



## Simultaneous estimation of amitriptyline and chlordiazepoxide by RP-HPLC method

Dr.R.Srinivasan\*, K.Lurdhu Mary, D.Rajesh Kumar, T.Parvathi

Siddhartha Institute of pharmaceutical sciences, Jonnalagadda, Narsaraopet, Guntur, (DT), India.

\*Corresponding author: Dr.R.Srinivasan

E-mail id: rangusha75@gmail.com

### ABSTRACT

A simple, accurate, rapid, precise and novel Reverse phase High Pressure liquid chromatographic method (RP-HPLC) has been developed and validated for simultaneous determination of Amitriptyline & Chlordiazepoxide. Amitriptyline and Chlordiazepoxide shows  $\lambda_{max}$  at 230nm and 259 nm respectively. The selected and optimized mobile phase was mixed phosphate buffer (pH 3.0) and Acetonitrile were mixed in the ratio of 55:45 and conditions were flow rate (1.0 ml/minute), wavelength (252 nm), Run time was 12 min. The retention time were found to be 2.857 min and 5.667 min for Amitriptyline & Chlordiazepoxide respectively. Linearity and range was found to be 25-150  $\mu\text{g/ml}$  for Amitriptyline and 10-60  $\mu\text{g/ml}$  for Chlordiazepoxide. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs. The method was validated for accuracy, precision, specificity, linearity, robustness, sensitivity, LOD and LOQ. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible.

**Keywords:** RP-HPLC, Amitriptyline, Chlordiazepoxide and acetonitrile

### INTRODUCTION CHROMATOGRAPHY

Russian botanist Michael Tswett invented chromatography as a separation technique. High performance liquid chromatography is defined as a separation of mixtures of compounds due to differences in their distribution equilibrium between two phases, the stationary phase packed inside columns and the mobile phase, delivered through the columns by high pressure pumps<sup>1,2</sup>.

### REVERSED PHASE CHROMATOGRAPHY

Reversed phase liquid chromatography (RPLC) is considered as the method of choice for the analysis of pharmaceutical compounds for several reasons, such as its compatibility with aqueous and organic solutions as well as with different detection systems and its high consistency and repeatability. The parameters that govern the retention in Reversed Phase systems are the following:

A. The chemical nature of the stationary phase surface.

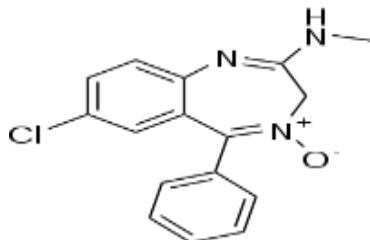
- B. The type of solvents that compose the mobile phase  
C. pH and ionic strength of the mobile phase<sup>3,4,5</sup>

## DRUG PROFILE

### CHLORDIAZIPOXIDE

IUPAC Name : 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine-4-oxide

#### Structural Formula

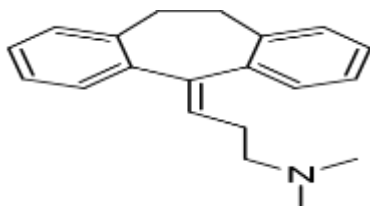


Chemical formula:  $C_{16}H_{14}ClN_3O$   
Molecular Mass : 299.75 g/mol  
Description : A white or almost white powder.  
Solubility : Soluble in water, sparingly soluble in methanol and partially insoluble in acetone.  
Category : Sedative/hypnotic drug and benzodiazepine<sup>6</sup>

### AMITRIPTYLINE

IUPAC Name: 3-(10, 11-dihydro-5H-dibenzo [a,d] cycloheptene-5-ylidene)-N,N-dimethylpropan-1-amine

#### Structural Formula



Chemical formula :  $C_{20}H_{23}N$   
Molecular Mass : 277.403 g/mol  
Description : A white to yellowish powder.  
Solubility : Soluble in alcohol and sparingly soluble in water.  
Category : Tricyclic antidepressant (TCA)<sup>6</sup>

## MATERIALS & METHODS

### ANALYTICAL METHOD DEVELOPMENT

#### CHROMATOGRAPHIC CONDITIONS

Mobile phase: Mixed phosphate buffer (pH 3.0) and Acetonitrile were mixed in the ratio of 55:45 and sonicated to degas

.Diluent : Mobile Phase  
Column : Phenomenex C18, 150X4.6mm, 5 $\mu$ .  
Flow : 1ml/min

