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Validated capillary electrophoresis method for the simultaneous determination of piperidine and diethylamine in Entacapone drug substance using indirect UV detection

Raja Gorla[1,2*], B. Sreenivasulu[1], N. Sreenivas[1] and Raghu Babu Korupolu[2]

[1]*APL Research Centre-II(A Division of Aurobindo Pharma Ltd), Survey No: 71&72, Indrakaran(V), Sangareddy (M), Medak Dist., Hyderabad, Andhra Pradesh, India*
[2]*Department of Engineering Chemistry, A. U. College of Engineering (A), Andhra University, Visakhapatnam, Andhra Pradesh, India*

ABSTRACT

A rapid and sensitive capillary electrophoresis (CE) method was developed for the determination of piperidine and diethylamine content in Entacapone drug substance using a background electrolyte(solution containing 10 mM of imidazole buffer adjusted to pH 5.0 with 1 molar HCl) along with constant applied voltage of 25kV at ambient temperature(25°c). A 56cm fused silica capillary was used as a stationary phase. Indirect UV detection mode was employed for the determination of analyte signal at 240nm against the reference signal at 210nm and this method requires 8min for data acquisition. Linearity, precision and accuracy were performed in the range of 0.03-0.12% w/w respectively for piperidine and diethylamine. The method was validated with respect to specificity, linearity, accuracy, precision, ruggedness, robustness, and stability in analytical solution. The limit of quantification and detection for piperidine was 3.16 µg/ml and 1.01 µg/ml respectively while for diethylamine was 3.12 µg/ml and 0.99 µg /ml respectively.

Key words: Capillary Electrophoresis, Entacapone, Piperidine and Diethylamine, Indirect UV detection.

INTRODUCTION

Entacapone is a potent and specific peripheral catechol-*O*-methyltransferase (COMT) inhibitor[1,2]. It has been shown to improve the clinical benefits of levodopa plus an aromatic L-amino acid decarboxylase inhibitor (AADC) when given to patients with Parkinson's disease and end-of-dose deterioration in the response to levodopa (the 'wearing off' phenomenon). The efficacy of entacapone is currently being assessed in patients with stable Parkinson's disease.

Enatacapone synthesis involves the condensation reaction of3,4-dihydroxy-5-nitrobenzaldehyde with N,N-diethyl-2-cyanoacetamide proceeds as per knoevenagel condensation. Piperidine (Fig.1)is used as base in this condensation. Diethylamine (Fig.1) used in the synthesis of N,N-diethyl-2-cyanoacetamide involves in the above condensation of Enatacapone(Fig.1) drug substance.

Piperidine [3,4]and diethylamine[5,6] has reported carcinogens and therefore the quantification of these was essential. For ions that do not fluoresce or absorb at useable wavelengths, sensitive detection can present a

challenge[18-20].A variety of techniques such as ion chromatography using conductivity detection[7,8],gas chromatography /mass spectrometry of a derivatized analyte[12,13], and HPLC using chemiluminescence detection[9-11],CE with laser induced fluorescence detection of a derivatized analyte[14].Considering this,the attention was focused on capillary electrophoresis using indirect UV detection which is rapid, sensitive and less expensive.

The aim of this study was to develop a CE method with indirect UV detection for the quantitation of piperidine and diethylamine from an API entacapone. This method was developed using imidazole(Fig.1) as a probe, and quantitation of the piperidine and diethylamine was carried out using pyrrolidine(Fig.1) as an internal standard(IS). This optimized CE method was validated to verify its performance characteristics according to ICH guidelines[21].The method was validated for specificity, linearity, precision and accuracy. Based on the validation results, the CE method developed was suitable for the quantitation of piperidine and diethylamine from an API entacapone.

MATERIALS AND METHODS

Chemicals and reagents

Piperidine, diethylamine, pyrrolidine and imidazole were purchased from Fluka(Sigma-Aldrich, st. louis, MO). HCl and Acetonitrile were supplied by E. Merck (Mumbai, India) and water was from Milli-Q purification system(Millipore, Billerica, MA). Entacapone and its related impurities were obtained from Aurobindo Pharma Ltd. (Hyderabad, India).

