



## **Spectrophotometric Determination of Amphetamine In Pure and Pharmaceutical Formulations**

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### **ABSTRACT**

A simple, sensitive and rapid spectrophotometric method has been developed for the estimation of Amphetamine (AM) in bulk and in pharmaceutical formulations using 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ). The proposed method is based on the formation of charge-transfer complex between AM and DDQ. AM in the presence of DDQ formed pale wine red color complex which showed a maximum absorbance at 465 nm against the blank. The limit of detection and quantitation were 0.1395 mg/ml and 0.4644 mg/ml respectively. The results of analysis were validated by recovery studies, accuracy, precision, LOD, LOQ, robustness and ruggedness, which indicated that the present method can be successfully applied for the determination of AM in pure and in pharmaceutical formulations.

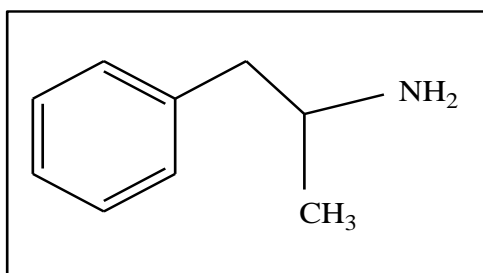
**Keywords:** Spectrophotometric method, Amphetamine, Charge-transfer complex, DDQ.

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## INTRODUCTION

Amphetamine (AM); 1-phenylpropan-2-amine (Figure. 1) is a potent central nervous system (CNS) stimulant of the phenylamine class and is a synthetic stimulant. It is used in the treatment of attention deficit hyperactivity disorder (ADHD), narcolepsy and obesity. AM is also used as an athletic performance enhancer, cognitive enhancer and recreationally as an aphrodisiac, euphoriant<sup>1-4</sup>. In the literature, several analytical methods have been reported for the determination of the AM, which include paper chromatography<sup>5</sup>, thin layer chromatography<sup>6-10</sup>, high performance liquid chromatography<sup>11-15</sup>, gas chromatography<sup>16-20</sup>, colorimetry<sup>21-24</sup> and spectrophotometry<sup>25,26</sup>. The survey of literature has shown that no spectrophotometric method was reported so far, for the determination of AM with DDQ. In the present paper, a new spectrophotometric method has been reported for the determination of AM based on charge-transfer complex reaction with DDQ, which gave a coloured solution. The proposed method is cost-effective, sensitive, rapid, accurate and reproducible and it can be used for the determination of AM in pure and in pharmaceutical formulations.



**Figure 1: Structure of AM**

## EXPERIMENTAL

### Instrumentation

A Shimadzu UV-Visible spectrophotometer (UV-160A) with a matched pair of 10 mm quartz cell was utilized for all measurements. Mettler Toledo analytical balance (accuracy 0.1 mg) was used for weighing all the samples.

### Materials and Reagents

AM was procured from Sigma-Aldrich. Formulations were purchased from local market. All the used chemicals were analytical reagent grade. Double distilled water is used throughout the experiment. A stock solution of AM was prepared by dissolving accurately weighed 100 mg of pure drug in 100 ml of methanol and sonicated to get required concentration of 1 mg/ml. From this, further dilutions were made with double distilled water to get required concentrations and used for current investigation.

## RESULTS AND DISCUSSION

### Absorption spectrum

Several aliquots of standard solutions of AM were taken in volumetric flasks, 2.8 ml of 0.2 % DDQ solution was added into each volumetric flask and solution was made up to the mark by using corresponding solvent. After the mixing of the above solutions, a pale wine red colour developed, which showed a maximum absorbance at 465 nm against blank (Figure. 2).