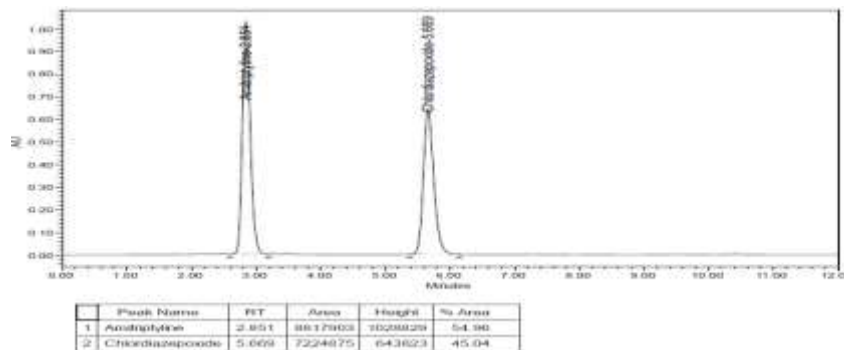
Injection volume : 20 $\mu$ l  
Wavelength : 252nm  
Column oven temperature : 25 $^{\circ}$ c  
Elution : Isocratic  
Run time : 12min

**RESULTS & DISCUSSION**

**SYSTEM SUITABILITY PARAMETERS**

All the system suitability parameters are within the acceptable range i.e. tailing factor is less than 2 for both Amitriptyline and Chlordiazepoxide. Resolution

between the two analyte peaks is 7.58 is within the acceptable range. Number of theoretical plate for the analyte peaks is more than 2000.



**LINEARITY**

The results reveal that method is linear.

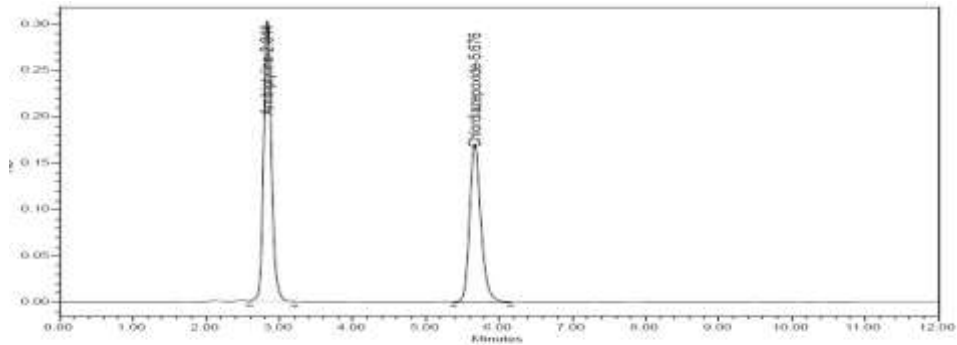
**Linearity of Amitriptyline**

S.NO	Concentration(µg/ml)	Area
1	25	2254766
2	50	4384905
3	75	6594695
4	100	8749662
5	125	10900930
6	150	13095293
	Slope	86749
	R <sup>2</sup>	1
	y-intercept	72807

**Linearity for Chlordiazepoxide**

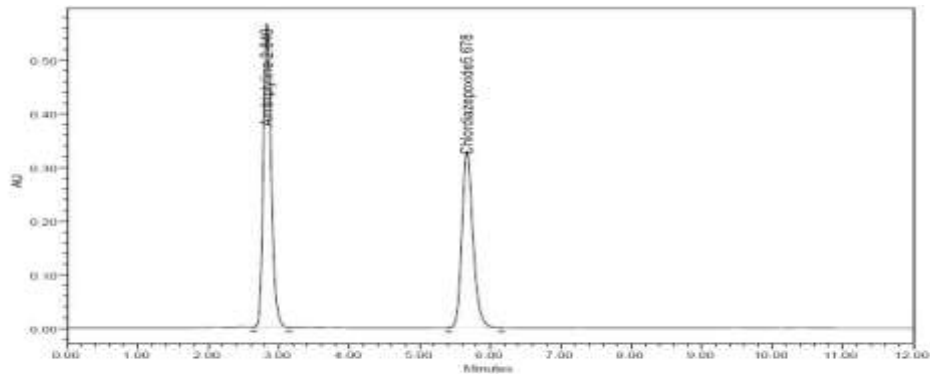
S.NO	Concentration(µg/ml)	Area
1	10	1828792
2	20	3600918
3	30	5397622
4	40	7183345
5	50	8910487
6	60	10742639
	Slope	177953
	R <sup>2</sup>	1
	y-intercept	48934

