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Analytical Method Development and Validation of Entacapone Drug by Modern LC-MS/MS Method to Quantify the Genotoxic Impurity

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ABSTRACT

A new high sensitive and specific LC-MS/MS (Liquid chromatography coupled with tandem mass spectrometric detection) method was developed and validated for the determination of 2-Chloro-N,N'-diethylacetamide (CDEA), a genotoxic impurity, in Entacapone drug substance. Hitherto there is a method known for identification of DECA in Entacapone. The successful separation of Entacapone and 2-Chloro-N,N'-diethylacetamide (CDEA) was achieved using Zorbax SB Aq column (Size: 4.6 × 250 mm, 5 µm particle size) with mobile phase consisting of 0.1% formic acid in water (50:50 v/v) as Mobile phase-A and Acetonitrile (100% v/v) as Mobile phase-B. High sensitive detection was achieved with "Applied Biosystems, Sciex, API-4000" Mass spectrometer and "API 4000, MDS Sciex, Toronto/Canada" Mass Detectors. As part of the method validation, system suitability, specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, precision, and stability of stock solutions were determined.

Keywords: Entacapone; Genotoxic impurity identification; Validation; LC-MS method

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INTRODUCTION

Entacapone is a catechol-O-methyltransferase inhibitor (COMT inhibitor), used for the treatment of Parkinson's disease in combination with other medications like carbidopa and levodopa. Entacapone is also used for the treatment of end-of-dose "wearing-off" in patients with parkinson's disease in combination with carbidopa and levodopa. It is a nitrocatechol-structured compound. Entacapone is chemically known as (E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N'-diethyl-2-propenamide. Its empirical formula is $C_{14}H_{15}N_3O_5$ and its molecular weight is 305.29 [1-6].Entacapone is commercially available in the form of tablets containing 200 mg of entacapone. Entcapone API is official in European Pharmacopoeia [7], British Pharmacopoeia [8], United States Pharmacopoeia [9] and Indian Pharmacopoeia [10]. The chemical name and structure of Entacapone is given in Figure 1.

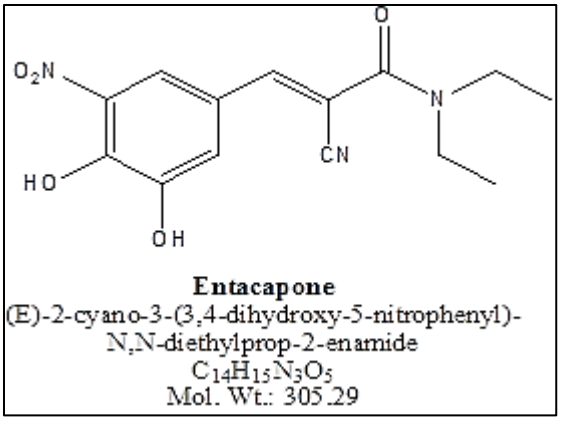


Figure 1: Chemical structure of Entacapone

Several synthetic methods were reported for the preparation of Entacapone [11-18]. Recently Veerareddy A and Reddy GS reported a new synthetic method for the preparation of Entacapone from 2-methoxy-4-iodophenol and 2-cyano-N,N-diethylacrylamide [19]. Since, Entacapone exists in high dose levels (200 mg) in commercial formulation, there is a need for identifying all the potential impurities which originates from different synthetic routes and their quantification with advance instrumental analytical methods. 2-Chloro-N,N'-diethylacetamide (CDEA) is the starting material for manufacture of N,N'-diethyl-2-cyano acetamide (DECA) which is used as key intermediate in the preparation of Entacapone drug substance [19]. CDEA is considered as potential genotoxic impurity based on structurally altering functional groups. There is a chance of carryover of CDEA into the final Entacapone drug substance and hence it is required to quantify the levels of CDEA in final Entacapone drug substance. None of the prior art references discloses method for the quantification of CDEA in Entacapone drug substance.

