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New Approach for the Determination of Tricyclic Antidepressant Amitriptyline Using *β*-Cyclodextrin-PEG System via Spectrophotomerty

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ABSTRACT

A new and simple procedure for the spectrophotometric determination of the tricyclic antidepressant drug amitriptyline is proposed. The method is based on enhancement of sensitivity of the $[AMIYTP]^+$ *β*-cyclodextrin and PEG molecules involved in formation of molecules inclusion complex, in presence of polyethylene glycol (PEG) medium. The mole ratio of $[AMIYTP]^+$ *β*-cyclodextrin and PEG molecules in inclusion complex were determined by the curve fitting method. The value of molar absorptivity of {[AMIYTP: (*β* CD)] PEG} complex in term of the drug lies in rage of (2.20 - 2.23) × 10^4 $L \cdot mole^{-1} \cdot cm^{-1}$ at absorption maximum 242 nm. The slope, intercept and correlation co-efficient were found to be 14.21, 0.0046, and 0.998, respectively. The effect of analytical variables on the determination of the drug and composition of the ion associated complex are discussed in the paper. This method is applicable in the determination of amitriptyline in the pharmaceutical preparations.

Keywords: Amitriptyline; PEG; *β*-Cyclodextrin; Spectrophotometric Determination; AMIYTP-*β*-Cyclodextrin Complex; Pharmaceuticals

1. Introduction

Amitriptyline [3-(10,11-dihydro-5H-dibenzol[a,d]cyclohept-5-ylidene) propyldimethylamine] constitute an important class of neurotherapeutics [1] belonging to the first generation of antidepressant drug [2,3] The former has a carbocyclic structure with an exocyclic double bond at C-5 which is substituted with an N, N-dimethyl-1-propanamino side chain (**Figure 1**). The value of angle of these molecules is important, the more nearly planar the greater the neuroleptic activity. It is believed that amitriptyline acts by blocking the receptors of neurotransmitters, naradrenaline in the synopsis in the central nervous system, which results an increase of concentration of both molecules with a subsequent—enhancement of their antidepressant patency [3]. However, this drug suffers from several drawbacks, such as antiarrhytmic, anticholinergic, cardiovascular and/or hyperthermia side effect [2,3], which may be reduced if the drugs are suitably vectored to the organism. In this field cyclodextrins (CDs) are considered as one of the most suitable artificial receptors for the vectorization of guest hydrophobic molecules (drug, dyes, detergents, pesticides, etc.) in aqueous media [4-6]. In fact, the use of CDs as a new family of pharmaceutical excipient and drug carriers has become an increasingly accepted method for many therapeutic molecules [7].

Various analytical methods have been reported for determination of amitriptyline including spectrofluorometric [8], fluorospectrophotometric [9-14] flow injection method [15] atomic absorption spectroscopic [16], conductometric [17,18], high performance liquid chromatographic [19,20], voltammetric [21] and chromatographic [22] methods. Some of these methods are not

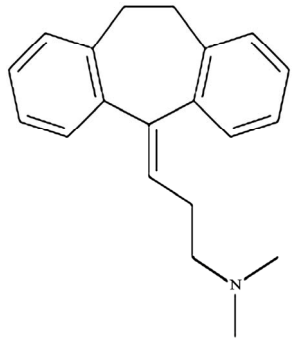

Figure 1. [AMIYTP] Cl Chemical Name: [3-(10,ll-dihydro-5H-dibenzol [a,d] cyclohept-5-ylidene) propyldimethylamine].

simple for the routine analysis and they need sophisticated instruments which are not commonly available in the routine laboratories and conventional batch process solvent extraction is a tedious and time consuming procedure. Therefore it seems necessary to develop a sensitive, simple and fast identification and determination of Amitriptyline. The proposed method is based on the enhancement of sensitivity of [AMIYTP: βCD] complex in presence of polythene glycol (PEG) medium. The optimization of analytical variables is discussed in this paper. The method is simple, sensitive and reproducible. This method has been applied for determination of amitriptyline in pharmaceutical preparations.