Level – I



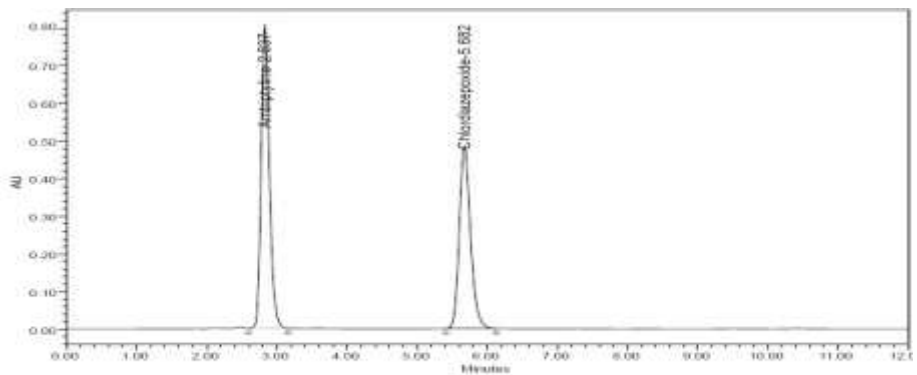
Peak Name	RT	Area	Height	% Area
1 Amitriptyline	2.844	2254766	303987	55.22
2 Chlordiazepoxide	5.676	1829792	170903	44.78

Level – II



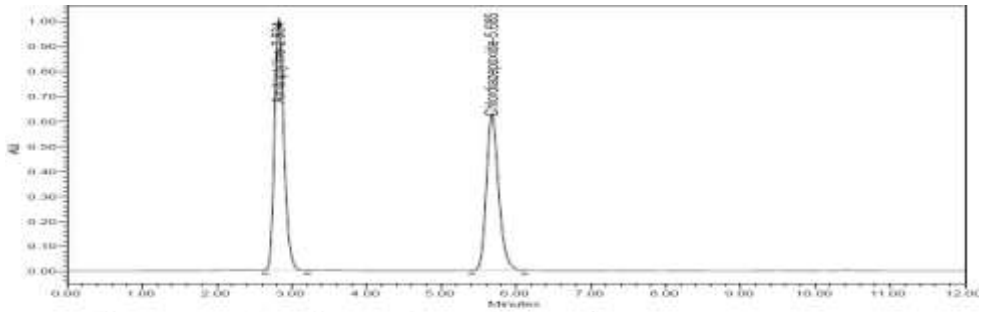
Peak Name	RT	Area	Height	% Area
1 Amitriptyline	2.840	4384905	569300	54.91
2 Chlordiazepoxide	5.678	3600918	330568	45.09

Level – III



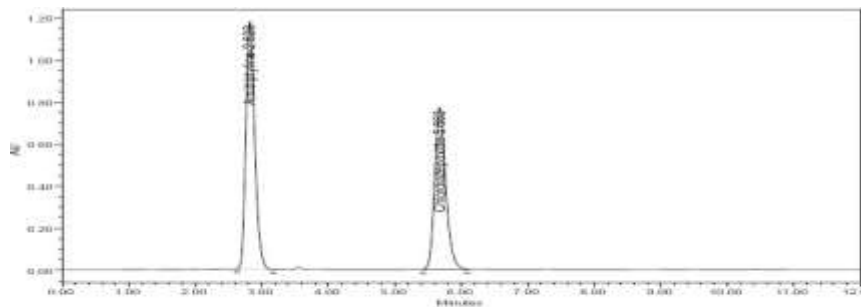
Peak Name	RT	Area	Height	% Area
1 Amitriptyline	2.837	6594895	806852	54.99
2 Chlordiazepoxide	5.682	5397622	485533	45.01

