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Stability indicating RP-LC method for determination of Pramipexole in bulk and pharmaceutical dosage forms

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ABSTRACT

An isocratic stability indicating liquid chromatographic method has been developed and validated for the determination of Pramipexole in bulk drug and its pharmaceutical dosage forms. Separation of the drug with degradation products was achieved using Prontosil,C18, 150 x 4.6mm; 5µm column as stationary phase and pH 3.0(±0.05) buffer: Acetonitrile (72:28,v/v) as mobile phase at a flow rate of 1.0 mL/min. UV detection was performed at 264 nm. The method is linear over the range of 0.96 – 89.7 µg/mL. The percent recovery of drug in dosage forms was ranged from 100 to 102.2. The method is simple, rapid, precise, selective and stability indicating and can be used for the assay in quality control and stability studies samples.

INTRODUCTION

Pramipexole dihydrochloride tablets contain pramipexole, a non-ergot dopamine having empirical formula $C_{10}H_{17}N_3S{\cdot}2HCL{\cdot}H_2O$ and used for treatment the signs and symptoms of idiopathic Parkinson's disease of moderate-to-severe primary Restless Legs Syndrome .Its chemical name is pramipexole dihydrochloride is (*S*)-2-amino-4,5,6,7-tetrahydro-6-(propylamino) benzothiazole dihydrochloride monohydrate. It is a white to off-white powder substance with a molecular weight of 302.27. Pramipexole is more than 20% soluble in water, about 8% in methanol, about 0.5% in ethanol and practically insoluble in dichloromethane [1-2].

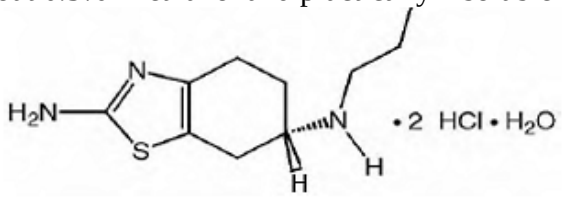


Figure-1 Pramipexole dihydrochloride monohydrate

It is not official in any pharmacopoeia and till now, few liquid chromatographic (LC) procedures have been reported for the determination of Pramipexole and its metabolites in biological fluids. Hence, simple, rapid and economical LC method required for quantitative estimation of Pramipexole in the presence of related impurities in bulk and pharmaceutical dosage forms. In the proposed method, related impurities were well separated and eluted with in 6 minutes. Finally the method was thoroughly validated for the assay of Pramipexole dihydrochloride Tablets.

MATERIALS AND METHODS

2.1 Instrumentation

The Waters LC system equipped with 2489 pump and 2996 Photodiode array (PDA) detector was used. The output signal was monitored and integrated using Waters Empower 2 software.

2.2 Solutions

2.2.1 pH 3.0 buffer solution

Weighed accurately 4.6 g of 1-octane sulphonic acid sodium salt and 9.1 g of potassium dihydrogen phosphate dissolved in 1000mL of milli-Q water and pH was adjusted to 3.0±0.05 with orthophosphoric acid and mixed well.

2.2.2 Mobile phase

A mixture of pH 3.0 buffer and acetonitrile in the ratio 72:28(v/v) was prepared and filtered through 0.45 µm nylon membrane filter and degassed for about 10 min.

2.2.3 Diluent:

pH 3.0 buffer and methanol mixed in the ratio 60:40(v/v) mixed well and filtered through 0.45µm nylon membrane filter.

2.2.4 Standard solution (60µg/ml)

60mg of Pramipexole dihydrochloride monohydrate working standard was transferred in to a 100 mL volumetric flask and to that 70 ml of diluent was added and sonicated to dissolve and diluted to volume with diluent. Solution was filtered through 0.45µm nylon membrane filter.

2.2.5 Test Solution

The number of tablets equivalent to 12 mg of Pramipexole(8 tablets of 1.5mg strength) were weighed and transferred in to a 200 mL volumetric flask and about 140 ml of the diluent was added and swirled the flask to disintegrate, sonicated for 40 min with intermediate shaking and diluted to the volume with the diluent. The solution was filtered through 0.45µm nylon membrane filter prior to use.

2.2.6 Preparation of Samples for Specificity Study

For **Acid degradation** Pramipexole sample was stressed with 1N HCl on mantel for 45min at 80°C and then neutralized by adjusting pH to 7.0 with 1 N NaOH. The solution was further diluted to the required concentration with the diluent.