2. Experimental

2.1. Reagents

All chemicals used were of analytical grade reagent (Merck). The standard solution of amitriptyline (l000 ppm) was prepared by dissolving its 1.000 g in 1 liter deionized double distilled water. The working solutions were prepared by the appropriate dilution of the stock solution. Solutions used were prior filtered. The β-cyclodextrin 1000 ppm (8.81×10^{-4} M) solution was prepared by dissolving its 0.1gm in 100 ml deionized double distilled water, and further diluted to 800 ppm (7.04×10^{-4} M) with double distilled water. The PEG solution 1000 ppm (2.5×10^{-3} M) was prepared by dissolving its 0.1 gm in 100 ml deionized double distilled water, and it was further diluted to 20 ppm (5×10^{-5} M) with deionized distilled water. All working solutions employed were prior filtered and degassed, by the degassing unit.

2.2. Equipment

Systronics UV-VIS double beam spectrophotometer –2201 matched with 1 cm quartz cell were employed to determine the absorbance. The absorbance of the solutions of different concentrations of amitriptyline are shown in **Figure 2**.

2.3. Procedure for determination of Amitriptyline

2 ml aliquots of the standard solution of amitriptyline having varying concentrations from 0.1 to 1.0 ppm were taken in 10 ml volumetric flasks. In each volumetric flask 2 ml of β-CDs solution and 2 ml of PEG solution were added. Then made up the solution up to the mark on volumetric flask with double distilled water. Then measured their absorbance at 242 nm against the reagent blank, and prepared the calibration graph by plotting absorbance versus concentration of amitriptyline, **Figure 2**. The similar procedure was repeated with sample solution, which was prepared from pharmaceutical product. The concentration of amitriptyline in the sample solution was computed by using the calibration curve prepared under similar condition.

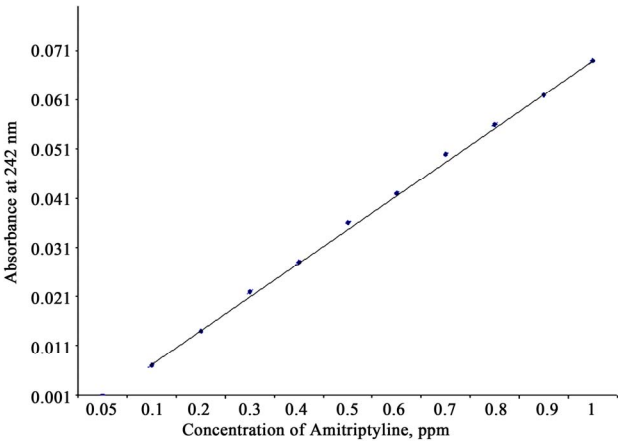

Figure 2. Calibration Graph for Determination of Amitriptyline 0.1 to 1.0 ppm (mg·L^{-1}).

3. Results and Discussion

3.1. Reaction Mechanism and Composition of Complex

Amitriptyline gives ion-associate species with β-CD and PEG system and the absorbance increases with increase in the concentration of amitriptyline. Amitriptyline cation reacts with β-CDs to give an inclusion complex. Their stoichiommetry are 1:1 indicating that the complex is formed by the association of a molecule of β-CD per each molecule of $[AMIYTP]^+$, as usually found for most cyclodextrin/drug complexes. Considering this 1:1 stoichiommetry the molecular encapsulation process can be represented as:

$$m[\mathrm{AMIYTP}]^+ \mathrm{Cl} + n\beta\mathrm{CD} \rightleftarrows \left\{[\mathrm{AMIYTP}]m(\beta\mathrm{CD})_n\right\}^{m+} \mathrm{Cl}$$

where n = 1 to 2.

This reaction has been used for the determination of cationic antidepressant drug Amitriptyline in presence of PEG molecule. The solubilization as well as the viscosity of an aqueous solution of polymer bound system is higher than the solution of pure polymer. The expected reaction in the PEG medium can be expressed as:

$$m[\mathrm{AMIYTP}]^+ \mathrm{Cl} + n\beta\mathrm{CD} + \chi\mathrm{PEG} \rightleftarrows \left\{[\mathrm{AMIYTP}]m(\beta\mathrm{CD})_n(\mathrm{PEG})\chi\right\}^{m+} \mathrm{Cl}$$

where, value of "χ" may vary from 3-4 and $[AMIYTP]^+$ = cation of the drug Amitriptyline.The mole ratio of $[AMIYTP]^+$ to β-CD and PEG molecules involved in formulation of molecules inclusion complex, were determined by the curve fitting method by plotting $\log(A_{eq.}/A_{max.} - A_{eq.})$ vs. Concentration. Where, $A_{eq.}$ = Absorbance of the complex when reagent was in equilibrium, and $A_{max.}$ = Absorbance when reagent was in constant excess. The value of slope for β-CD and PEG were found to be in ratio 0.140 and 0.436, respectively **Figures 3** and **4**.