Level – IV



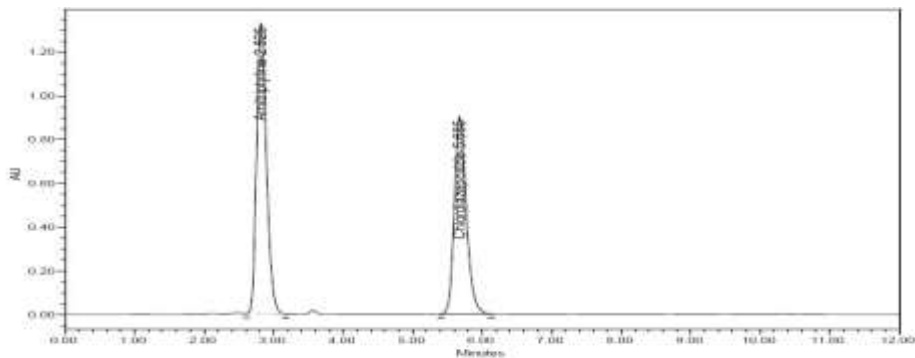
Peak Name	RT	Area	Height	% Area
1 Amitriptyline	2.834	8749862	1010996	54.92
2 Chlordiazepoxide	5.665	7183345	831902	45.08

Level – V



Peak Name	RT	Area	Height	% Area
1 Amitriptyline	2.828	10900930	1191573	55.02
2 Chlordiazepoxide	5.663	8910487	770999	44.98

Level – VI



Peak Name	RT	Area	Height	% Area
1 Amitriptyline	2.825	13095293	1336670	54.93
2 Chlordiazepoxide	5.665	10742639	904119	45.07

## PRECISION

The system meets the required system precision

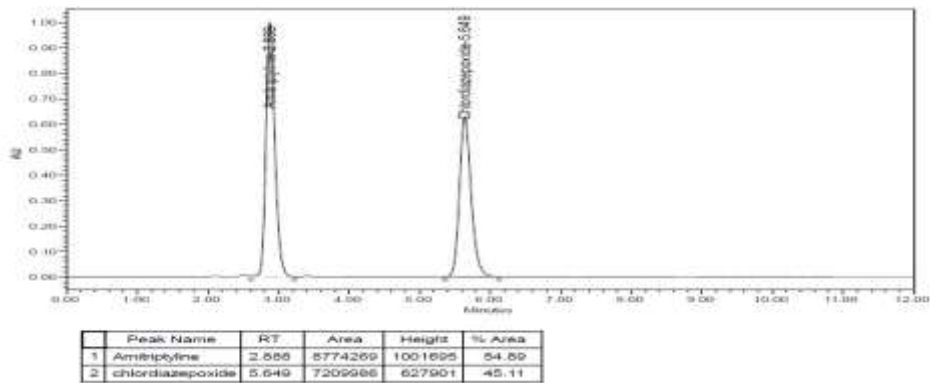
### Precision for Amitriptyline

S.NO	RT	Area
1	2.888	8774269
2	2.88	8786169
3	2.875	8802689
4	2.871	8812564
5	2.868	8809470
6	2.861	8844757
<b>Avg</b>	2.873	8804986
<b>SD</b>	0.00945	24339.9
<b>%RSD</b>	0.33	0.28

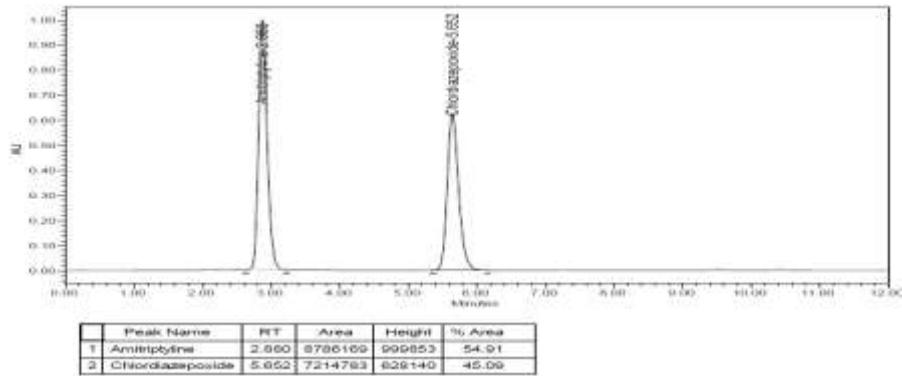
### Precision for Chlordiazepoxide

S.NO	RT	Area
1	5.649	7209986
2	5.652	7214783
3	5.654	7209881
4	5.658	7225436
5	5.663	7225811
6	5.663	7253389
<b>Avg</b>	5.6565	7223214
<b>SD</b>	0.005822	16407.58
<b>%RSD</b>	0.10	0.23