For **Alkali degradation** Pramipexole sample was stressed with 0.1 N NaOH on mantel for 45min at 80°C and then neutralized by adjusting pH to 7.0 with 0.1N HCl. The solution was further diluted to required concentration with the diluent.

For **Oxidative degradation** Pramipexole sample was stressed with 1% H_2O_2 for 20 minutes on Bench top. The solution was further diluted to required concentration with the diluent.

For **Water degradation** Pramipexole sample was stressed with water by heating on mantel at 100ºC for 2 hours. The solution was further diluted to required concentration with the diluent.

For **Humidity degradation** Pramipexole sample was stressed at 25°C/90% RH for 288 hours.

For **Photolytic stress** the samples were exposed to UV light at 288 nm for 54 hours and visible light for 288hours meeting the specification of ICH i.e. UV (200 watt/m^2) and Visible (1.2 million Lux hours).

For **Thermal degradation** samples were exposed to temperature at 105ºC for 24 hrs.

The Photolytic, Humidity and Thermal stress sample solutions were prepared to required concentration with the diluent.

2.2.7 Chromatographic Conditions

A Prontosil, C18 (150 x 4.6 mm; 5 µm packing) column was used for analysis at column temperature 40ºC. The mobile phase was pumped through the column at a flow rate of 1.0 mL/min.The sample injection volume was 20µL. The photodiode array detector was set to a wavelength of 264 nm for the detection.

RESULTS AND DISCUSSION

3.1 Method development

3.1.1 Separation of known degradation impurities

To develop a suitable rapid, rugged and robust LC method for the determination of Pramipexole, different mobile phases and columns were employed to achieve the best separation and resolution. The method development was started with a Peerless basic, C18 (150 x 4.6 mm; 5 µm packing) column using a mobile phase containing pH 3.0 buffer and Acetonitrile in the ratio 60:40 with 1.0 mL/min, where elution was found to be very broad. Early elution with slight separation was observed with mobile phase consisting of above components in the ratio 65:35. Finally the mobile phase with the ratio 72:28 was found to be appropriate with good separation and symmetrical peak shape to get Pramipexole peak RT about 3.9 min with 1.0 ml/min using Prontosil, C18 (150 x 4.6 mm; 5 µm packing) column (Figure-2). Under the last condition all related compounds were eluted with in 6 min and well separated. The chromatogram of Pramipexole sample spiked with the related impurities using the proposed method is shown in Figure -3. In the proposed method the resolution is more than 2 between the Pramipexole and impurity-A. System suitability results of the method are presented in Table 1. Pramipexole and its related compounds show significant UV absorbance at wavelength 264 nm. Hence this wavelength has been chosen for detection in the analysis of Pramipexole.

3.1.2 Column Selection [21-22]

Based on the retention and separation of the compounds Pramipexole, C18 (150 x 4.6 mm; 5 µm packing) column was selected as suitable column for the analysis of Pramipexole.

3.2 Method Validation [23-26]

The developed LC method was extensively validated for assay of Pramipexole dihydrochloride Tablets using
the following parameters.

3.2.1 SPECIFICITY

Placebo Interference

A study to establish the interference of placebo was conducted. Assay was performed on placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of placebo solutions showed no peaks at the retention time of Pramipexole peak. This indicates that the excipients used in the formulation do not interfere in the estimation of Pramipexole in Pramipexole dihydrochloride tablets.

Interference from degradation products

A study was conducted to demonstrate the effective separation of degradants from Pramipexole peak. Separate portions of Drug product, Drug substance and Placebo were exposed to the following stress conditions to induce degradation. Stressed samples were injected into the HPLC system with PDA detector by following test method conditions. All degradant peaks were resolved from Pramipexole peak in the chromatograms of all samples. The chromatograms of the stressed samples were evaluated for peak purity of Pramipexole using Empower 2 software. In all the forced degradation samples, Pramipexole peak purity angle was less than purity threshold. From the above results it is clear that the method can be used for determining the stability of Pramipexole as bulk and pharmaceutical formulations. Figure-4 shows the separation of Pramipexole from its degradation products.

3.2.2 Linearity of Detector Response

Linearity of detector response was established by plotting a graph to concentration versus average area and determining the correlation coefficient. A series of solutions of Pramipexole standard were prepared in the concentration range of 0.98 µg/mL to 89.7 µg/mL. A graph was plotted to concentration in µg/mL on the abscissa versus response on the ordinate. The detector response was found to be linear (Figure- 5) with a correlation coefficient of 0.999.