Curve-fitting method suggested the molar ratio of β-CD and PEG and drug cation in the complex to be in 1:3 ratios. As per the curve-fitting method, the composition of the inclusion complex is expected to be as:

$$\left[\text{AMIYTP}\right]^+\text{Cl} + \beta\text{CD} + 3\text{PEG} \rightleftarrows \left\{\left[\text{AMIYTP}\right]\left(\beta\text{CD}\right)\left(\text{PEG}\right)_3\right\}^+\text{Cl}$$

3.2. Absorption Spectra

The $\{[AMIYTP](\beta CD)(PEG)_3\}^+$ Cl complex exhibit the absorption maximum at 242 nm. The position of λ_{max} is changed when the PEG is added and absorbance was increased. The absorption maxima of only $\{[AMIYTP](\beta CD)\}$ complex were found to be at 250 nm. The position of λ_{max} is change when the PEG is added and the absorbance was increased. The $\{[AMIYTP](\beta CD)(PEG)_3\}^+$Cl complex exhibits the absorbance maximum at 242 nm **Figure 5**. In presence of the drug/PEG, hyperchromic and hypsochromic shift was observed due to formation of molecular inclusion complex.

3.3. Optimum Concentration Range, Detection Limit and Statistics

The absorbances for the different concentration of amitriptyline are shown in **Figure 2**. The method followed linearity in the range 0.1 - 1.0 ppm of amitriptyline with the slope, intercept and correlation coefficient of 14.21, 0.00458, and 0.998 respectively. The value of molar absorptivity [calculated by taking the molar concentration of amitriptyline and path length of the cell to be 1 cm] with amitriptyline drug was found in the range (2.20 - 2.23) × 10^{-4} L·mole^{-1}·cm^{-1} at the absorption maximum 242 nm. The detection limit (absorbance > 3 × SD) of the method was 0.034 ppm. The relative standard deviation for the analysis of seven different solutions containing 0.5 ppm amitriptyline was found to be ±0.023%.

3.4. Application of the Method

The proposed method was applied for the determination of amitriptyline in commercial pharmaceuticals tablets Amil-25 (From Mano Pharma India) and Amitriptyline Hydrochloride tablet USP/IP—25 mg (From (Iron) drugs and pharmaceuticals Pvt. Ltd. India). Sample solutions were prepared from the tablets. Five tablets of each produce were weighed and powdered. The powder equivalent to their single tablet was filtered and evaporated to dryness. Then the residue was dissolved in distilled water and made up the volume up to 100 ml in a volumetric flask. These stock solutions were further diluted to contain the requisite concentration. Then followed the procedure as described earlier for the proposed method, and it was compared with the results obtained from the official method, **Tables 1** and **2**.

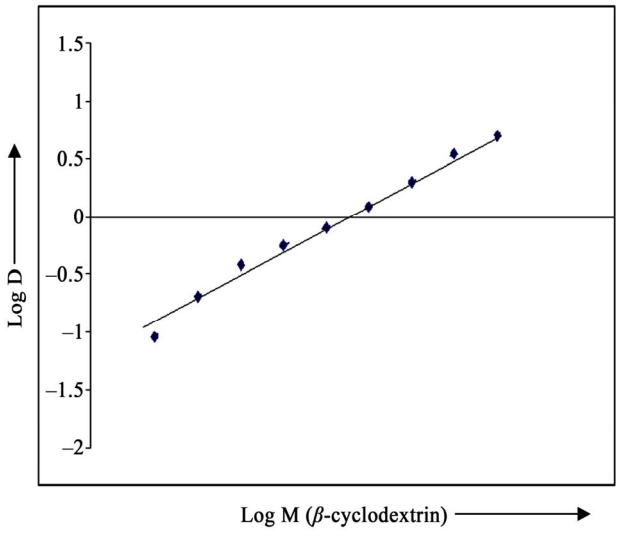

Figure 3. Curve fitting method for determination of molar ratio of β-cyclodextrin in ion-associate complex.