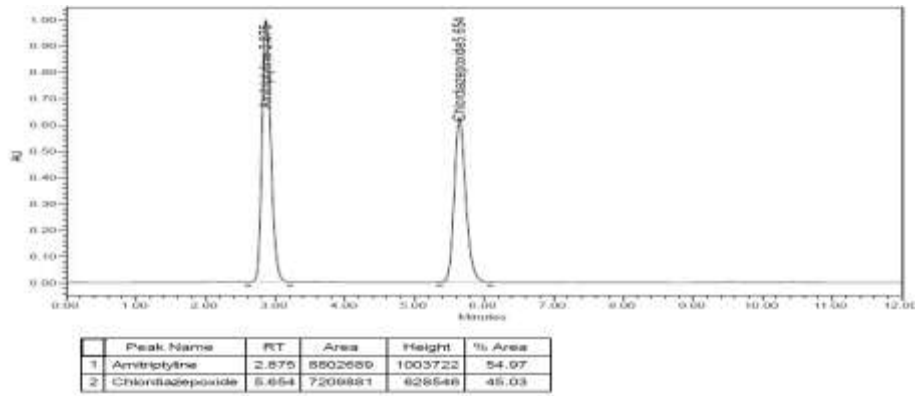
## INJECTION 1



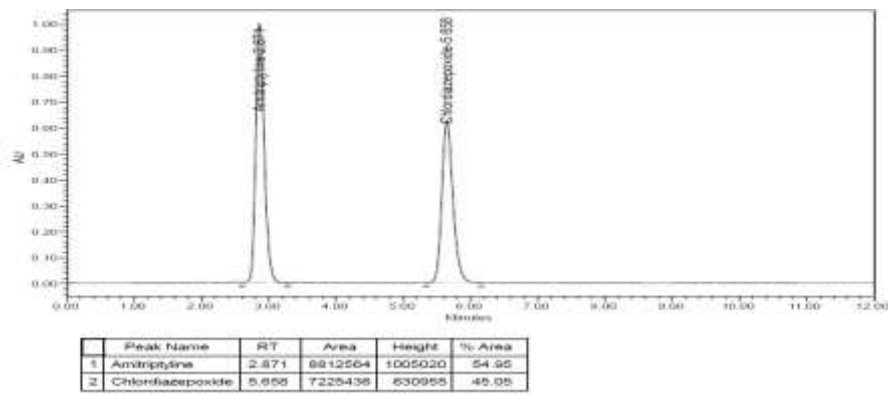
**INJECTION 2**



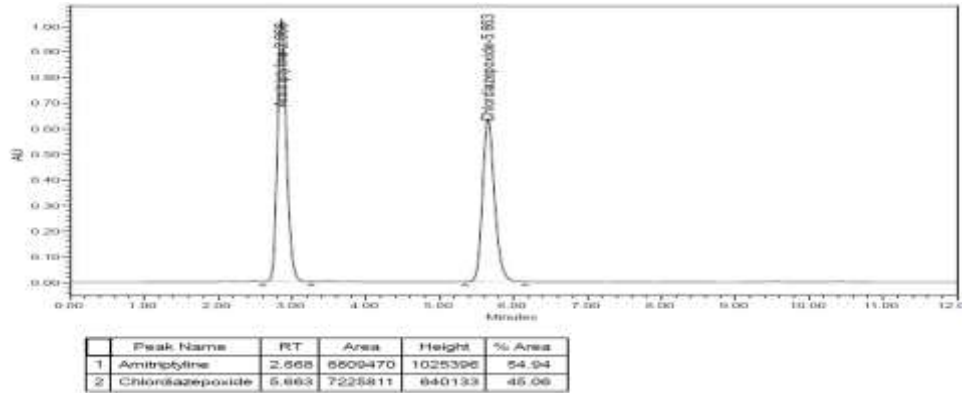
**INJECTION 3**



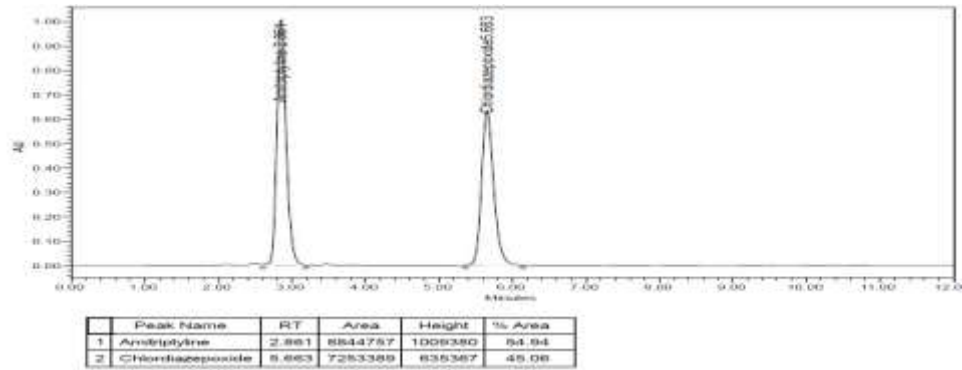
**INJECTION 4**



**INJECTION 5**



**INJECTION 6**



**ACCURACY**

	<b>Amitriptyline</b>	<b>Chlordiazepoxide</b>
Accuracy Standard	8697387.333	7120981
Accuracy sample	8741043	7152532
50% spike	13004523	10630299
	13002122	10682120
	12994129	10644044
Avg	1300258	10652154.33
Amount recovered	48.97	49.15
% recovery	97.94%	98.3%
100% spike	17357836	14284734
	17359635	14279482
	17340742	14251650
Avg	17352737.67	14271955.33
Amount recovered	99.01	99.98
% recovery	99.01%	99.98%



150% spike	21550743	17737960
	21588163	17755457
	21660536	17817914
Avg	21599814	17770443.67
Amount recovered	147.84	149.1
%recovery	98.56%	99.4%

**ASSAY**

	Amitriptyline	Chlordiazepoxide
Standard	8724698	7178519
	8718294	7170924
	8713949	7151390
Avg	8718980.333	7166944
Sample	8725464	7151796
	8716128	7149115
Avg	8720796	7150456
LC	25	10mg
Standard weight	25.2	10.1
Sample weight	158.4	158.4
Standard purity	99.8	99.75
Avg weight	156.4	156.4
Amount/tablet	24.8mg	9.92mg
% assay	99.35%	99.25%

**SYSTEM SUITABILITY PARAMETERS**

S.NO	Parameters	Amitriptyline	Chlordiazepoxide
1	Theoretical plates	3911	4265
2	LOD(µg/ml)	3.70370	1.21
3	LOQ(µg/ml)	11.55990	3.79870
4	Tailing factor	1.24	1.52