Different analytical methods were reported for determination of Entacapone [7-10,20-23]. The reported methods describe about the characterization of Entacapone, degradation studies, estimation of assay and impurity profile for both drug substance and drug product as well in the combination drug product. But quantification of Genotoxic impurity i.e., CDEA was not reported. Hence, the objective of the present work was to develop and validate a new LC-MS method for quantification of CDEA in Entacapone and validate the method according to ICH guidelines [24-27] and US FDA guidance [28]. The chemical name and structure of CDEA is given in Figure 2.

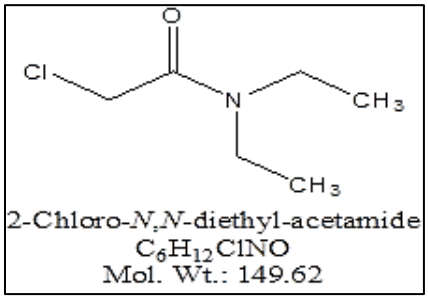


Figure 2: Chemical structure of 2-chloro-N,N'-diethylacetamide (CDEA)

EXPERIMENTAL SECTION

Standards and Reagents

Entacapone and 2-Chloro-N,N-diethylacetamide (CDEA) were obtained from Suven Life sciences Ltd., Hyderabad, India. In addition, analytical reagent grade Formic acid, Methanol and Acetonitrile was purchased from Merck. HPLC grade water was used from Milli-Q water purification system. The chemical names of the Entacapone and CDEA were given in Figures 1 and 2.

Instrumentation and Software

The Liquid chromatography coupled with tandem mass spectrometric detection was used for the method development and validation. LC-MS/MS conditions and detection conditions were provided in Tables 1 and 2.

Table 1: LC-MS/MS conditions

Instrumentation	LC-MS/MS (Liquid chromatography coupled with tandem mass spectrometric detection)
HPLC System	Agilent 1100 series
Mass spectrometer	Applied Biosystems, Sciex, API-4000
MS Detectors	API 4000, MDS Sciex, Toronto/Canada
Software	ANALYST version 1.6
Column	Zorbax SB Aq, 4.6 × 250 mm, 5 µm
Mobile Phase	Mobile phase A: 0.1% formic acid in water (50:50 v/v)
	Mobile phase B: Acetonitrile (100% v/v)
Ionization	Electrospray Ionization (ESI)
Ion source	Turbo spray
Scan mode	MRM (multiple reaction monitoring)
Ions	CDEA: m/z 150.10 à 75.89
Response	Peak area
Flow	1200 µL/min
Retention time	3.6 (± 0.3) min

Table 2: Detection conditions

Mass parameters	CDEA
MRM Transitions	150.104 → 75.89
Resolution — Q1	Low
Resolution — Q3	Unit
Declustering potential (DP) volts	57
Entrance potential (EP) Volts	10
Collision energy (CE) Volts	31
Collision cell exit potential (CXP) Volts	4
Dwell time (msec)	200 msec
Ionisation / Polarity	ESI-MS/MS+ve
Collision gas (CAD)	6
Curtain gas (CUR)	12
Gas-1	25
Gas-2	30
Ion spray voltage (IS) Volts	5500
Temperature (TEM) °C	300

Preparation of Solvents and Reagents

Diluent: Water / Acetonitrile solution (50:50, v/v).
Mobile phase A: 0.1% Formic acid in water v/v.
Mobile phase B: Acetonitrile (100% v/v).
Injector Wash Solvent: Methanol (100% v/v).
CDEA stock solution (Solution A: 1 mg/mL): Weighed accurately about 50 mg of CDEA, dissolved in 10 mL of diluent and made up the volume to 50 mL.

CDEA Stock solution (A-1: 10 µg/mL): Pipetted 1 mL of solution A into 100 mL volumetric flask and made up the volume with diluent.

CDEA Stock solution (A-2: 100 ng/mL): Pipetted 1.0 mL of solution A-1 into 100 mL volumetric flask and made up the volume with diluent.

CDEA Stock solution (A-3: 20 ng/mL): Pipetted 2.0 mL of solution A-2 into 10 mL volumetric flask and made up the volume with diluent.

Entacapone stock solution (B: 1 mg/mL): Weighed accurately about 50 mg of Entacapone, dissolved in 10 mL of diluents and made up the Volume to 50 mL.

