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Chemistry

Established New HPLC Method for Cleaning Validation of Pramipexol Dihydrochloride Monohydrate Active Pharma Ingredient

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Original Research Article

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Abstract: The analytical method has been developed to evaluate the efficacy of the cleaning procedure of all the equipment involved in the production of final active ingredients. The choice of the methodology is based upon the production method and on the intrinsic properties of the products. For this validation HPLC method has been chosen. The HPLC chromatographic separations were achieved on (100×4.6 mm), 3.5µm, column make: Phenomenex, employing acetonitrile and 0.4% orthophosphoric acid aqueous solution in the ratio of 35:65 as mobile phase with flow rate 1.0 mL/min was chosen. The column temperature was maintained at 25^{o}C and a detector wavelength of 254 nm was employed. The method was successfully validated by establishing System Suitability, Specificity, Linearity, Accuracy, and limit of detection and Limit of quantification for Pramipexol Dihydrochloride Monohydrate.

Keywords: HPLC, cleaning validation, Pramipexol Dihydrochloride Monohydrate, LOQ, LOD.

INTRODUCTION

Pramipexole is a is a non-ergoline dopamine agonist indicated for treating early-stage Parkinson's disease (PD) and restless legs syndrome [1,2]. Chemically it is (S)-2-amino-4,5,6,7-tetrahydro-6- (propylamino)benzothiazole. Pramipexole is a none-argot dopamine agonist with high relative in vitro specificity and full intrinsic activity at the D2 subfamily of dopamine receptors, binding with higher affinity to D_3 than to D2 or D4 receptor subtypes.

Pramipexole dihydrochloride, a non-ergot dopamine agonist approved in the US 1997, is used as an antidyskinetic for treatment of Parkinson's disease [4, 5]. It is also sometimes used off-labelas a treatment forcluster headacheand to counteract the problems with sexual dysfunction experienced by some users of theselective, serotonin reuptake inhibitor (SSRI) antidepressants. Pramipexole is a category of Non ergot dopamine receptor agonist [3,4]. It can improves the ability to move and decrease shakiness (tremor), stiffness, slowed movement, and unsteadiness. The molecular formula is C10H17N3S.2HCl.H2O. This corresponds to a molecular weight of 302.2 g/mol [5, 6].

Cleaning validation is documented proof with high measure of assurance that one can always clean a system or piece of equipment to predetermined and suitable limits [7]. Cleansing validation is especially applicable to the cleansing of method manufacturing apparatus in pharmaceutical enterprise. It is integral to have effective cleaning programs in place because of regulatory requirements [8]. Cleansing is among the imperative strategies in pharmaceutical manufacturing. Equipment contamination may just come from any of the substances which have been in contact with the equipment surfaces [9,10]. It is crucial to restrict carryover of trace quantities of either active or different substances from one batch to yet another in order to preclude go-illness of the following product Consequently[11-13], equipment used in pharmaceutical manufacturing has got to be cleaned meticulously, and the cleansing approach used ought to be validated. In the pharmaceutical enterprise, just right Manufacturing Practices (GMP) require that the cleaning of drug manufacturing equipment be validated [14]. Many unique validation methods can exhibit that the manufacturing gear is cleaned and just about free from residual energetic drug components and all cleaning agents [15, 16]. Common analytical procedures in the validation procedure incorporate HPLC, spectrophotometry (UV/Vis) and TOC. HPLC and UV/Vis are categorized specific methods that identify and measure appropriate active and substances.

In the present study, a novel HPLC method was developed and successfully validated for Pramipexol Dihydrochloride Monohydrate. As on date, there were no research articles for cleaning validation of Pramipexol Dihydrochloride Monohydrate.

MATERIALS AND METHODS

Standards, reagents and samples

The analytical standard of Pramipexol Dihydrochloride (99.8%) was obtained from Sigma Aldrich. The HPLC grade solvents i.e., Ortho phosphoric acid and acetonitrile were purchased from Rankem, New Delhi.

Experimental

HPLC Chromatographic Parameters

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 100 mm x 4.6 mm and particle size 3.5 µm (Phenomenex) Column oven temperature was maintained at 25°C. The injected sample volume was 20µL. Mobile Phases A and B was Acetonitrile and 0.4% ortho phosphoric acid (35:65 (v/v)). The flow- rate used was kept at 1.0 mL/min with a detector wavelength at 254 nm. The retention time of Pramipexol Dihydrochloride Monohydrate about 4.2 min.

