA BY LCMS METHOD

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ABSTRACT

An accurate and precise developed LCMS method was validated for the determination of pregabalin in human plasma. Validation was carried out according to US FDA guidelines. Validation data showed were within the limits. No matrix effect was found in different sources of human plasma tested. Dilution integrity, Lower limit of quantitation were also within the limit. The Mean extraction recovery of pregabalin was satisfactory.

Keywords: Pregabalin, dilution integrity, matrix effect, LC-MS-MS.

INTRODUCTION

Pregabalin is chemically (S)-3-aminomethyl-5-methyl hexanoic acid, is a structural analogues of Gamma aminobutyric acid (GABA). The search for the reliable range of a method and continuous application of this knowledge is called validation. It can also be defined as the process of documenting that the method under consideration is suitable for its intended purpose.

Method validation[1-5] involves all the procedures required to demonstrate that a particular method for quantitative determination of the concentration of an analyte (or a series of analytes) in a particular biological matrix is reliable for the intended application. Validation is also a proof of the repeatability, specificity and suitability of the method. Bioanalytical methods must be validated if the results are used to support the registration of a new drug or a new formulation of an existing one. Validation is required to demonstrate the performance of the method and reliability of analytical results. If a bioanalytical method is claimed to be for quantitative biomedical application, then it is important to ensure that a minimum package of validation experiments has been conducted and yields satisfactory results. The guideline[6-9] for industry by FDA states that the fundamental parameters of validation parameters for a bioanalytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility and stability. Typical method development and establishment for bioanalytical method includes determination of (1) selectivity, (2) accuracy, (3) precision, (4) recovery, (5) calibration curve, and (6) stability. For a bioanalytical method to be considered valid, specific acceptance criteria should be set in advance and achieved for accuracy and precision for the validation of the QC samples.

EXPERIMENTAL

Recovery

Recovery of the developed method can be evaluated by analyzing six replicates of analyte along with internal standard by comparing the analytical results for extracted samples at three concentrations (equivalent to LQC, MQC and HQC) with unextracted samples that represent 100% recovery. The percentage recovery of analyte and internal standard (IS) were calculated using appropriate chromatographic conditions.

LOWER LIMIT OF QUANTIFICATION (LLOQ)/ SENSITIVITY

Sensitivity was determined by limit of quantification by analyzing six replicates of lower limit of quantification (LLOQ) that can be measured with acceptable accuracy and precision.

MATRIX EFFECT

It had been noted that co eluting, undetected endogenous matrix components might reduced the ion intensity of the analyte and adversely affect the reproducibility and accuracy of the LCMS/MS assay. In order to determine whether this effect (matrix effect) was present or not, 6 different plasma pools were extracted and then spiked with standard solution concentration equal to LQC (post extracted spiked sample). Samples were prepared at low quality control level (LQC) in different human plasma sources analysed with 3 replicates of comparison samples in a single run. Percentage nominal concentrations were calculated for each matrix.

DILUTION INTEGRITY

Dilution integrity test was done by taking **1.8** times more the ULOQ concentration in the ratio of **50:50 and 25:75** with matrix blank. This test was performed using 6 replicates. Concentration obtained was multiplied with dilution **factor 2 or 4 to** get the actual concentration.