Linearity solutions: Individual linearity solutions from Level 1 (0.250 ng/mL) to Level 7 (1.500 ng/mL) were prepared by pipetting 0.125 mL to 0.750 mL of Stock solution A-3 and diluted to 10 mL with diluents.

RESULTS AND DISCUSSION

HPLC Method Development and Transfer to LC-MS/MS Method

The method reported by D Purnachand et al. [23] was verified for detection of CDEA in Entacapone drug substance by HPLC method as all reported impurities were well quantified in single method. Similar chromatographic conditions were adopted for initial method verification. The peak for CDEA was identified at RT 9.150 min and well separated from Entacapone drug substance peak. However, limit of quantification (LOQ) for CDEA impurity using this method was 24 ppm. Since, CDEA is a genotoxic impurity and considering the maximum daily dose of the Entacapone, LOQ for CDEA should be less than 1.25 ppm. Hence it was concluded that, by using HPLC, CDEA cannot be quantified below 24 ppm. Hence, an alternative method shall be developed with higher sensitivity.

Since, the HPLC method was unable to detect the CDEA to an acceptable level, decided to develop the new method with high sensitive detector like LC-MS/MS (Liquid chromatography coupled with tandem mass spectrometric detection). Several experimentations were conducted to optimize the method parameters and finally optimized the conditions provided at Tables 1 and 2. The chromatograms obtained for CDEA standard and Sample was shown in Figure 3.

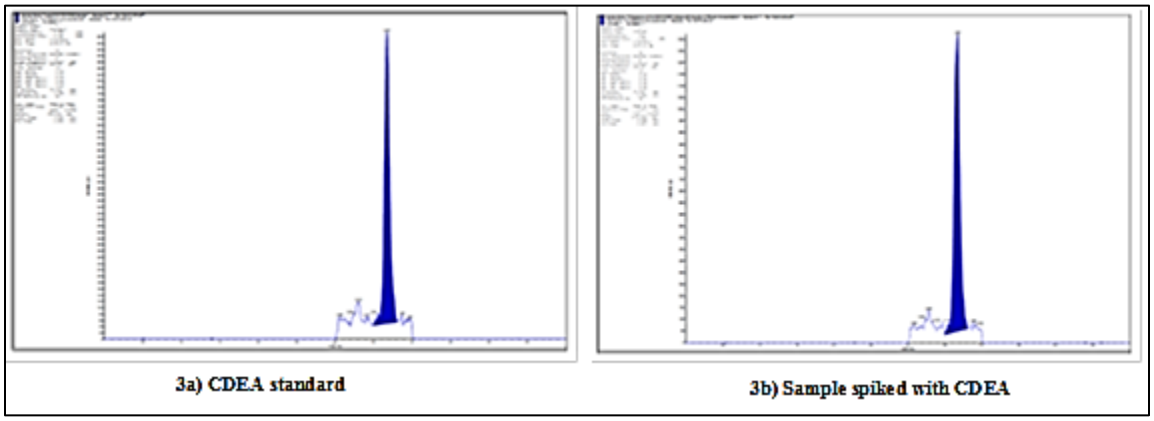


Figure 3: Chromatogram of CDEA standard and sample

Method Validation

System suitability:

A system suitability test (SST) was performed as routine at the beginning of each day with six replicate injections of CDEA at a concentration of 100% standard level (0.750 ng/mL) solution. The purpose of the SST was to check the chromatographic conditions by evaluation of the retention time of analyte, the peak shape. The fluctuation of the retention time was evaluated as RSD for each batch considering all analyzed samples. The RSD values for area response and retention time were within the acceptance criteria of NMT 5% for peak Retention Time and NMT 10% for peak area. The system suitability test (SST) results were given in Table 3.

