INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 5, Issue 6 - 2018

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2018.05.06.017

Pharmaceutical Properties And Spectral Studies of Celiprolol ((3-[3-Acetyl-4-(3-T-Butylamino-2-Hydroxypropoxy)Phenyl]-2,1-Diethylurea Hydrochloride)

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Introduction

Celiprolol is a medication in the class of beta blockers, used in the treatment of high blood pressure. It is a β_1-selective adrenoceptor antagonist (beta-blocker), which acts as a weak agonist at β_2-adrenoceptors [1]. It lowers blood pressure in hypertensive patients at rest and on exercise. The effects on heart rate and cardiac output are dependent on the pre-existing background level of sympathetic tone. Under conditions of stress such as exercise, Celiprolol attenuates chronotropic and inotropic responses to sympathetic stimulation. However, at rest minimal impairment of cardiac function is seen [2]. A recent clinical trial has suggested a use for this medication in the prevention of vascular complications of a rare inherited disease called vascular Ehlers–Danlos syndrome. This study demonstrated decreased incidence of arterial rupture or dissection (a specific type of arterial rupture in which the layers of the vessel separate prior to complete failure of the artery wall) [3-6].

Celiprolol is absorbed from the gastro intestinal tract (GIT) in a nonlinear fashion; the percentage of the dose absorbed increases with increasing dose. Occasional side effects with Celiprolol are usually mild and transient have occurred. These include headache, dizziness, fatigue, nausea, somnolence and insomnia (sleep disturbances). Additional side effects associated with β_2 agonist activity, tremor and palpitations, have been reported. These effects usually do not require withdrawal of therapy. Depression, asthmatic dyspnoea and hypersensitivity pneumonitis have been reported rarely.

Materials and Methods

Stock solution of the Drug

About 14.9 mg of Celiprololwas weighed accurately and transferred into a 20 mL volumetric flask containing 2 mL of methanol. The contents were sonicated for 5 min and then the volume made up with a further quantity of methanol to get an approximate concentration of 741.28µg/ml. The stock is then stored in the refrigerator below 10^0C until further use.

Stock solution of the internal standard

About 80.0 mg of Metaprolol was weighed accurately and transferred into a 5ml volumetric flask containing 2mlof methanol. The solution was sonicated for 5 min and then the volume made up with a further quantity of the methanol to get an approximate concentration of 15.936µg/ml. Store this stock solution below 10^0C in a refrigerator.

Calibration Curve dilutions (CC Spiking solutions)

The calibration curve dilutions were prepared from Celiprolol stock solution as per the table 5.5 in the concentration range of 0.68to40.03µg/mL using a mixture of methanol and water (50:50) as the diluent. These dilutions (CC spiking solutions) were subsequently used for spiking the screened blank plasma.

Method Development

For optimum detection and quantitation of Celiprolol in human plasma by liquid chromatography, it was necessary to maintain the chromatographic condition throughout the experimentation. For developing a new simple suitable analytical method for the estimation of Celiprolol in human plasma, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all the other conditions constant. The following studies were conducted for this purpose. A non-polar Phenomenex ODS-2, C_{18}column (150 X 4.6 mm id) was chosen as the stationary phase for this study.

The mobile phase and the flow rate

A number of trials were made to find out the ideal solvent system (mobile phase) for eluting the drug Celiprolol and its internal standard Metoprolol. To effect ideal separation of the drug under isocratic conditions, mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases on a C_{18} stationary phase. Preliminary trails confirm that methanol was the better solvent for elution of the standard drug and internal standard with high resolution.

The mobile phase containing Methanol as high percentage and water, Acetonitrile with minimum usage doesn't improve the separation of the standard drug. Hence different ratios of Methanol with different buffer solutions were tried. Better peak resolution with less tailing and high theoretical plates was observed with the 30:70 (v/v) ratio of 20mM Sodium acetate buffer (pH 4.0 ± 0.05) and methanol. Hence this binary solution was used as mobile phase for the separation and estimation of Celiprolol. Flow rate of the mobile phase was tested from 0.8ml/min to 1.2ml/min in order to get sharp symmetric peaks with high resolution, minimum usage of the solvents and low pump pressure. A mobile phase flow rate of 1.0mL/min was found to be suitable in the present study.

Detection wave length

The sensitivity of the HPLC Method depends upon the proper selection of the detection wavelength. An ideal wavelength is one that give good response to the detector. The UV absorption spectrum of working standard solution of the Celiprolol was recorded separately on UV-Visible spectrophotometer and found the maximum absorbance wavelength at 210nm.Hence the response of the detector was recorded continuously at maximum wavelength 210nm.