Results and Discussion

The assay was found to be linear for pregabalin concentrations in the range 50 to 10000 ng/mL. The precision and accuracy were studied satisfactory at four QC concentrations for pregabalin. The results of stability studies showed that no significant degradation was observed under the test conditions which indicate that compounds are highly stable in plasma. The values obtained for the stability studies are within the acceptance criteria. Recovery of Pregabalin was evaluated by comparing mean analyte responses of six processed samples of low (LQC), medium (MQC) and high (HQC) quality control samples to mean analyte responses of six appropriately diluted pure diluted solutions. Mean recovery values are 88.92, 88.25 and 84.81 % at low, medium and high quality control levels respectively. Mean recovery value for the internal standard was 89.43% and it is within the limit. The results of recovery studies were presented in Table 1. Sensitivity was determined by limit of quantification by analyzing six replicates of lower limit of quantification (LLOQ) that can be measured with acceptable accuracy and precision. A calibration curve standards and lower limit of quantification samples (LLOQ) were processed and analysed in a single run. At the time of analysis, the samples were removed from the deep freezer and kept in the room temperature and allowed to thaw. Lower limit of quantitation for Pregabalin coefficient of variation was 6.766 and a percentage of nominal concentration was 109.07% which is within the limit. Results are presented in Table 2. It had been noted that co eluting, undetected endogenous matrix components might reduced the ion intensity of the analyte and adversely affect the reproducibility and accuracy of the LCMS/MS assay. In order to determine whether this effect (matrix effect) was present or not, 6 different plasma pools were extracted and then spiked with standard solution concentration equal to LQC (post extracted spiked sample). Samples were prepared at low quality control level (LQC) in different human plasma sources analysed with 3 replicates of comparison samples in a single run. Percentage nominal concentrations were calculated for each matrix. The Matrix effect was found to be 104.84% for Pregabalin. The Results are presented in Table 3. The calculated concentrations of **50:50, 25:75**, include the dilution factor, yielded coefficients of variation of **0.50%**and **1.03%** respectively for DQC. Percentages of nominal concentration are **99.72** and **99.77%** respectively and it is within the limit. The Results are presented in Table 4.

Table: 1 Recovery of Pregabalin

Sample Name	Extracted sample Response	Un extracted sample Response	% Recovery
	6912	7680	
	7765	8533	
LQC	7903	8815	
	8181	9037	
	7508	8763	88.92 %
	7949	9149	
Mean	7703	8663	
SD	446.44	527.81	
%CV	0.602	6.092	
	250914	285486	
	283355	314839	
MQC	234636	272928	
	280869	323712	
	264981	287898	88.25 %
	265775	305946	
Mean	263421	298468	
SD	18410.72	19461.52	
%CV	6.989	6.520	
	611211	712699	
	657090	782436	
HQC	645259	799875	
	647679	731593	
	569744	702434	84.81 %
	605876	676956	
Mean	622809	734332	
SD	33271.13	47738.47	
%CV	5.342	6.500	

Table: 2 Lower Limit of Quantitation (LLOQ)

S. No	Cal. Concentration (2.001 ng/mL)	Accuracy
1	47.132	94.25
2	55.523	111.03
3	57.082	114.15
4	55.150	110.29
5	55.913	111.81
6	56.415	112.82
Mean	54.535	
SD	3.6899	
%CV	6.766	
% Nominal	109.07	

Table: 3 Matrix Effect of Pregabalin

Matrix ID	Response of standard solution	LQC Response of Post Extracted sample	Matrix factor
	9158	8733	104.87
MT-110/09	9346	8875	105.31
	9417	9037	104.20
	9170	8733	105.00
MT-114/09	9157	8875	103.18
	9977	9037	110.40
	8870	8733	101.57
MT-115/09	9284	8875	104.61
	9551	9037	105.69
	9293	8733	106.41
MT-124/09	9351	8875	105.36
	10111	9037	111.88
	9334	8733	106.88
MT-123/09	9600	8875	108.17
	9922	9037	109.79
	8863	8733	101.49
MT-125/09	9473	8875	106.74
	9478	9037	104.88

Table: 4 Dilution Integrity

50:50 Dilutions (18002.5776 ng/mL)					25:75 Dilutions (18002.5776 ng/mL)			
Sl. No	**Obtained conc. (ng/mL)**	**Dilution factor**	**Final conc. (ng/mL)**	**Accuracy**	**Obtained conc. (ng/mL)**	**Dilution factor**	**Final conc. (ng/mL)**	**Accuracy**
1	8886.821	2	17773.642	98.728	4453.467	4	17813.868	98.95176
2	8985.286	2	17970.572	99.822	4552.963	4	18211.852	101.1625
3	9011.21	2	18022.420	100.110	4468.687	4	17874.748	99.28994
4	8982.745	2	17965.490	99.794	4525.634	4	18102.536	100.5552
5	8994.28	2	17988.560	99.922	4432.242	4	17728.968	98.48016
6	9001.259	2	18002.518	100.000	4511.274	4	18045.096	100.2362
Mean	**8976.934**		**17953.867**		**4490.711**		**17962.845**	
SD	**45.368**		**90.736**		**46.494**		**185.977**	
%CV	**0.50538497**		**0.505385**		**1.035**		**1.035**	
% Nominal	**99.729**		**99.72942**		**99.779**		**99.779**	

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