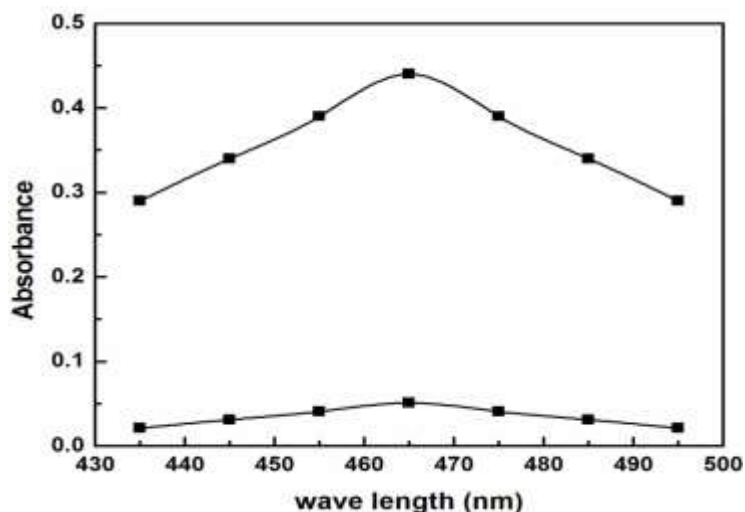


Figure 2: Absorption spectrum of AM with DDQ

### Effect of DDQ concentration

To know the effect of DDQ concentration, standard solution of AM was allowed to react with different volumes of 0.2 % DDQ, viz. 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8 and 3.2 ml. The results showed that the maximum absorbance was obtained with 2.8 ml. Hence, 2.8 ml 0.2 % DDQ was used for the determination of the drug.

### Effect of the concentration of drug

The absorbance spectrum was measured for various concentrations of AM solutions in the range of 4-44  $\mu\text{g/ml}$  with addition of 2.8 ml of 0.2 % DDQ solution at wave length of 465 nm against blank. The obtained results obeyed Beer's law.

### Effect of the solvent

Different non-polar solvents were investigated in order to select the suitable solvent for the analysis of drug. These solvents include chloroform, methanol, 1, 2-dichloroethane, acetonitrile and acetone. It is found that methanol is considered to be an ideal solvent for this experiment and that exhibited a maximum optical density when compared to other solvents.

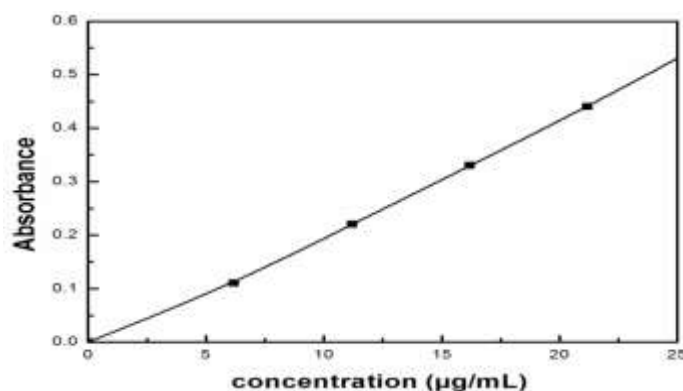
### Analytical method validation

#### Linearity

Linearity of the AM solution was studied and calibration plots were constructed with obtained results (Figure. 3). The linearity of calibration graphs was proved by the high values of the correlation coefficient and the small values of the y-intercept of the regression equation. The apparent molar absorptivity's of the resulting coloured complexes and relative standard deviation of response factor for proposed spectrophotometric method were also calculated. The obtained results like Beer's law limit, Sandell's sensitivity, molar absorptivity, correlation coefficient were reported in Table 1.

**Table 1: Spectral characteristics of the drug with reagent**

$\lambda_{max}$ (nm)	Beer's law limit ( $\mu\text{g/ml}$ )	Molar absorptance ( $\text{L}\cdot\text{mol}^{-1}\text{cm}^{-1}$ )	Sandell's sensitivity y	Correlation coefficient ( $r^2$ )	Slope (m)	Intercept (c)	% RSD	Colour	L O D	L O Q
410	6-27	$2.698 \times 10^4$	0.0017	0.9925	0.25	0.044	0.1	Yellow	0.1	0.3
					4	7	724	Red	18	94
									3	0



**Figure 3: Calibration plot of AM**

### Robustness and ruggedness

In the evaluation of the robustness, some parameters like drug concentration, reagent concentration, wavelength range and shaking time were interchanged. The capacity remains unaffected. Method ruggedness was expressed as RSD % of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical difference between different analysts and instruments suggesting that the developed methods were robust and rugged.