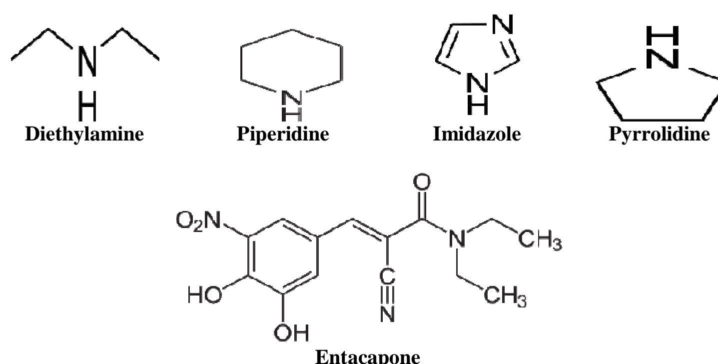

Fig. 1. Structure of the diethylamine and piperidine analytes, the pyrrolidine internal standard, the imidazole probe, and the Entacapone drug substance

Preparation of solutions

Internal standard solution

Weigh and transfer about 100mg of Pyrrolidine in 100ml of acetonitrile.Further,10ml of this solution to 1000ml with acetonitrile

Standard solution

Accurately weigh and transfer 100mg each of Diethylamine and Piperidine in 100ml of internal standard solution.Further,2ml of this solution to 200ml with internal standard solution, and filtered through 0.22-um or finer porosity membrane filter

Sample solution

Weigh and transfer accurately 100mg of drug substance in 10ml of internal standard solution and filtered through 0.22-um or finer porosity membrane filter.

Instrumentation and method conditions

An Agilent instrument CE system equipped with a diode array detector along with chemstation software for data acquisition and processing was used. Separation was carried out in fused silica capillary with extended light path length(Agilent, Boblingen, Germany) of effective length of 56cm and i.d. of 50um.

The back-ground electrolyte used was 10mmole imidazole buffer adjusted to pH-5.0 with 1molar HCl solution. The standard and sample were introduced by hydrodynamic pressure of 50 mbar for 10 s and the separation was carried out with constant applied voltage 25 kV and capillary temperature was thermostated to 25°c.Before introducing the sample, the capillary was conditioned with back-ground electrolyte for 3min at the inlet pressure of 5 bar. The analyte signal was detected by indirect UV photometric method, the wavelength was set at 240nm against reference signal at 210nm.Flush the capillary with back-ground electrolyte for 10min before starting the experiment every day. New capillaries were rinsed with water for 5mins and followed by background electrolyte for 15min.

RESULTS AND DISCUSSION

Method development

The selection of suitable background electrolyte, its optimum concentration, and its pH are the critical parameters of an electrophoretic system to make the separation efficient, selective and sensitive. In CE, the electrophoretic mobility of the co-ion relative to that of the analyte determines the shape of the migrating analyte zone as a result of electromigration dispersion [16]. This relationship applies to indirect photometric detection[15]. Piperidine and diethylamine are strong bases having pKa values of 11.1 and 10.93 and entacapone is a weak acid and it is not ionisable at lower pH. Therefore, a probe with a buffering capacity at moderate pH was desired. Using imidazole probe[17] at pH 5.0 adjusted with diluted acetic acid was tried ,but some interferences and desired resolution between piperidine, diethylamine and internal standard pyrrolidine was not achieved. Instead of acetic acid, adjusted with diluted HCl at pH-5.0, it shows positive results regarding resolution between the peaks and good peak shapes. In order to optimize the separation, the probe concentration was varied from 5 to 50mM. The best peak shape for piperidine and diethylamine was achieved at a imidazole concentration of 10mM. As the piperidine and diethylamine is present in a very trace quantity, the concentration of the analyte is very less in sample solution compared to the electrolyte probe concentration. Therefore the difference between the analyte and probe concentration is maximum [16]. On the basis of above discussion the detection relies on the use of UV active buffer component with the same charge as electrolyte(Imidazole buffer). Migration time of pyrrolidne is 4 to 6min.The relative migration time of diethylamine and piperidine with respect to pyrrolidine is 1.02 and 1.04 respectively.