3.3.3) Precision of test Method

The precision of the test method was conducted by assaying six samples of Pramipexole dihydrochloride tablets. The Average % assay of Pramipexole in Pramipexole tablets was found to be 100.0% with an RSD of 0.6%. The results are given in Table -2.

3.3.4) Accuracy

A study of recovery of Pramipexole from spiked placebo was conducted at six different spike levels i.e. 6%25%,50%,75%,100% and 150%. Samples were prepared by mixing placebo with Pramipexole dihydrochloride monohydrate raw material equivalent to that of the target initial concentration of Pramipexole. Sample solutions were prepared in triplicate for each spike level and

assayed as per proposed method. The % recovery and correlation coefficient were calculated and given in Table- 3. The mean recoveries of Pramipexole from spiked were found to be in the range of 100.0-102.2%.

3.3.5) Ruggedness

A study to establish the stability of Pramipexole in standard and test solutions were conducted on bench top and Refrigerator at Initial, 1 day and 2 days. The assay of Pramipexole in standard and test solutions was estimated against freshly prepared standard each time. The difference in % assay of standard and test solutions from initial to 1 day and 2 days was calculated and given in Table- 4. From this study, it was established that the test preparation and standard are stable for a period of 2 days on bench top and Refrigerator.

3.3.6) Robustness

A study to establish the effect of variation in mobile phase organic phase composition, variation in mobile phase pH of buffer solution, variation in column oven temperature and variation in Flow rate was conducted. Standard and test solutions prepared as per proposed method were injected into HPLC system. The system suitability parameters and % assay were evaluated and given in Table-(5A-5D). From the above study the proposed method was found to be Robust.

Figure -2: Typical LC chromatogram of formulated pramipexole dihydrochloride tablets (1.5mg)

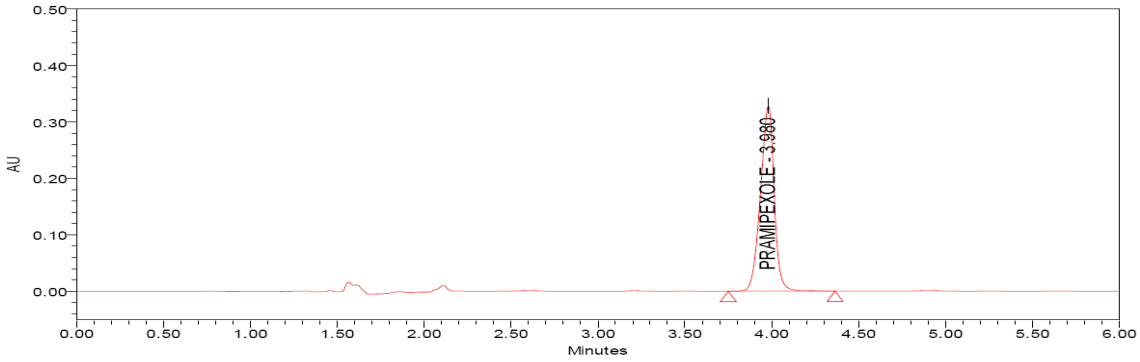


Figure -3 : Typical LC chromatogram of formulated pramipexole spiked with impurity

