AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

A Validated RP-HPLC Method for the Simultaneous Estimation of Dextromethorphan Hydrobromide and Chlorpheniramine Maleate in Syrup Formulation

Krushna D. Khalode*[1], Shekhar B. Waikar[1], Suhas P. Padmane[1]

1. Gurunanak College of Pharmacy, Nari, Kamptee road, Nagpur 440026, Maharashtra, India

ABSTRACT

The proposed method is a simple, accurate, precise, specific and rapid method for the simultaneous estimation of dextromethorphan hydrobromide (DXM) and chlorpheniramine maleate (CPM) in bulk and syrup formulation. Stationary phase consist of Eclipse-XDB C18 column(150×4.6mm, 5µm) and mobile phase with gradient mode consisting of phosphate buffer (adjusted to pH 3.0 with o-phosphoric acid): acetonitrile (80:20 v/v) was used. The flow rate was set at 1.0 ml/min and UV detection was carried out at 272 nm. The retention time of DXM and CPM were 9.05 min and 7.53 min respectively. The % recovery of DXM and CPM was found to be 99.58 ±1.33 and 98.24 ±1.97 respectively. DXM and CPM drugs were found to be linear over the concentration range of 2-50 µg/ml and 0.8 - 20 µg/ml respectively. The proposed method can be useful in the quality control of DXM and CPM in bulk drug and drug products.

Keywords: Chlorpheniramine maleate, Dextromethorphan hydrobromide, RP-HPLC, Validation.

*Corresponding Author Email: krish1304@yahoo.com

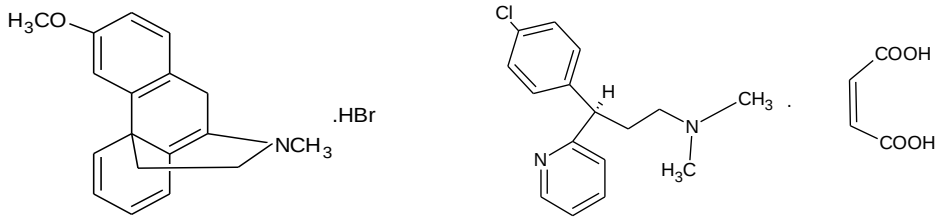
Received 26 July 2012, Accepted 6 August 2012

Please cite this article in press as: Khalode KD *et al.*, A Validated RP-HPLC Method for the Simultaneous Estimation of Dextromethorphan Hydrobromide and Chlorpheniramine Maleate In Syrup Formulation. American Journal of PharmTech Research 2012.

INTRODUCTION

Dextromethorphan hydrobromide (DXM) is antitussive (cough suppressant) drug used for the pain relief and in psychological conditions. It acts on cough centre to elevate the threshold for coughing [1.] Chemically, it is morphinan, 3-methoxy-17-meth (9, 13, 14)-, hydrobromide. Chlorpheniramine maleate (CPM) is an antihistamine drug that is widely used in pharmaceutical preparations for symptomatic relief of common cold and allergic diseases [2]. Chemically, it is 3-(4- chlorophenyl)-N, N-dimethyl-3-pyridin-2-ylpropan-1-amine.The structures of both the drugs are shown in figure (1). In the literature, several UV and HPLC methods have been reported for the estimation of DXM and CPM individually and in combination with other drugs. Literature survey revealed that different methods have been reported for the determination of DXM in bulk drug and in dosage forms in combination with other drugs. Spectrophotometry[3], RP-HPLC [4], electrophoresis [5], liquid chromatography [6], methods have been reported for the estimation of dextromethorphan hydrobromide in pharmaceutical formulations. For CPM in bulk drug and in dosage forms in combination with other drugs. Spectrophotometry [7] and RP-HPLC [8] methods have been reported for the estimation of chlorpheniramine maleate in pharmaceutical formulations. A variety of methods in the literature for the determination of some of the compounds but none describe the determination of these two compounds.

However, no RP-HPLC method has been developed for the simultaneous determination of DXM and CPM in combined liquid dosage form. The present study describes a precise, accurate, specific and sensitive RP-HPLC method for the simultaneous estimation of DXM and CPM in syrup formulation.