Method Validation

Specificity

Sample spiked with piperidine and diethylamine at 0.05(%w/w) was analysed as per method (Fig 2). Further, known related substances of Entacapone along with piperidine and diethylamine were spiked in the sample to check their interference if any with piperidine and diethylamine peak. The area of the analyte peak in the sample spiked with piperidine and diethylamine and other known related impurities remains same compared to the area in the sample spiked with piperidine and diethylamine indicating that there is no interference of Entacapone related substances with piperidine and diethylamine peak proving that the method is specific for the determination of piperidine and diethylamine in Entacapone drug substance.

From the above study we can conclude that, unless and until the charge to mass ratio of any other impurity is similar to that of analyte, it will not appear at the migration time window of the analyte. Therefore, on the basis of this study it is concluded that the peak is homogeneous and pure.

The specificity experiment results are given in Table 1.

Precision

The system precision was demonstrated by performing six replicate introduction of piperidine and diethylamine standard solution(10ug/ml) into capillary electrophoresis system and the relative standard deviation of response for six replicate measurements of piperidine was found to be 1.7% and diethylamine was found to be 2.8%

Method precision was demonstrated by preparing six replicate sample preparations by spiking at 0.05(%w/w) concentration of piperidine and diethylamine in a single lot of Entacapone. These were analysed as per the method and the content of piperidine and diethylamine was determined. The relative standard deviation for six replicate measurements of piperidine was found to be 3.8% and diethylamine was found to be 2.1%.The achieved precised values are presented in Table 2.

Intermediate precision was demonstrated by preparing six replicate sample preparations by spiking known concentration of piperidine and diethylamine in a single lot of Entacapone by different analyst on a different day using a another lot of capillary. The relative standard deviation for six replicate measurements of piperidine was found to be 3.5% and diethylamine was found to be 2.8%