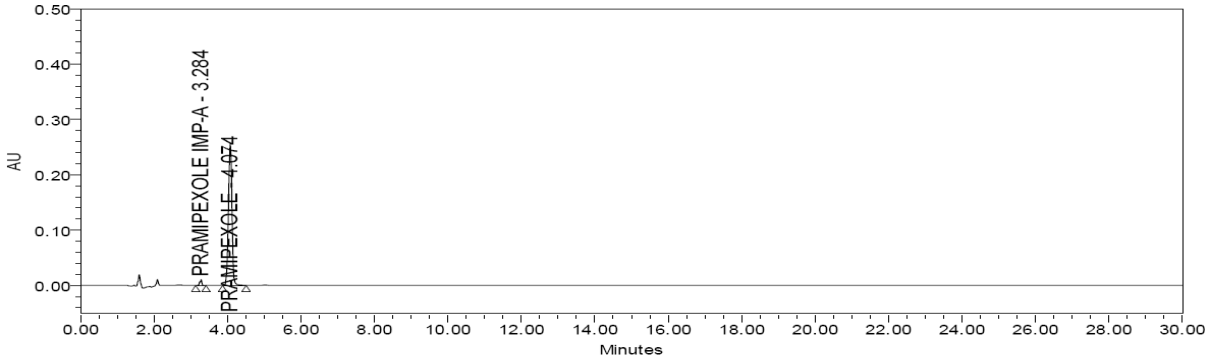
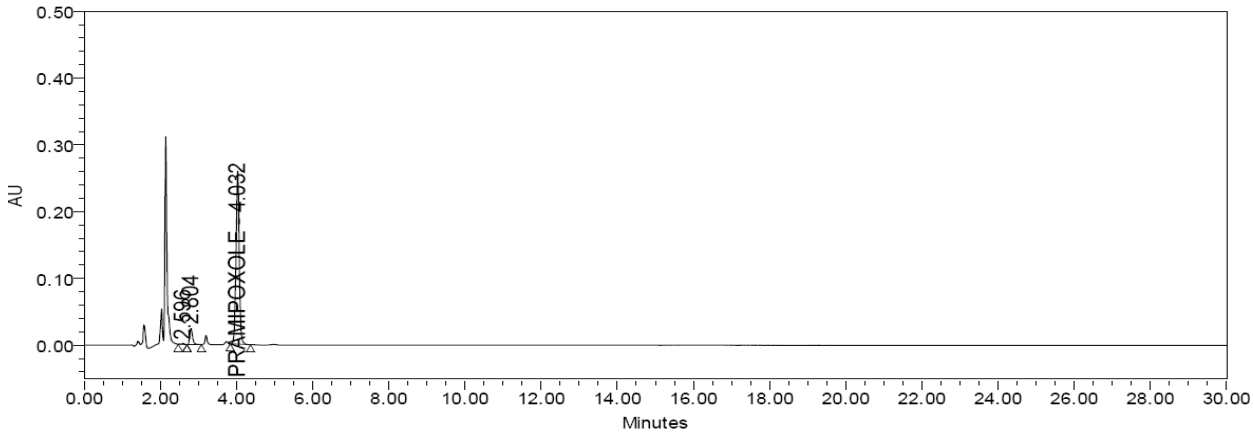
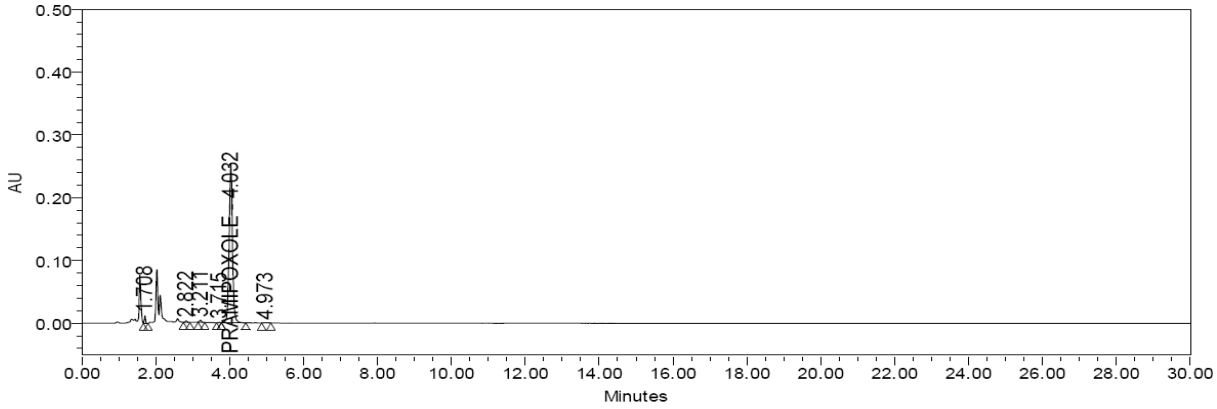