(a) (b)

Figure 1: **Structure of (a) DXM and (b) CPM**

MATERIALS AND METHODS

Pure drugs of Dextromethorphan hydrobromide and Chlorpheniramine maleate were procured as gift samples from Lupin Pharmaceuticals Ltd. (Aurangabad, India) and Zim Laboratories Ltd. (Nagpur, India) respectively. HPLC grade acetonitrile, ortho-phosphoric acid and water and AR grade potassium dihydrogen phosphate were used throughout the study. The mobile phase was

filtered through Millipore Nylon filter (0.45 µ) and vacuum degassed. All the samples were filtered through Millipore Nylon filter (0.22 µ). Two syrup formulations containing DXM 10 mg and CPM 4 mg per 5 ml (Piriton-CS and Dilo-DX, both from GlaxoSmithKline) were purchased from the local pharmacy.

Instruments

Agilent HPLC 1200 series comprising of quaternary gradient pump G1311A with on Line degasser G1322A, variable wavelength UV-VIS detector G1314B, manual Rheodyne injector, 7725 I with 20 µl loop and Eclipse XDB C_{18} column (150×4.6mm, 5µm) was employed. Other equipments used were digital balance (Shimadzu AUY-220, Japan), Sonicator (PCI Services, Mumbai) and Digital hot air oven (Meta-Lab Scientific Industries, Mumbai) and Millipore filtration assembly.

Preparation of standard solution of DXM

Stock solution of DXM was prepared by dissolving 25.0 mg of DXM in 25 mL of mobile phase (1 mg/mL). From this solution 1.0 mL was diluted to 10.0 mL with mobile phase to get concentration of 100µg/mL Further 2.0 mL of this solution was diluted to 10.0 mL with mobile phase to get 20µg/mL

Preparation of standard solution of CPM

Stock solution of CPM was prepared by dissolving 10.0 mg of CPM in 25 mL of mobile phase (1 mg/mL). From this solution 1.0 mL was diluted to 10.0 mL with mobile phase to get concentration of 40µg/mL further 2.0 mL of this solution was diluted to 10.0 mL with mobile phase to get working standard solution 8µg/mL

Preparation of mix standard solution

Mixed standard solution of DXM and CPM was prepared by transferring 1.0mL each of DXM and CPM stock standard solution to 10.0 mL volumetric flask and volume was adjusted to 10.0 mL with mobile phase (Concentration of DXM and CPM was 100µg/mL and 40µg/mL respectively).2.0mL of this solution was further diluted to 10.0mL with mobile phase to get concentration of DXM and CPM as 20µg/mL and 8µg/mL respectively.

Preparation of Sample Solution

An accurately weighed quantity of syrup equivalent to 10 mg of DXM and 4 mg of CPM was transferred to a 50 ml volumetric flask, dissolved in adequate quantity of mobile phase and finally volume was adjusted to 50.0 ml with mobile phase. 1.0 mL of this solution was further diluted 10.0mLwith mobile phase to obtain a concentration of 20 µg/ml of DXM and 8 µg/ml of CPM. The solution was filtered through 0.22 µ membrane filter.

Chromatographic Conditions

The mobile phase consisted of mixture of potassium dihydrogen phosphate buffer (20 mM adjusted to pH 3 with o-phosphoric acid) and acetonitrile (80:20 v/v). (Sol.-A): buffer and (Sol.-B): Acetonitrile was used. The gradient program was initiated with solvent A (At t=0 the mobile phase consisted of 80% A and 20% B and it changed with a linear gradient during 3 min to 72% A and 28% B, At min 3, from 3-6 min. constant flow rate, it changed to 58% A and 42% B for 6 min. and at t=14 min, it returns to the initial conditions (80% A and 20% B)) flow rate used was 1.0 ml/min and wavelength UV detector was set at 272 nm. All analysis were performed at ambient temperature of 25^{o}. The mixed standard solution of DXM and CPM and sample solution of syrup were analysed using the optimized chromatographic conditions and yielded the chromatograms as shown in figure 2 and 3.

VALIDATION OF METHOD[9]

Accuracy

The accuracy of an analytical method is expressed as percent recovery of standard added drug in fixed quantity of prenalysed syrup sample. An accurately weighed five quantities of syrup equivalent to 5 mg of DXM and 2 mg of CPM were transferred to a different 50 ml volumetric flask. To each flask standard DXM and CPM drugs were added, dissolved in adequate quantity of mobile phase and volume of each flask was adjusted to 50.0 ml with mobile phase. The solutions were further diluted appropriately with mobile phase to obtain a concentration in the range of 70 to 130 % on label claim basis. The solutions were filtered and analyzed using a set chromatographic method.