Method Validation

Method validation ensures analysis credibility. In this study, the parameters Specificity and Selectivity, linearity, precision, accuracy, Limits of Detection (LOD) and Quantification (LOQ) were considered. The accuracy of the method was determined is to verify the recovery and the release efficacy of the swabs and rinse used in the cleaning operation. Linearity was determined by different known concentrations (2.5, 5.0, 10.0, 15.0 and 20.0 µg/mL) which were prepared by diluting the stock solution. The Limit of Detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control sample. The Limit of Quantification (LOQ, µg/mL) was determined as the lowest concentration of given Pramipexol Dihydrochloride Monohydrate giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSIONS

Specificity; Selectivity

Procedure

To demonstrate the discrimination of the analyte in presence of others. Test samples containing each analyte then test sample without analyte (blank).

Take 10mg of each product in each 100ml volumetric flask and bring to volume with methanol. Take 10ml of each solution in each 100ml volumetric flask and bring to volume with methanol. Separately, inject once 20µl of each solution.

Selectivity

Take 10 ml of each solution in a 100 ml volumetric flask end bring to volume to 100 ml with methanol. (This solution contains 10 ppm of each substance).

Inject six times 20µl of this solution.

Since one product is utilized for this validation, six results of precision were used instead.

Linearity

Procedure

The linearity was determined according to the ICH guidelines. The chosen concentration as 100% was 10 µg/ml of each product. The scheme carried out was the following:

Dilution scheme:	sample weight in 100ml	Solution A
	1ml solution A in 100ml	Solution B

Test solution

25% solution

Take 25mg of each product in a 100ml volumetric flask and bring to volume with methanol. (Sol A).Take 1ml in a 100ml volumetric flask and bring to volume with methanol.

50% solution

Take 50mg of each product in a 100ml volumetric flask and bring to volume with methanol (Sol A1). Take 1ml in a 100ml volumetric flask and bring to volume with methanol.

100% solution

Take 100mg of each product in a 100ml volumetric flask and bring to volume with methanol. (Sol A2) Take 1ml in a 100ml volumetric flask and bring to volume with methanol.

150% solution

Take 150mg of each product in a 100ml volumetric flask and bring to volume with methanol. (Sol A3) Take 1ml in a 100ml volumetric flask and bring to volume with methanol.

200% solution

Take 200mg of each product in a 100ml volumetric flask and bring to volume with methanol. (Sol A4) Take 1ml in a 100ml volumetric flask and bring to volume with methanol.

The linearity solutions were injected thrice and détails were given Table 1 and représentative chromatogram

was showéd in Figure. 1.

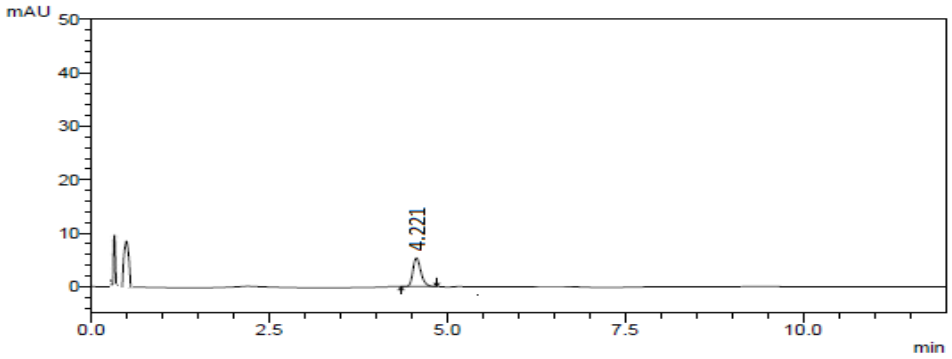

Fig-1: Representative chromatogram of linearity standard solution

Table-1: Linearity details of pramipexol dihydrochloride monohydrate

SET	Percent%	Weight (mg)	Area Injection 1	Normalized Area	AV. peak area	RSD %
1	25%	25.2	112386	111494	113910	2.29
2		25.2	114471	113563		
3		24.9	116206	116673		
1	50%	53.6	184243	171868	179596	3.73
2		50.0	183803	183803		
3		50.1	183484	183118		
1	100%	100.0	336253	336253	335177	0.39
2		100.0	335534	335534		
3		100.2	334411	333744		
1	150%	143.8	472722	493104	479895	2.43
2		150.0	475557	475557		
3		150.2	471652	471024		
1	200%	200.2	623123	622500	626995	0.74
2		200.1	626991	626678		
3		200.1	632124	631808		

Slope	29509
Intercept	37271
R^2	1.00

Precision: Repeatability

This was determined on 6 different solutions having a concentration of 10µg/ml of each product (100%).