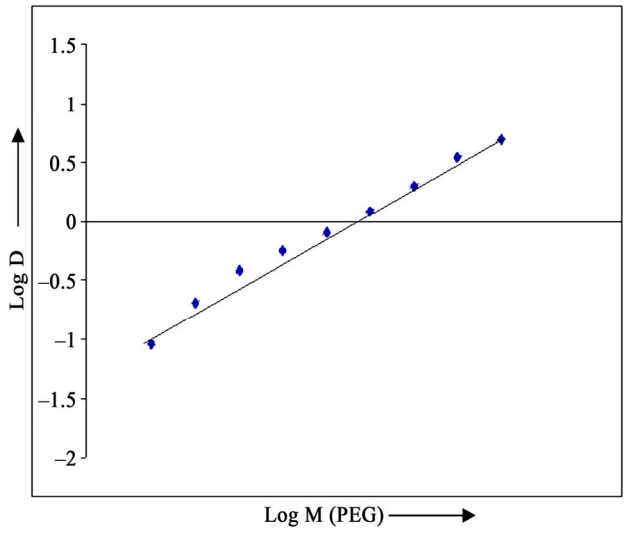

Figure 4. Curve fitting method for determination of mole ratio of the PEG in the ion-associate complex.

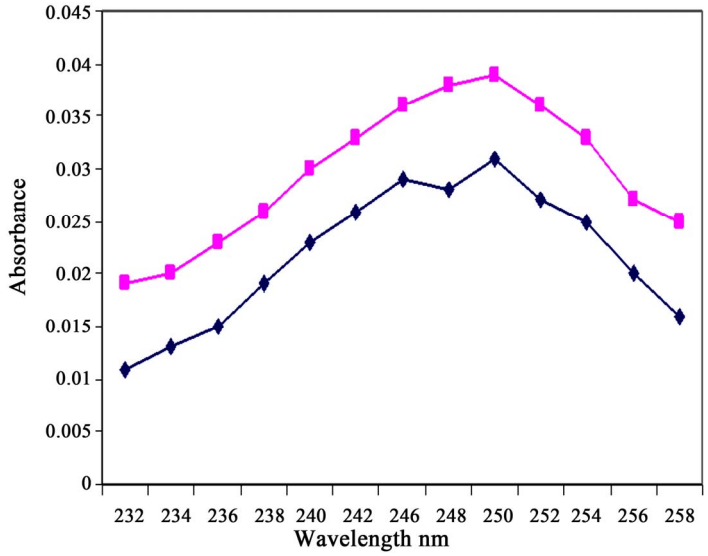

Figure 5. Absorption spectra of {[AMIYTP](β-CD)}$^{+}$Cl and {[(AMIYTP](β-CD)] $(PEG)_3$}$^{+}$Cl Ions—associate complexes.

Table 1. The result of analysis of Amitriptyline tablets by proposed and official method.

S.No.	Pharmaceutical Product	Recovery, mg		Error, %
		Official Method	Proposed Method	
1.	Amit 25, 25 mg	25.3 ± 0.03	24.87 ± 0.16	**1.6%**
2.	Amitriptyline Hydrochloride tablet 25mg	25.2 ± 0.25	24.72 ± 0.32	**1.9%**

Table 2. Comparison with other established spectrophotometric method.

S.No.	Method	λ_{max}, (nm)	Working Range, (ppm)	R.S.D* (%)	Correlation Co-efficient
1.	Extraction spectrophotometer ammonium reinkate method	523	0.1 - 6.0	0.8	0.994
2.	Proposed spectrophotometric {AMIYTP(βCD)$(PEG)_3$}$^{+}$Cl ion associate complex method	242	0.1 - 1.0	2.0	0.998

4. Conclusion

The method was successfully applied for the determination of amitriptyline in the pharmaceutical preparations. The method is very simple as there is no requirement of prior separation or extraction of the complex and the reagents are cheap and commonly available in routine laboratories. The results obtained from the proposed method were comparable with the established methods. The method has good potential in simplicity and sensitivity.

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