Precision

The precision of an analytical method was performed by five replicate estimations of the same homogeneous sample. An accurately weighed five quantities of syrup equivalent to 10 mg of DXM and 4 mg of CPM were transferred to a different 50 ml volumetric flasks, dissolved in adequate quantity of mobile phase and volume of each flask was adjusted to 50.0 ml with mobile phase. The solutions were further diluted appropriately with mobile phase to obtain a concentration of 20 µg/ml of DXM and 8 µg/ml of CPM. The solutions were filtered and analysed using a set chromatographic method.

Specificity

Accurately weighed six quantities of syrup equivalent to about of 20 mg DXM (also equivalent to 8 mg CPM), were transferred to 50.0 mL volumetric flasks, dissolved in adequate quantity of mobile phase and volume of each flask was adjusted to 50.0 ml with mobile phase. All these

solutions were stored for 24 hrs under following different conditions.

1. Normal
2. 1.0 mL of 0.1N NaOH at 50^0C
3. 1.0 mL of 0.1N HCl at 50^0C
4. 1.0 mL of 0.1N 3% H_2O_2 at 50^0C
5. At 60^0C (Thermal)
6. In Sunlight

Linearity

Mixed stock standard solution of DXM and CPM having concentration of 1 mg/ml and 0.4 mg/ml respectively, was prepared in methanol. Five ml of this solution was diluted to 50 ml with mobile phase (100µg/ml). Seven aliquot portions of this solution (0.2, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 ml) was further diluted to 10 ml with mobile phase to get concentration range of 2-50 µg/ml and 0.8-20 µg/ml for DXM and CPM respectively. All the solutions were analysed using a set chromatographic method and the responses were measured as peak area. The calibration curves were obtained by plotting peak area verses concentration.

System Suitability

For system suitability parameters, seven replicate injections of mixed standard solution were injected and parameters such as the resolution, capacity factor, tailing factor, theoretical plate, retention volume and asymmetry factor of the peaks were calculated.

Robustness

As defined by the International Conference on Harmonisation, the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in method parameters and provides an indication of its reliability during normal usage.

Ruggedness: (intermediate precision)

The study of ruggedness was carried out by means of two different conditions.

Inter-day study:

The study was performed by applying the proposed method on same sample of syrup formulation on different days.

Intra-day study:

The study was performed by applying the proposed method on same sample of syrup formulation on same day at two hours interval.

RESULTS AND DISCUSSIONS

Method Development

Several mobile phase compositions were tried to resolve the peaks of DXM and CPM. The optimum mobile phase containing phosphate buffer and acetonitrile 81:19 (v/v) (pH =3), adjusted with o-phosphoric acid) was selected because it could resolve the peaks of DXM (R_t = 7.54 min) and CPM (R_t = 9.05 min) respectively. Quantification was achieved with UV detection at 272 nm on the basis of peak area at 1.0 ml/min flow rate. A typical HPLC chromatogram obtained during simultaneous determination of DXM and CPM.