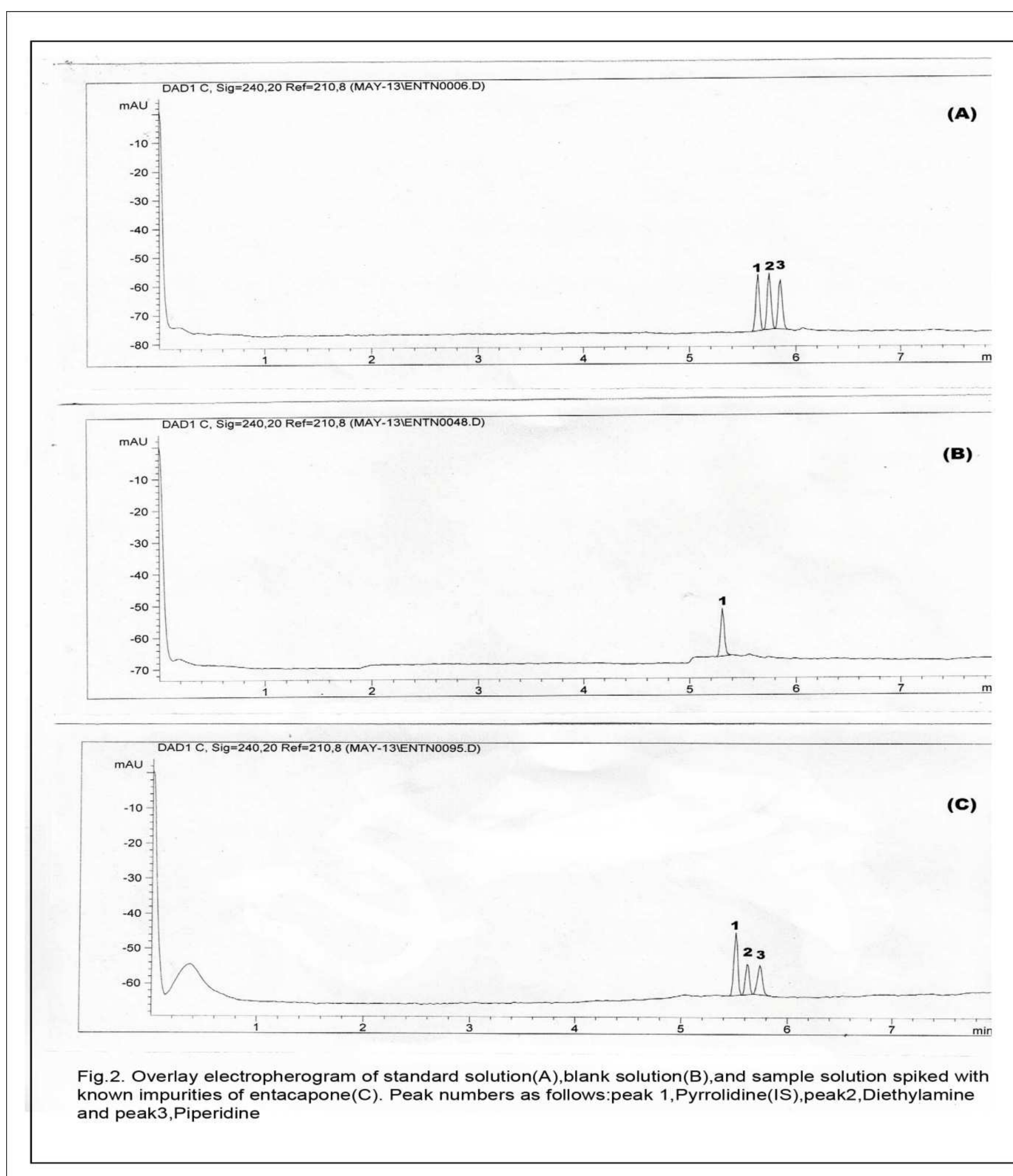

Fig.2. Overlay electropherogram of standard solution(A),blank solution(B),and sample solution spiked with known impurities of entacapone(C). Peak numbers as follows:peak 1,Pyrrolidine(IS),peak2,Diethylamine and peak3,Piperidine

Linearity

The linearity of response for piperidine and diethylamine was determined by injecting piperidine standard solution in the range of about 3.16 to 12.14 µg/ml and diethylamine standard solution in the range of about 3.12 to 11.99 µg/ml(from at about 0.03% to 0.12% w/w). The detector response for piperidine and diethylamine was found to be linear over the specified range as determined from the correlation coefficient value of 0.998 and 0.998 for piperidine and diethylamine respectively. The equation representing the calibration curve for piperidine was y = 0.1070 x –

0.0645 and for diethylamine was y = 0.0868 x - 0.0265.The Statistical analysis for linearity data is tabulated in Table 3.

Table 1. Specificity

Sample S. No	Piperidine Content(%w/w)		Diethylamine Content(%w/w)	
	Without spiking of related substances	Spiked with related substances	Without spiking of related substances	Spiked with related substances
1	0.050	0.058	0.048	0.052
2	0.051	0.057	0.049	0.052
3	0.053	0.063	0.049	0.051
Mean	0.051	0.059	0.049	0.052
SD*	0.002	0.003	0.001	0.001
(%)RSD^	3.0	5.4	1.2	1.1

^Relative standard deviation
**Standard deviation*

Table 2.Statistical data of Precision obtained for determination of Piperidine and Diethyl amine