### Accuracy

The accuracy of an analytical method is the nearness in conformity between the established exact value or reference value and the experimental value. Accurate results proved that the recovery values in drug and in pharmaceutical forms are within the acceptance criteria and details were presented in Table 2 and 3.

### Precision

Precision of a method is a measure of the ability to create reproducible results. It is evaluated using six separate determinations. The intra and inter day precision were evaluated and found RSD % less than 1.0, which indicates that there is no significant variations for intra and inter day analysis. These results were presented in Table 2 and 3.

**Table 2: Evaluation of accuracy and precision results of the proposed method in bulk form**

Taken mg/ml	Intra day				Inter day			
	*Found mg/ml	Recovery %	± SD	% RSD	*Found mg/ml	Recovery %	± SD	% RSD
2	1.98	99.00	0.010	0.51	1.97	98.67	0.006	0.29
4	3.97	99.25	0.010	0.25	3.97	99.33	0.012	0.29
6	5.98	99.67	0.010	0.17	5.96	99.33	0.010	0.17

\*Average of six determinations

**Table 3 Evaluation of accuracy and precision results of the proposed method in pharmaceutical dosage form**

Pharma- ceutical formulation	Taken mg/ml	Intra day				Inter day			
		*Found mg/ml	Recovery %	± SD	% RSD	*Found mg/ml	Recovery %	± SD	% RSD
adderall	4	3.94	98.50	0.020	0.51	3.96	99.08	0.006	0.15
Adderal XR	6	5.97	99.56	0.012	0.19	5.97	99.50	0.010	0.17
mydayis	8	7.92	99.00	0.040	0.51	7.96	99.50	0.026	0.33

\*Average of six determinations

### Recovery

Recovery studies were carried out by using standard addition method. The results are summarized in Table 4.

### Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

The LOD was calculated by using the following formula.

$$LOD = \frac{3.3s}{S}$$

Where  $s$  = Standard deviation of the response

$S$  = Slope of the calibration curve

### Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The LOQ was calculated by using the following formula.

$$LOQ = \frac{10s}{S}$$

Where  $s$  = Standard deviation of the response

$S$  = Slope of the calibration curve

## APPLICATIONS

The proposed method is applied for the determination AM in available pharmaceutical formulations in market drug and the results are given in Table 3.

Blood and urine samples were collected from healthy volunteers. The samples were centrifuged at 3000 rpm min<sup>-1</sup> for 10 minutes. The solutions were filtered and preserved in the absence of light at 4<sup>0</sup> C. To this, various concentrations of AM samples were added and analyzed by the developed method. The results are given in Table 4.

**Table 4 Method accuracy from recovery studies**

Sample	Added mg/ml	*Found mg/ml	Recovery%	±SD	%RSD
Blood samples	0.2	0.20	98.50	0.000	0.00
	0.4	0.40	99.25	0.001	0.25
	0.6	0.59	99.06	0.003	0.42
	0.8	0.79	98.63	0.007	0.89
Urine samples	0.4	0.40	99.08	0.002	0.39
	0.6	0.60	99.39	0.002	0.35
	0.8	0.79	99.29	0.005	0.59
	1.0	0.98	98.33	0.012	1.17

**\*Average of six determinations**

## CONCLUSION

In the present reported method, the drug AM was estimated in bulk, in pharmaceutical formulations and in biological fluid samples. The linearity of the proposed method was good from the result of correlation coefficient. The developed method is novel, simple, specific, accurate, precise and reproducible. Specificity and selectivity, LOD, LOQ, molar absorptivity and Sandell's sensitivity values indicate that the proposed analytical method can be successfully utilized for the estimation of AM in bulk, in pharmaceutical formulations and in biological fluid samples.

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