Dilution Scheme: 100mg in 100ml Solution A
1ml solution A in 100ml Solution B

Precision Solution

Take 100mg of each product in a 100ml volumetric flask and bring to volume with methanol. Take 1ml in a 100ml volumetric flask and bring to volume with methanol. The détails were given Table 2.

Table-2: Repeatability detials of pramipexol dihydrochloride monohydrate

Injection	Weight (mg)	factor N	area	Area N	Date
1	100.1	0.9990	1222456	1221235	10/08/2018
2	100.1	0.9990	1219156	1217938	
3	99.9	1.0010	1221557	1222780	
4	100	1.0000	1220371	1220371	
5	99.9	1.0010	1217157	1218375	
6	100.1	0.9990	1217690	1216474	
Average		1226147			
s		5099.9			
RSD %		0.42%			
Confidence		4081			

Precision: Intermediate

This was determined on 6 different solutions having a concentration of 10µg/ml of each product, performed on different days and using fresh mobile phase.

Dilution scheme: 100mg in 100ml olution A
1ml solution A in 100ml Solution B

Precision Solution

Take 100mg of each product in a 100ml volumetric flask and bring to volume with methanol. Take 1ml in a 100ml volumetric flask and bring to volume with methanol. The Intermediate détails were given Table 3 and Table 4.

Table-3: The intermediate details of pramipexol dihydrochloride monohydrate on first day

Injection	Weight (mg)	factor N	area	Area N	Date
1	99.9	1.0010	317596	317914	12/108/2016
2	100.1	0.9990	322522	322200	
3	100.2	0.9980	320552	319912	
4	99.8	1.0020	322662	323309	
5	99.8	1.0020	322549	323195	
6	100.2	0.9980	322230	321587	

Table-4: The intermediate details of pramipexol dihydrochloride monohydrate on second day

Injection	Weight(mg)	factor N	area	Area N	Date
1	100.1	0.9990	1222456	1221235	110/08/2016
2	100.1	0.9990	1219156	1217938	
3	99.9	1.0010	1221557	1222780	
4	100	1.0000	1220371	1220371	
5	99.9	1.0010	1217157	1218375	
6	100.1	0.9990	1217690	1216474	
7	100.1	0.9990	1222456	1221235	11/08/2016
8	100.1	0.9990	1219156	1217938	
9	99.9	1.0010	1221557	1222780	
10	100	1.0000	1220371	1220371	
11	99.9	1.0010	1217157	1218375	
12	100.1	0.9990	1217690	1216474	
Average		329892			
s		9744			
RSD %		2.95%			
confidence		5513			

Accuracy

The purpose of determining accuracy is to verify the recovery and the release efficacy of the swabs and rinse used in the cleaning operation. The determination of the recovery factor is obtained using the following Scheme:

- Transfer a known quantity of product, possibly dissolved in a volatile solvent, upon a surface which is similar to that used in the production plant. It is important to take care to distribute the product homogenously on the surface.
- Carefully eliminate the solvent from the surface, to prevent loss of product from the surface.
- Proceed to the mechanical cleaning of the surface (swab) or rinse as is described in the protocol using the identified solvent.
- For standard solutions one may use the means of peak areas obtained in Precision-Intermediate precision results.
- Extract with the swabs and determine the quantity of substance removed according to the analytical method. The percentage recovery obtained represents the recovery factor of the solvent to be used in the final calculation of the residual quantity of substance present in the equipment used for synthesis.
- Repeat in triplicate the operation described with all surfaces with which product has come in contact.

Solution to be used: Use 1 ml of each solutions (Sol A1 50%; Sol A2 100%; Sol A3 150%) prepared for the determination of linearity at 50%, 100%, 150%. The Swab and Rinse details were given Table 5 and Table 6.