Retention time of drug and internal standard

In the optimized conditions, Celiprolol and Metoprolol separately with high resolution and obey the system suitable criteria. Prominent fixed retention time of 4.08min for Celiprolol and 5.38min for Metoprolol was observed. The typical standard chromatogram was shown in figure 5.4

Data acquisition and processing

The chromatograms were obtained and data were processed by the peak area ratio method using the LC solution software. The concentrations of the unknown samples were calculated from the following equation of the regression analysis of the spiked plasma calibration graph using $1/X^2$ as weighting factor.

$$Y = m X + C$$

X = Concentration of Analyte / Concentration of Internal standard
Y = Area of Analyte / Area of Internal standard (area ratio)
m = Slope of the calibration curve
C = Y- intercept value

Results and Discussion

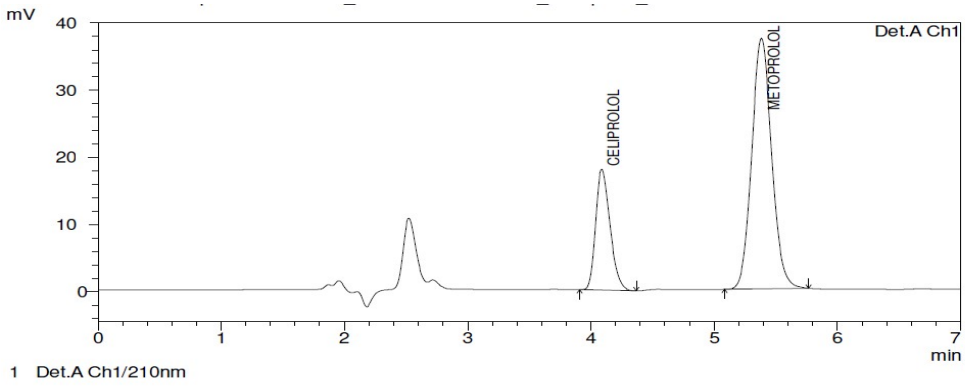

Figure 1: Chromatogram of Celiprolol (drug) and Metoprolol (IS) extracted from human plasma

Table 1: Precision and Accuracy of QC samples for Celiprolol

P & A ID		Observed Concentration (ng/ml)			
	QC ID	**HQC**	**MQC**	**LQC**	**LLOQ QC**
PA-01	1	1782.28	1074.09	109.90	35.41
	2	1826.58	996.68	104.18	37.80
	3	1905.35	972.36	109.65	37.36
	4	1824.21	1023.56	101.22	35.41
	5	1811.34	1088.97	102.34	33.67
	6	1798.65	1062.21	108.76	36.41
N		**6**	**6**	**6**	**6**
Average		**1824.73**	**1036.31**	**106.01**	**36.01**
Standard Deviation		**42.812**	**46.248**	**3.891**	**1.509**
CV (Precision %)		**2.35**	**4.46**	**3.67**	**4.19**
Nominal Concentration		**1853.19**	**1000.72**	**105.85**	**35.54**
Accuracy (%)		**98.46**	**103.56**	**100.14**	**101.33**
PA02	13	1812.58	1092.35	111.77	36.01
	14	1868.59	1019.60	106.57	38.67
	15	1945.36	992.78	111.95	38.15
	16	1821.29	1021.92	101.06	35.35
	17	1792.32	1077.54	101.27	33.32
	18	1766.45	1043.20	106.81	35.76
N		**6**	**6**	**6**	**6**
Average		**1834.433**	**1041.231**	**106.571**	**36.210**
Standard Deviation		**64.057**	**37.746**	**4.786**	**1.957**
CV (Precision %)		**3.49**	**3.63**	**4.49**	**5.41**
Nominal Concentration		**1853.19**	**1000.72**	**105.85**	**35.54**
Accuracy (%)		**98.99**	**104.05**	**100.68**	**101.89**
PA 03	19	1786.48	1076.62	110.16	35.50
	20	1860.00	1014.91	106.08	38.49
	21	1918.90	979.28	110.43	37.63
	22	1812.37	1016.91	100.56	35.18
	23	1786.41	1073.98	100.93	33.21
	24	1762.74	1041.01	106.59	35.68
N		**6**	**6**	**6**	**6**
Average		**1821.149**	**1033.785**	**105.792**	**35.948**
Standard Deviation		**58.245**	**37.722**	**4.295**	**1.880**
CV (Precision %)		**3.20**	**3.65**	**4.06**	**5.23**
Nominal Concentration		**1853.19**	**1000.72**	**105.85**	**35.54**
Accuracy (%)		**98.27**	**103.30**	**99.94**	**101.16**