Figure -4: HPLC chromatograms of pramipexole and its degradation products

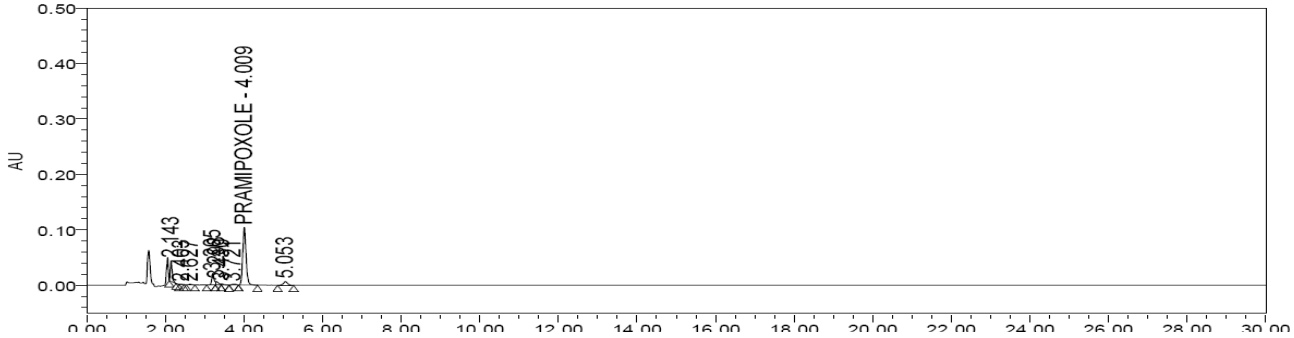
Acid Degradation



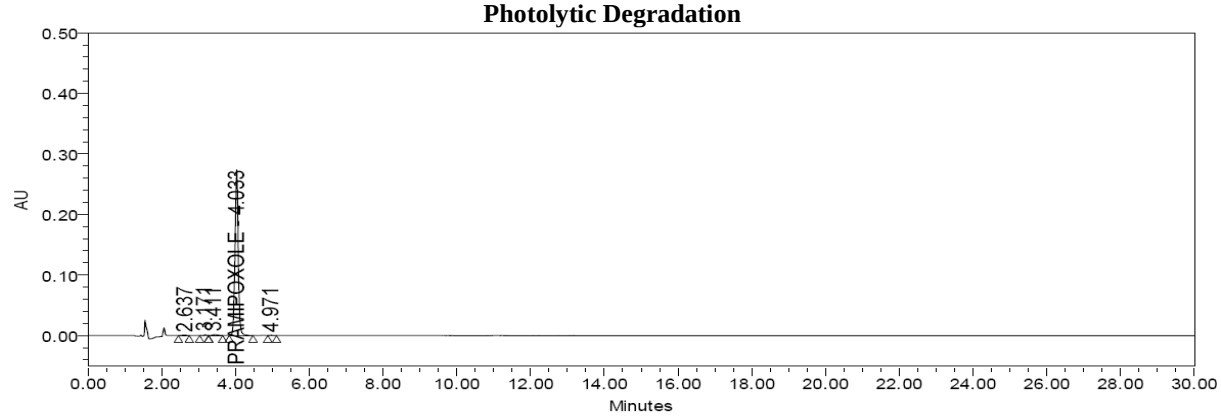
Alkali Degradation



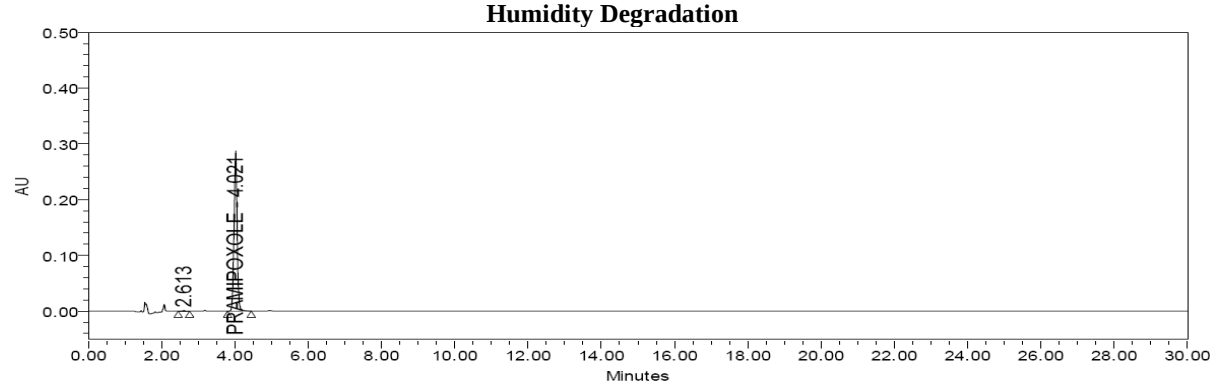
Oxydative Degradation



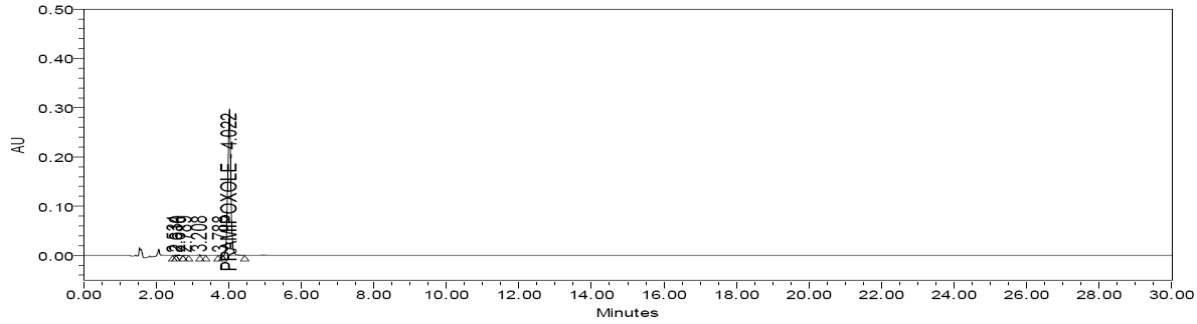
Photolytic Degradation



Humidity Degradation



Heat Degradation



Water Degradation

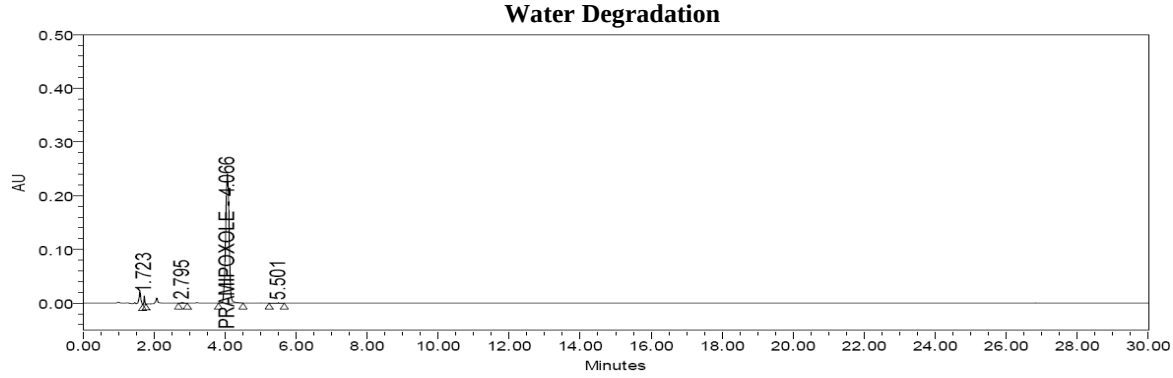


Table 1: system suitability report

Compound	Tailing Factor [a]	Theoretical plates[a]	Resolution[a]	%RSD[a]
Pramipexole	1.0	13025	4.5	0.1

[a] *Number of samples analyzed are six*

Table 2: results for precision of test method

Sample No.	%Assay
1	100.1
2	100.7
3	99.9
4	100.7
5	99.2
6	99.4
MEAN	**100.0**
%RSD	**0.6**

Table 3: accuracy in the assay determination of pramipexole

Sample No.	Spike level	'mg' added	'mg' found (recovered)	% Recovery	Average %recovery
1.	6%	0.76	0.76	100.0	100.0
2.		0.76	0.76	100.0	
3.		0.76	0.76	100.0	
4.	25%	3.01	3.03	100.7	100.7
5.		3.01	3.03	100.7	