System precision			Method precision	
Injection ID / Sample ID	Corrected area ratio of Piperidine	Corrected area ratio of Diethyl amine	Piperidine Content (%w/w)	Diethyl amine Content (%w/w)
1	0.93531	1.02147	0.050	0.048
2	0.94068	1.06747	0.051	0.049
3	0.97084	1.05762	0.053	0.049
4	0.96411	0.98679	0.053	0.047
5	0.96772	1.01661	0.054	0.046
6	0.94183	1.02119	0.056	0.050
Mean	0.95342	1.02853	0.053	0.048
SD	0.016	0.029	0.002	0.001
%RSD	1.7	2.8	3.8	2.1
95%Confidence interval	(±)0.017	(±)0.030	(±)0.002	(±)0.001

. **Table 3.The Statistical Evaluation of Linearity data and Determine the Limit of Quantification and Limit of Detection**

Statistical parameter	Results	
	Piperidine	Diethyl amine
Correlation coefficient(r)	0.998	0.998
Concentration range(µg/ml)	3.16 – 12.14	3.12 – 11.99
Intercept	-0.0645	-0.0265
Slope	0.1070	0.0868
STEYX(SD)	0.0261	0.0248
Limit of Quantification(µg/ml)	3.16	3.12
Limit of Detection(µg/ml)	1.01	0.99
Precision for Limit of Quantification (%)RSD	4.1	6.7
Precision for Limit of Detection (%)RSD	5.9	8.3

Table 4. Recovery Results from spiking of sample with Piperidine and Diethyl amine

Accuracy (Average of triplicates)	Level-I	Level-II	Level-III	Overall recovery (%Average of 9 replicates)
Piperidine				
Added(µg/g)	301.85	504.14	756.21	99.4
Found(µg/g)	290.91	511.68	759.49	
Recovery(%)	96.4	101.5	100.4	
RSD(%)	1.1	1.0	1.6	
Diethyl amine				
Added(µg/g)	298.27	498.16	747.24	95.4
Found(µg/g)	254.19	505.04	744.63	
Recovery(%)	85.2	101.4	99.7	
RSD(%)	3.0	0.4	1.8	

Accuracy
A known amount of piperidine and diethylamine was added to Entacapone sample solution at concentration levels i.e, about 0.03(LOQ),0.05 and 0.075 % w/w (with respect to Entacapone) in triplicate at each level. The samples were analyzed as per proposed method. The average recovery values are reported in Table 4.

Sensitivity
The Limit of detection and quantification for piperidine and diethylamine was determined from linearity data and verifying the predicted LOD and LOQ values by showing precision at these concentrations. Experimentally determined LOQ and LOD for piperidine are 3.16 μg/mL and μg/mL respectively while for diethylamine are 3.12 μg/mL and 0.99 μg/mL respectively.

Stability in analytical solution
A sample solution spiked with piperidine and diethylamine was prepared and kept at ambient room temperature. The sample solution was analyzed initially and at regular time intervals. The sample was found to stable for 5 hours at room temperature.

Robustness
Robustness of the method was verified by deliberately altering the critical method parameters from that of actual conditions. The altered conditions include change in temperature, buffer pH, and applied voltage. The results obtained from robustness experiments indicated that the method parameters were suitably optimized to tolerate minor variations

CONCLUSION

Indirect photometric detection is a useful detection technique for nonabsorbing analytes. For an indirect photometric detection method to perform optimally, the analyst must consider several key parameters, including peak shape, peak efficiency, detection sensitivity, baseline noise, and buffering of electrolyte to ensure method ruggedness. These principles impose a number of criteria in designing a suitable indirect photometric detection method. First, the probe should be chosen for closeness of its mobility to the mobilities of critical analytes, and it should provide the maximum absorbance change when displaced by an analyte ion. Second, co-ionic impurities in the electrolyte should be avoided because these cause system peaks and decrease in detection sensitivity and they always contribute to unstable baseline. Considering all the above parameters, a rapid and simple CE method for the simultaneous determination of piperidine and diethylamine has been developed. This CE method provides excellent reproducibility, good linearity, accuracy and appropriate sensitivity. These results indicate that the proposed method is robust and rugged and can be useful for routine analysis of piperidine and diethylamine content in Entacapone drug substance.

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