Table 2: Short-term stability of Celiprolol and Metoprolol

	Drug		Internal Standard	
Injection No.	**AQS MQC Response**		**AQS MQC Response**	
	Fresh Stock	**Room Temp Stock**	**Fresh Stock**	**Room Temp Stock**
1	4118847	4343029	814480	839471
2	4306866	4448822	854202	871161
3	4278486	4484124	842281	867267
4	4172991	4407018	820960	861525
5	4834034	4576093	860434	885064
6	4816978	4297260	858756	843432
N	**6**	**6**	**6**	**6**
Average	**4421367.0**	**4426057.7**	**841852.2**	**861320.0**
SD	**320479.82**	**100245.32**	**19846.63**	**17282.31**
% CV	**7.25**	**2.26**	**2.36**	**2.01**
% Stability	**100.44**		**103.58**	
% Change (100 - % Stability)	**-0.44**		**-3.58**	
Stock Concentration µg/ml	**75.61**	**75.36**	**200.79**	**198.33**
Correction Cc	**1.0034**		**1.0124**	

A rapid, sensitive and rugged solid-phase extraction high performance liquid chromatography method was developed for determination of Celiprolol in human plasma. Optimization of chromatographic conditions were intended to take into account the various goals of the method development and to weigh each goal (resolutions, run time, sensitivity, peak symmetry, etc) accurately, according to the requirements can be used for the estimation of Celiprolol in plasma samples. The optimized conditions obtained a mobile phase of 20mMSodium acetate buffer (pH 4.0) and Methanol in the ratio of 30: 70 (v/v), at a flow rate of 1ml/min on Phenomenex ODS-2, $C_{18,}$ (4.6 X 150 mm, 5µ) column, and UV detection at 210nm. In this condition, retention time was found to be 4.08min for Celiprololand 5.38min for Metoprolol. The optimized conditions for estimation provided a well-defined separation between the drug, internal standard and endogenous components. The blank plasma samples showed no interference at retention time of the drug and internal standard.

The method was validated for selectivity, sensitivity, linearity, precision, accuracy and stability. The selectivity of the method was evaluated by comparing the chromatograms obtained from the samples containing Celiprolol and the internal standard with those obtained from blank samples. Sensitivity was determined in terms of LLOQ (lower limit of quantification) where the response of LLOQ was at least five times greater than the response of interference in blank matrix at the retention time or mass transitions of the analyte.

The optimized method for the estimation of the drug was precise as it showed < 10 % coefficient of variation at all concentrations. The blank plasma samples obtained from volunteers were analyzed and the chromatograms were recorded. Endogenous interferences were not detected at the retention time of selected drug and internal standard. The peak purity test method using UV detector was employed for selectivity studies. Some additional peaks were also observed in the sample chromatograms. These peaks, however, did not interfere with the drug and internal standard peaks. These observations showed that the developed assay method is specific and selective.

For linearity, different concentrations of standard solutions were prepared to contain 34.02µg/ml to 2001.44 ng/ml of Celiprolol containing 200µg/ml of Metoprolol. These solutions were analyzed and the peak areas and response factors were calculated. The calibration curve was plotted using response factor vs. concentration of the standard solutions. Standard curve was determined by applying the simplest model that adequately describes the concentration-response relationship using appropriate weighing and statistical tests for goodness of fit. The regression coefficient (r^2) ranged from 0.9943 to 0.9975 for Celiprolol. No significant changes in the chromatographic parameters were observed when changing the experimental conditions (Analysts, instruments, source of reagents and column of similar type) and optimized conditions (pH, mobile phase ratio and flow rate). System suitability

parameters such as column efficiency (theoretical plates), resolution factor and peak asymmetry factor of the optimized methods was found satisfactory.

The high percentage of recovery of Celiprolol was found, indicates that the proposed method is highly accurate. The percentage accuracy and precision studies obtained were less than 15% for QC sample and less than 20% for LOQ QC samples revealed that developed method was accurate and precise as per the FDA guidelines. The limit of detection (LOD) value was found to be very less. This observation showed that the developed method has adequate sensitivity. These values, however, may be affected by the separation conditions (e.g., column, reagents and instrumentation), instrumental changes (e.g., pumping systems and detectors) and use of non- HPLC grade solvents and may result in changes in signal to noise ratios. The stability of the drug spiked human plasma samples at three levels were studied for three freeze thaw cycles. The mean concentrations of the stability samples were compared to the theoretical concentrations. Similarly, short term (8 hours), long term (11 days) and standard solution stability were evaluated. The stability of the internal standards was also performed. The results showed that the selected drug was stable in plasma for about 11days when stored at frozen state. Results indicates that the developed method was sensitive and precise. Hence, this developed assay method can be applied for estimation of Celiprolol in different bioavailability and bioequivalence studies.

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DOI: 10.22192/ijcrcps.2018.05.06.017	

How to cite this article:
Shafia Hoor F. (2018). Pharmaceutical Properties And Spectral Studies of Celiprolol ((3-[3-Acetyl-4-(3-T-Butylamino-2-Hydroxypropoxy)Phenyl]-2,1-Diethylurea Hydrochloride). Int. J. Curr. Res. Chem. Pharm. Sci. 5(6): 101-106.
DOI: http://dx.doi.org/10.22192/ijcrcps.2018.05.06.017