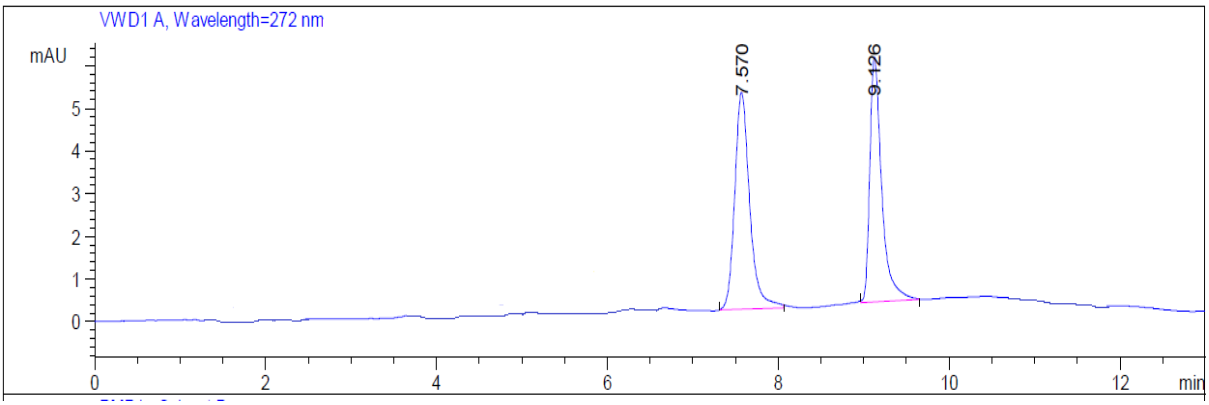


Figure 2: **Chromatogram of mixed standard solution of drugs**

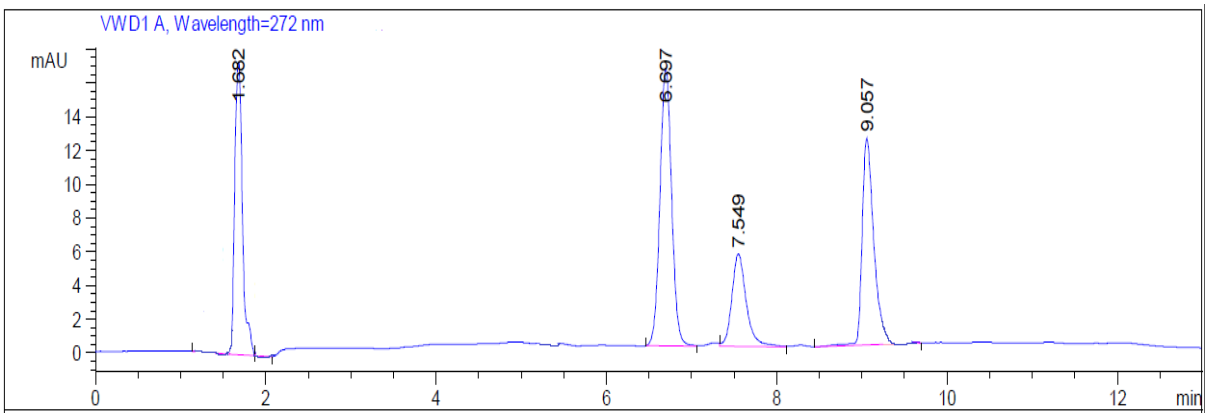


Figure 3: **Chromatogram of sample solution.**

Method Validation

Accuracy

Recovery studies were carried out by applying the standard addition method. Known amounts of standard DXM and CPM corresponding to 70%, 85%, 100%, 115% and 130% of the label claim were added to sample of liquid dosage form separately. The average % recoveries for DXM and CPM in marketed formulation were found to be between 99.58 and 98.24 respectively. The results revealed that there was no interference of excipients. The results of accuracy are shown in Table 1.

Table 1: Results of recovery studies

Wt. of syrup/ 5.0 ml (mg)	Wt. of pure drug		Area		Amt. of drug		% drug recovered	
	DXM	CPM	DXM	CPM	DXM	CPM	DXM	CPM
4180.2	2.0	0.8	85.90	46.30	1.92	0.85	97.0	98.83
4200.7	3.5	1.4	105.86	55.80	3.59	1.43	100.3	102.86
4210.6	5.0	2.0	122.8	70.64	4.96	1.94	99.2	97.5
4170.6	6.5	2.6	140.01	74.86	6.36	2.61	98.0	100.3
4220.1	7.0	3.2	144.62	83.68	6.73	3.15	97.0	98.43
Mean± SD							98.24±1.33	99.58±1.97
% RSD							1.35	1.97

Precision

From the standard stock solutions, mixed standards containing DXM and CPM were prepared. Standard solutions (n=3) were injected using a universal rheodyne injector with injection volume of 20 µl. The intra-day and inter-day precisions were assessed by analyzing standard solutions. The % RSD was found to be 0.21 and 0.34 for both the drugs. The lower values of % RSD indicate that the method was precise. The results of precision are shown in Table 2.