6.		3.01	3.03	100.7	
7.	50%	6.01	6.03	100.3	100.2
8.		6.02	6.03	100.2	
9.		6.01	6.01	100.0	
10.	75%	9.02	9.04	100.2	100.4
11.		9.00	9.07	100.8	
12.		9.02	9.05	100.3	
13.	100%	12.02	12.28	102.2	102.2
14.		12.02	12.27	102.1	
15.		12.02	12.28	102.2	
16.	150%	18.03	18.15	100.7	100.9
17.		18.00	18.18	101.0	
18.		18.01	18.19	101.0	

Table 4: Ruggedness
Stability data of pramipexole in standard and test solutions

BENCH TOP STABILITY						
Time in Days	**% Assay of Standard preparation**	**Difference from Initial**	**% Assay of test preparation**		**Difference from Initial**	
			Test - 1	**Test - 2**	**Test - 1**	**Test – 2**
Initial	93.5*	NA	101.2	100.5	NA	NA
1	93.1	0.4	100.8	99.8	0.4	0.7
2	93.2	0.3	99.9	99.5	1.3	1.0
REFRIGERTOR STABILITY						
Time in Days	**% Assay of Standard preparation**	**Difference from Initial**	**% Assay of test preparation**		**Difference from Initial**	
			Test - 1	**Test - 2**	**Test - 1**	**Test – 2**
Initial	93.5*	NA	101.2	100.5	NA	NA
1	93.2	0.3	100.9	100.2	0.3	0.3
2	93.1	0.4	100.3	100.8	0.9	0.3