Table 3: System suitability results

Day of analysis	Mean peak area response	RSD for peak area response	Mean retention time	RSD for retention time
Day 1	6152.833	2.735	3.631	0.516
Day 2	4575.167	6.295	3.656	0.17

Specificity/selectivity:

In order to determine the specificity, one diluent, each one injection of three different preparations of Entacapone sample, three preparations of Entacapone sample spiked with 100% standard solution containing CDEA equivalent to 0.750 ng/mL and three preparations of 100% concentration standard solution containing CDEA equivalent to 0.750 ng/mL was analyzed in triplicates. No interfering peak was observed at the retention time of CDEA in diluent. All peaks were identified and integrated in the spiked samples. Specificity results were given in Table 4.

Table 4: Specificity results

S. No	Sample name	Peak area response of CDEA	Retention time of CDEA for Peak identification
1	Diluent-1	0	3.6
2	Entacapone Sample-01	3844	
3	Entacapone Sample-02	3828	
4	Entacapone Sample-03	3623	
5	Entacapone Sample-01 (Spiked with CDEA, 0.750 ppm Concentration)	9515	
6	Entacapone Sample -02 (Spiked with CDEA, 0.750 ppm Concentration)	8767	
7	Entacapone Sample -03 (Spiked with CDEA, 0.750 ppm Concentration)	8880	
8	CDEA standard-01	5845	
9	CDEA standard -02	5496	
10	CDEA standard -03	5735	

Limit of detection (LOD) and Limit of quantitation (LOQ):

In order to determine the limit of detection, a standard solution containing CDEA equivalent to 0.083 ng/mL was analyzed in triplicate. The Signal-to-Noise ratio (S/N ratio) calculated were more than 3 for each injection and the peaks were well detected. The theoretically calculated LOD value (from the signal to noise ratio method) for S/N=3 was 0.073 ng/mL. In order to determine the limit of quantitation, a LOQ standard solution containing CDEA equivalent to 0.250 ng/mL was analyzed in six replicates. The S/N ratio calculated was more than 10 for each injection and the RSD of the peak areas was 9.892%. The theoretically calculated LOQ value (from the signal to noise ratio method) for S/N=10 was 0.217 ng/mL, LOD and LOQ results for CDEA was given in Table 5.

Table 5: Limit of detection (LOD) and Limit of quantification (LOQ) results

S. No	Sequence	Area Response	S/N ratio
Limit of Detection (LOD)			
1	Diluent	0	0
2	LOD Sample-1	963	3.7
3	LOD Sample-2	1018	3.4
4	LOD Sample -3	1124	3.1
Limit of Quantification (LOQ)			
1	Diluent-1	0	0
2	LOQ Sample-1	2009	12.6
3	LOQ Sample-2	1995	10.3
4	LOQ Sample-3	2193	10.2
5	LOQ Sample-4	1713	10.4
6	LOQ Sample-5	1915	13.8
7	LOQ Sample-6	2271	11.9
Mean		2016	11.533
SD		199.426	1.483
RSD (%)		9.892	12.857

Accuracy (% recovery):

The accuracy of the method for CDEA was demonstrated by spiking Entacapone sample with CDEA at three different levels. The levels in Entacapone for CDEA corresponding to LOQ in triplicates and each in triplicates at 100%, and 200% of the specification level. The recovery of CDEA was found to be in between the predefined acceptance criteria of ± 20% for 100% and 200% solutions and ± 30% of LOQ solution. The obtained % recovery values for CDEA at LOQ, 100% and 200% concentration level were given in Table 6. Hence it was concluded that the method was found to be accurate.

Table 6: Accuracy (%recovery) results

Sample No.	Entacapone sample		LOQ Solution		100% Solution		200% Solution	
	NA		0.250 ng/mL		0.750 ng/mL		1.501 ng/mL	
	Estimated Conc.	% Recovery	Estimated Conc.	% Recovery	Estimated Conc.	% Recovery	Estimated Conc.	%Recovery
Solution-1	0.581	NA	0.805	113.467	1.257	98.089	1.876	90.251
Solution-2	0.447	NA	0.759	95.067	1.305	104.489	1.983	97.38
Solution-3	0.536	NA	0.753	92.667	1.211	91.956	1.879	90.451
Mean	0.521	NA	0.772	100.4	1.258	98.178	1.913	92.694
SD	0.068		0.028		0.047		0.061	
RSD (%)	13.081		3.683		3.737		3.186	