Table 2: Results for precision study

Wt. of syrup/5ml (mg)	Area		Amt. of drug		% label claim	
	DXM	CPM	DXM	CPM	DXM	CPM
4180.2	125.37	67.77	30.08	9.18	100.09	100.70
4200.7	124.55	66.40	34.92	10.92	99.77	101.34
4210.6	122.06	64.28	40.02	12.29	99.98	101.27
4170.6	121.98	63.72	44.89	13.93	99.67	101.60
4220.1	124.12	63.09	49.78	15.92	99.57	101.50
Mean± SD					99.81±0.21	101.28±0.35
% RSD					0.21	0.34

Specificity

Accurately weighed six quantities of syrup equivalent to about of 20 mg DXM (also equivalent to 8 mg CPM), were transferred to 50.0 mL volumetric flasks, dissolved in adequate quantity of mobile phase and volume of each flask was adjusted to 50.0 ml with mobile phase. All these solutions were stored for 24 hrs under following different conditions. The results are shown in Table. 3

Linearity

The range of 2-50 µg/ml for both the drugs. The linear regression equations for CPM and DXM were found to be y =6.239x and y = 5.476x respectively. The regression coefficient values (r^2) were found to be 0.9998 and 0.9989 respectively. The graph is as fallows in Figure 4

Table 3 :For specificity study

Sr. no.	Sample	% label claim	
		DXM	CPM
1	Normal	99.6	101.1
2	Acid	99.5	98.5
3	Alkali	99.0	98.7
4	Oxide	99.78	98.25
5	Heat	99.8	99.25
6	Sunlight	100.2	101.0

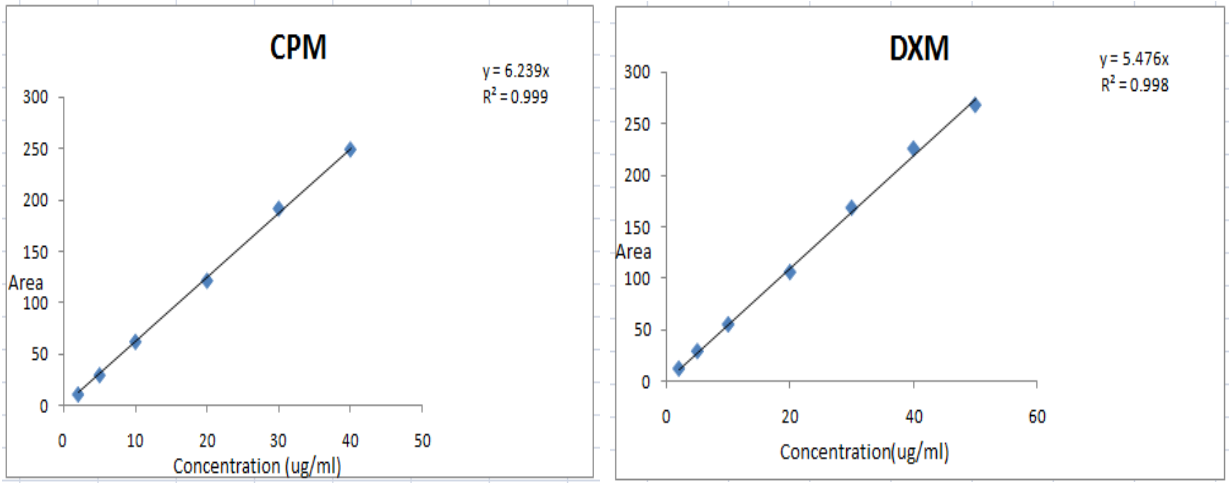


Figure 4: Graph for linearity for CPM and DXM

System Suitability Parameters

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions. The results are as follows Table 4.

Table 4: **System suitability of DXM and CPM**

	Retention time		Capacity factor		Symmetry		Area	
	DXM	CPM	DXM	CPM	DXM	CPM	DXM	CPM
Mean	9.063	7.533	2.63	2.02	0.55	0.76	123.16	64.90
± SD	±0.054	±0.064	±0.026	±0.024	±0.010	±0.008	±1.52	±0.91
% RSD	0.595	0.849	0.988	1.188	1.81	1.09	1.23	1.40

Robustness

Robustness can be termed as method should not be affected by small changes. We can study robustness by varying chromatographic condition a deliberate change in detection wavelength by ±2 nm.

Ruggedness: (intermediate precision)

The studies were carried out i.e. days (intra-day and inter-day).