** Potency of Pramipexole on as is basis*

Table 5: Robustness data of pramipexole in test solutions

TABLE: 5A

Effect of variation in Mobile phase composition(Acetonitrile)					
System Suitability	**Organic phase ratio (Acetonitrile)**				
	100%	90%	110%	**Acceptance criteria**	
USP Tailing factor for Pramipexole Peak	0.9	1.0	1.1	NMT 2.0	
USP Plate count for Pramipexole Peak	13094	13852	12438	NLT 2500	
%RSD of Pramipexole peak area from five replicate injections of standard solution	0.1	0.2	0.1	NMT 2.0%	
Organic phase ratio (Acetonitrile)	**% Assay of test preparation**		**Avg. Assay**	**Difference**	
	Trail - 1	**Trail - 2**			
100%(Organic phase)	100.6	101.0	100.8	NA	
90%(Organic phase)	100.5	99.9	100.2	0.6	
110%(Organic phase)	100.0	99.8	99.9	0.9	

TABLE: 5B

Effect of variation in pH of Mobile phase					
System Suitability	**Variation in buffer solution pH**				
	pH 2.8	pH 3.0	pH 3.2	**Acceptance criteria**	
USP Tailing factor for Pramipexole Peak	1.1	1.0	1.0	NMT 2.0	
USP Plate count for Pramipexole Peak	9749	10156	11427	NLT 2500	
%RSD of Pramipexole peak area from five replicate injections of standard solution	0.2	0.4	0.5	NMT 2.0%	
Variation in buffer solution pH	**% Assay of test preparation**		**Avg. Assay**	**Difference**	
	Trail - 1	**Trail - 2**			
pH 3.0	100.6	99.4	100.0	NA	
pH 2.8	100.1	100.6	100.4	0.4	
pH 3.2	99.8	100.2	100.0	0.0	

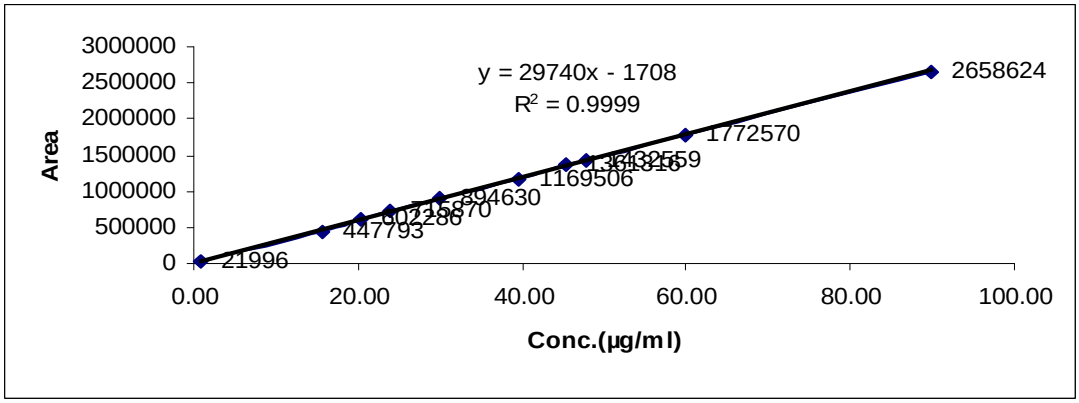
TABLE: 5C

Effect of variation in Flow rate					
System Suitability	**Variation in Flow rate (mL/min)**				
	0.8	1.0	1.2	**Acceptance criteria**	
USP Tailing factor for Pramipexole Peak	1.1	0.9	1.0	NMT 2.0	
USP Plate count for Pramipexole Peak	12051	12975	11366	NLT 2500	
%RSD of Pramipexole peak area from five replicate injections of standard solution	0.1	0.8	0.6	NMT 2.0%	
Variation in Flow rate (mL/min)		**% Assay of test preparation**		**Avg. Assay**	**Difference**
		Trail - 1	**Trail - 2**		
1.0		99.9	100.3	100.1	NA
0.8		100.5	99.5	100.0	0.1
1.2		100.2	99.8	100.0	0.1