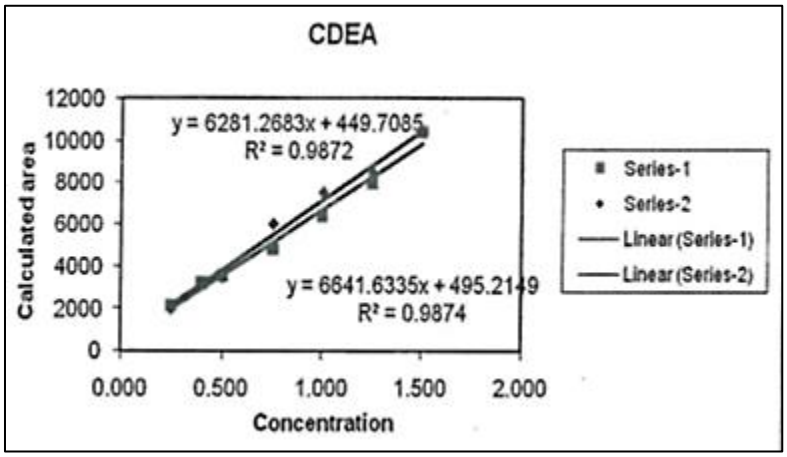
Linearity and range:

For the CDEA profiles in Entacapone, a minimum 0.250 ng/mL (LOQ) to 1.500 ng/mL (200% of the specified limits) was considered as the range. A linear relationship for CDEA was evaluated across the range of analytical procedure containing seven non-zero standards. Linearity and range results were given in Table 7 and linearity graph is given in Figure 4.

Table 7: Linearity and range results

Level Number	Linearity and Range-1 (Series-1)			Linearity and Range-2 (Series-2)		
	Concentration	Area	% Accuracy	Concentration (ng/mL)	Area	% Accuracy
Level 1	0.25	2176	109.95	0.251	2004	90.528
Level 2	0.4	3231	110.699	0.401	3207	101.816
Level 3	0.5	3569	99.32	0.501	3467	89.3
Level 4	0.749	4831	93.118	0.752	6017	110.559
Level 5	0.999	6422	95.172	1.003	7549	105.891
Level 6	1.249	7995	96.178	1.254	8458	95.601
Level 7	1.499	10388	105.553	1.504	10396	99.12
Regression coefficient (R^2)		0.9872			0.9874	
Slope		6281.268			6641.634	
Intercept		449.709			495.215	

Figure 4: Linearity curve in the range of LOQ to 200% of the specification limit



System precision:

To evaluate the system precision, ten Injections of working standard (100% level: 0.750 ng/mL) solution were injected from single preparation. Calculated the %RSD for ten replicate injections and results were in compliance to acceptance criteria and the RSD was 4.565%.

Method precision:
To evaluate the method precision, six different solutions were prepared by spiking CDEA into Entacapone at specification limit concentration and injected. CDEA peak area of each preparation was calculated individually. The % RSD of area response of CDEA was 3.920%. Hence, the method was precise.

Stability of system suitability solution and CDEA stock solution:
The stability of the system suitability solution and CDEA stock solution (solution A) over the time were evaluated up to 25 hours at room temperature. The test was performed by fresh preparation of system suitability solution. The percent (%) change for system suitability solution and CDEA stock solution (solution A) after 25 hours when compared with freshly prepared solutions were -3.529% and +7.771% respectively. Hence, the system suitability solution and CDEA stock solution (solution A) can be used up to 25 hours after preparation.

CONCLUSION

The method validated in this study was suitable for its intended purpose, which is the quantification of CDEA in Entacapone using Liquid Chromatography with Tandem Mass Spectrometric detection. The method was validated in the range of 0.250 - 1.500 ng/mL (0.250 - 1.500 ppm) for CDEA in Entacapone. The method was specific, accurate, linear and precise over the range. The stock solutions were stable up to 25 hours at room temperature. The proposed method can be successfully applied for the quantification of CDEA in Entacapone in routine analysis in quality control.

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