Table-5: Swab table

%	Mg Product	Volume	ml deposited	Volume extracted	Theoretic µg/ml
50%	50	100	1	100	5
100%	100	100	1	100	10
150%	150	100	1	100	15

Table-6: Rinse table

%	Mg Product	Volume	ml deposited	Volume extracted	Theoretic µg/ml
50%	50	100	1	100	5
100%	100	100	1	100	10
150%	150	100	1	100	15

Table-7: Pramipexol dihydrochloride monohydrate swab - glass lined

	50%	100%	150%		
Weight (mg)	53.6	100.0	143.8		
Total dilution	100	100	100		
µg/ mL	5.36	10.00	14.38		
µg deposited	536	1000	1438		
Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery%	AV recovery%
50% A	5.36	172733	5.236	97.687	95.195
100% A	10.00	311244	9.435	94.347	
150% A	14.38	443793	13.453	93.551	
50% B	5.36	175245	5.312	99.108	94.573
100% B	10.00	309127	9.371	93.706	
150% B	14.38	431241	13.072	90.905	
50% C	5.36	171119	5.187	96.775	94.815
100% C	10.00	312418	9.470	94.703	
150% C	14.38	441027	13.369	92.968	
Mean recovery:			94.86%		
RSD recovery:			0.33%		

Accuracy: Glass lined

50% solution

Swab - Take 1 ml of solution A1. Extract the swab with 100 ml of methanol
Rinse - Take 1 ml of solution A1. Rinse with 100 ml of methanol

100% solution

Swab - Take 1 ml of solution A2. Extract the swab with 100 ml of methanol
Rinse - Take 1 ml of solution A2. Rinse with 100 ml of methanol

150% solution

Swab - Take 1ml of solution A3. Extract the swab with 100 ml of methanol
Rinse - Take 1ml of solution A3. Rinse with 100 ml of methanol
The Swab and Rinse recovery details were given Table 7 and Table 8.

Table-8: Pramipexol dihydrochloride monohydrate rince - glass lined

	50%	100%	150%		
Weight (mg)	53.6	100.0	143.8		
Total dilution	100	100	100		
µg/ mL	5.36	10.00	14.38		
µg deposited	536	1000	1438		
Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery%	AV recovery%
50% A	5.36	184363	5.589	104.265	101.845
100% A	10.00	336777	10.209	102.087	
150% A	14.38	470514	14.263	99.184	
50% B	5.36	182657	5.537	103.300	101.115
100% B	10.00	333592	10.112	101.122	
150% B	14.38	469283	14.225	98.925	
50% C	5.36	183455	5.561	103.751	101.104
100% C	10.00	331653	10.053	100.534	
150% C	14.38	469762	14.240	99.026	
Mean recovery:			101.35%		
RSD recovery:			0.42%		

Accuracy: Steel

50% solution

Swab - Take 1 ml of solution A1. Extract the swab with 100 ml of methanol
Rinse - Take 1 ml of solution A1. Rinse with 100 ml of methanol

100% solution

Swab - Take 1 ml of solution A2. Extract the swab with 100 ml of methanol
Rinse - Take 1 ml of solution A2. Rinse with 100 ml of methanol

150% solution

Swab - Take 1ml of solution A3. Extract the swab with 100 ml of methanol
Rinse -Take 1ml of solution A3. Rinse with 100 ml of methanol
The Swab and Rinse recovery details were given Table 9 and Table 10.

Table-9: Pramipexol dihydrochloride monohydrate swab - steel

	50%	100%	150%		
Weight (mg)	53.6	100.0	143.8		
Total dilution	100	100	100		
µg/ mL	5.36	10.00	14.38		
µg deposited	536	1000	1438		
Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery%	AV recovery%
50% A	5.36	171768	5.207	97.142	94.213
100% A	10.00	307975	9.336	93.356	
150% A	14.38	437100	13.250	92.140	
50% B	5.36	171481	5.198	96.979	97.782
100% B	10.00	311265	9.435	94.354	
150% B	14.38	483938	14.670	102.014	
50% C	5.36	194529	5.897	110.014	103.579
100% C	10.00	309427	9.380	93.796	
150% C	14.38	507249	15.376	106.928	
Mean recovery			98.52%		
RSD recovery			4.80%		

Table-10: Pramipexol dihydrochloride monohydrate rinse – steel

	50%	100%	150%		
Weight (mg)	53.6	100.0	143.8		
Total dilution	100	100	100		
µg/ mL	5.36	10.00	14.38		
µg deposited	536	1000	1438		
Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery%	AV recovery%
50% A	5.36	184077	5.580	104.103	101.465
100% A	10.00	334932	10.153	101.528	
150% A	14.38	468529	14.202	98.766	
50% B	5.36	186793	5.662	105.639	101.971
100% B	10.00	334352	10.135	101.352	
150% B	14.38	469276	14.225	98.923	
50% C	5.36	185139	5.612	104.704	101.663
100% C	10.00	333552	10.111	101.109	
150% C	14.38	470477	14.262	99.176	
Mean recovery:			101.70%		
RSD recovery:			0.25%		