CONCLUSION

A novel RP- HPLC method has been developed for the simultaneous estimation of DXM and CPM in marketed formulations. The method gave good resolution for both the drugs. The developed method was validated. It was found to be novel, simple, precise, accurate, and sensitive. The good % recovery in liquid forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of DXM and CPM in combined liquid dosage form. It can be also used in the quality control in bulk manufacturing.

ACKNOWLEDGMENT

The authors are thankful to Gurunanak College of pharmacy for providing facilities to carry out research work and grateful to Lapin Pharmaceuticals Ltd. (Aurangabad, India) and Zim Laboratories (Nagpur, India), for providing gift samples of DXM and CPM respectively.

REFERENCES

1. British Pharmacopoeia (veterinary published) by medicine and health care product regulary (MHRA) London, 2009;2:1849-1852.
2. British Pharmacopoeia (veterinary published) by medicine and health care product regulary (MHRA) London, 2009;2:1289-1290.
3. Vijayalakshmi R, Bhargavi S, Dhanaraju M. Simultaneous UV spectrophotometric determination of cetrizine and dextromethorphan in tablet dosage form. J Chemistry 2010; 7(S1): S314-S318.
4. Rauha J, Salmies H, Aalto M. Development simultaneous determination of bromohexine hydrochloride methyl and propyl p-hydroxybenzoate and determination of dextromethorphan hydrobromide in cough-cold syrup by high-performance liquid chromatography. J Pharma Biomedical Analysis 1996;15: 287-283.
5. Fuad Al-Rimawi. Development & validation of Normal-phase LC method for simultaneous analysis of pseudoephedrine hydrochloride, Dextromethorphan hydrobromide, chlorpheniramine maleate and paracetamol in tablet formulations. Saudi Pharma J 2010;18: 103-106.
6. Yuming Dong, Xiaofeng Chen, Yonglei Chen, Xingguo Chen, Zhide Hu. Separation and determination of pseudoephedrine, dextromethorphan, diphenhydramine and chlorpheniramine in cold medicines by nonaqueous capillary electrophoresis. J Pharma

Biomedical Analysis 2005; 39: 285–289.

7. Maithani M, Raturi R. Pharmacie Globale. Development and validation of a rp-hplc method for the determination of chlorpheniramine maleate and phenylephrine in pharmaceutical dosage form. IJCP school of Pharma Sci 2010;1:5(5).
8. Murtha J, Julian T, Radebaugh G. Simultaneous determination of pseudoephedrine hydrochloride, chlorpheniramine maleate and dextromethorphan hydrobromide by Second-Derivative photodiode array spectroscopy. J Pharma Sci 1988; 77: 715-717.
9. ICH, Q2A and Q2B, Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Geneva 2005:1-13.
10. United State Pharmacopoeia, vol. I, Asian edition, United Pharmacopoeial Convention, Inc., Rockville, 2009;1225: 736.
11. Beckett AH, Stenlake JB, Practical Pharmaceutical Chemistry, 4th edition, part II, CBS Publisher and Distributor, New Delhi, 1997:157-174.
12. Indian pharmacopoeia, Government of India ministry of health and family welfare, published by the I.P. commission Ghaziabad (2007),vol.II:395-396, 301.
13. Sharma BK. Instrumental Methods of Chemical Analysis, Goel Publishers, New Delhi, (1999: 1.
14. Kalsi PS. Spectroscopy of Organic Compounds,3rd Edn., New Age International Pvt. Ltd., New Delhi 1988:13.
15. H.H Willard., LL (Jr). Merritt, J.A. Settle, Instrumental Methods of Analysis, 7th Edn. CBS Publishers and Distributors, Delhi, 2001: 1.
16. L.R. Snyder, J.J. Kirkland, Introduction to Modern Liquid Chromatography, 2nd Edn., John Wiley and Sons Publication, 1989:84-90.
17. G.H. Jeffery, J. Bassett, J. Mendham ,R.C. Denney, Vogel's Text Book of Qualitative Analysis, 5th Edn. Longman Scientific &Technical Publication. 1988:708.
18. Sethi PD. Quantitative Analysis of Pharmaceutical Formulations.3rd Edn. CBS Publishers and Distributors, New Delhi,1997: 51.