**CONCLUSION**

For RP-HPLC method mixture of P<sup>H</sup> 4.0 Mixed phosphate buffer and Acetonitrile in the ratio of 55:45 was selected as a mobile phase and equal proportions of Buffer and acetonitrile. Which gives good resolution and good peak shapes for Amitriptyline and Chlordiazepoxide. The flow rate was set at 1.0 ml/min, and the detection was carried out with UV detector at 252nm. Phenomenex C18 column, 150 × 4.6mm, 5µm column was used for the separation. At the optimum conditions mentioned above. The total run

time required was 12 mins. The linearity and range was established over the range of LOQ – 1000 % concentration range Amitriptyline and Chlordiazepoxide. The correlation coefficient of Amitriptyline and Chlordiazepoxide was found to be 1.

From the overall results obtained it was concluded that the developed method was more accurate, precise, specific and robust with ±5°C in temperature, ±0.1ml/min in flow rate, ±5% variation in organic phase.

## REFERENCES

- [1] Connors K.A, "A Text Book of Pharmaceutical Analysis", Wiley-Inter science, Singapore, 1999; 175-82.
- [2] Willard-H.H., Lynne L.M. Jr., John A., Dean F.A.," Instrumental Methods of Analysis", 7<sup>th</sup> Edn., CBS Publishers and Distributors, New Delhi; 1-12, 580-610, 614-652.
- [3] Davidson A.G, "Basis of Spectrophotometry", 4<sup>th</sup> Ed., Part-2, CBS Publishers, New Delhi, 2002; 264-74.
- [4] Fronk A.S.,"Handbook of Instrumental Techniques for Analytical Chemistry", 1<sup>st</sup> Edn., Pearson Education, 2004.
- [5] Skoog D.A., Holler F.J., Nieman D.A.," Principle of Instrumental Analysis", 6<sup>th</sup> ed Reprint, Thomson Brooks/Cole publication, 2004 ; 300-351.(UV)

\*\*\*\*\*