TABLE: 5D

Effect of variation in Column oven temperature					
System Suitability	**Variation in Column oven temperature**				
	40°C	35°C	45°C	**Acceptance criteria**	
USP Tailing factor for Pramipexole Peak	0.9	1.1	1.0	NMT 2.0	
USP Plate count for Pramipexole Peak	11151	11957	12530	NLT 2500	
%RSD of Pramipexole peak area from five replicate injections of standard solution	0.5	0.4	0.2	NMT 2.0%	
Variation in Column oven temperature		**% Assay of test preparation**		**Avg. Assay**	**Difference**
		Trail - 1	**Trail - 2**		
40°C		99.6	100.2	99.9	NA
35°C		100.1	99.3	99.7	0.2
45°C		99.4	99.8	99.6	0.3

Figure 5: linearity of detector response graph



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REFERENCES

[1] KS Wong, CS Lu, DE Shan, CC Yang, Journal of the, **2003**.
[2] S.C.Sweetman., Ed., Martindale-The complete Drug Reference", 33rd Edn. Pharmaceutical Press, London (UK), **2002**, 1176
[3] G. Srinubabu, K. Jaganbabu, B. Sudharani, K. Venugopal, G. Girizasankar and J. V. L. N. S. Rao **,** *Chromatographia* ,Volume 64, Numbers 1-2, 95-100, DOI: 10.1365/s10337-006-0829-3
[4] *Biomedical Chromatography*, Volume 23, Issue 2, pages 212–218, February **2009**.
[5] D.B. Pathare, A.S. Jadhav and M.S. Shingare, Department of Chemistry, Dr. B.A. Maratha Wada University, Aurangabad 431 004, India, Received 11 January **2006**;
[6] *Journal of Pharmaceutical and Biomedical Analysis*, Volume 41, Issue 4, 16 June **2006**, Pages 1152-1156, D.B. Pathare, A.S. Jadhav and M.S. Shingare
[7] Srinivas vishnumulaka, Narasimha Rao medicherla, Allam apparao and Gedelasrinibabu, *E-Journal of Chemistry*, Vol. 5, No. 1, pp. 58-67, January **2008**
[8] Vedavati M. Panditrao, Aniket P. Sarkate, Jaiprakash N. Sangshetti, Pravin S. Wakte and Devanand B. Shinde, *J. Braz. Chem. Soc.*, Vol. 00, No. 00, 1-6, **2011**.
[9] B.M. Gurupadayya, V. Vishwajith and N. Srujana, *World Journal of Chemistry* 4 (2): 157-160, **2009**.
[10] Syeda Humaira , Akalanka Dey, S. Appala Raju, Syed Sanaullah .*Der Pharmacia Lettre*, **2010**, 2(4): 315-325
[11] Wakode Rajeshri1and Amrita Bajaj, *J. Chem. Pharm. Res.*, **2010**, *2(2): 136-146*
[12] C.vinodhini, *International Journal of Research in Pharmaceutical and Biomedical Sciences*, ISSN: 2229-3701
[13] Indian Pharmacopeia, Vol I, II, 1996 & Addendum **2000** and Vet, Government of India, Ministry of Health and Family Welfare, Controller of Publications, Delhi, **2000**.
[14] British Pharmacopeia, Vol. I & II and Vet 1993, HMSO, London, **1999**.
[15] United States Pharmacopeia USP XXIV & NF IXX, 2000 and 4 Addendum, USP convention Inc., Rockville, **2002**.
[16] European Pharmacopeia, 3rd Edition, **1997** and Supplement, Council of Europe, Strasbourg, 1999.
[17] Japan Pharmacopoeia, XIIIth Edition, **1996**.
[18] Chattan, L.G., Ed., Pharmaceutical Chemistry, Vol I & II, Moral Dekker Inc, New York, **1966**.
[19] Lachman, L., Liberman, H.A., and Kaning, J.L., The Theory and Practice of Industrial Pharmacy, 2nd Edn. London.
[20] The Drugs and Cosmetics Act and Rules, Government of India Publications, **1984**.
[21] The Merck Index, 12th Edition, Merck & Co Inc, New York, **1996**.
[22] The Merck index, 13th edition**,** Merck Research Laboratories., Division of Merck and CO, INC**,** White House station, NJ, **2003**
[23] ICH stability testing of new drug substances and products (Q1AR2), International Conference on Harmonization, IFPMA, Geneva, **2003**.
[24] ICH Draft Guidelines on Validation of Analytical Procedures: Text and methodology (Q2R1), IFPMA, Switzerland, **1995**
[25] Leenata P.Mandpe and Varsha B.Pokharkar ,*Der Chemica Sinica*, **2011**, 2 (2): 230-239
[26] Joshi Pryanka and Kumar Mukesh ,*Der Pharmacia Sinica*, **2011**, 2 (3): 211-219