Accuracy: Rubber

50% solution

Swab - Take 1 ml of solution A1. Extract the swab with 100 ml of methanol
Rinse - Take 1 ml of solution A1. Rinse with 100 ml of methanol

100% solution

Swab - Take 1 ml of solution A2. Extract the swab with 100 ml of methanol
Rinse - Take 1 ml of solution A2. Rinse with 100 ml of methanol

150% solution

Swab - Take 1ml of solution A3. Extract the swab with 100 ml of methanol
Rinse - Take 1ml of solution A3. Rinse with 100 ml of methanol
The Swab and Rinse recovery details were given Table 11 and Table 12.

Table-11: Pramipexol dihydrochloride monohydrate swab – rubber

	50%	100%	150%		
Weight (mg)	53.6	100.0	143.8		
Total dilution	100	100	100		
µg/ mL	5.36	10.00	14.38		
µg deposited	536	1000	1438		
Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery %	AV recovery%
50% A	5.36	172139	5.218	97.352	94.164
100% A	10.00	311350	9.438	94.379	
150% A	14.38	430563	13.052	90.762	
50% B	5.36	175714	5.326	99.373	96.434
100% B	10.00	310125	9.401	94.008	
150% B	14.38	455034	13.793	95.921	
50% C	5.36	184342	5.588	104.253	96.972
100% C	10.00	310505	9.412	94.123	
150% C	14.38	438993	13.307	92.539	
Mean recovery:			95.86%		
RSD recovery:			1.55%		

Table-12: Pramipexol dihydrochloride monohydrate rinse – rubber

	50%	100%	150%
Weight (mg)	53.6	100.0	143.8
Total dilution	100	100	100
µg/ mL	5.36	10.00	14.38
µg deposited	536	1000	1438

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery %	AV recovery%
50% A	5.36	183353	5.558	103.693	101.132
100% A	10.00	333760	10.117	101.173	
150% A	14.38	467410	14.169	98.530	
50% B	5.36	184549	5.594	104.370	101.660
100% B	10.00	334800	10.149	101.488	
150% B	14.38	470216	14.254	99.121	
50% C	5.36	183778	5.571	103.934	101.291
100% C	10.00	335427	10.168	101.678	
150% C	14.38	466140	14.130	98.262	
Mean recovery:			101.36%		
RSD recovery:			0.27%		

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The limit of quantification is at least 1 ppm. Dilute 10 ml of linearity solution A at 100% in 100 ml of methanol. Inject six times 20µl of this solution. The LOQ and LOD details were given in Table 13 and Table 14 and représentative LOQ chromatogram was showed in Figure 2.

Table-13: Loq details of pramipexol dihydrochloride monohydrate

SET	Area	Found(ppm)	Recovery %
1	33412	1.013	101.282
2	33872	1.027	102.676
3	35352	1.072	107.162
4	34064	1.033	103.258
5	33319	1.010	101.000
6	33808	1.025	102.482
Average	33971.17	1.03	102.98
Std Dev	733.45	0.02	2.22
RSD	2.16%	2.16%	2.16%

Table-14: Lod détails of pramipexol dihydrochloride monohydrate

Sample	Area	Found (ppm)	Recovery %
Pramipexol Dihydrochloride Monohydrate	10235	0.27	98.64

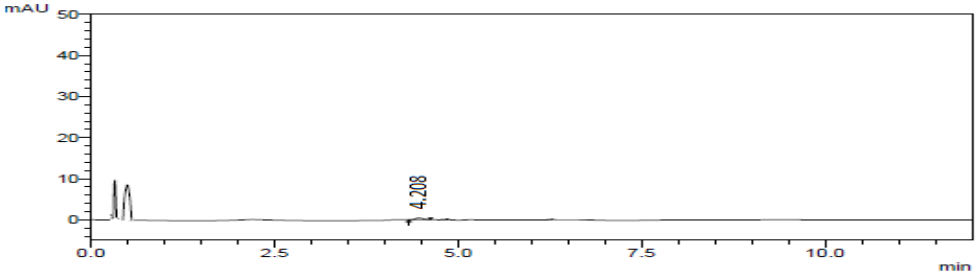

Fig-2 : LOQ Level chromatogram of Pramipexol Dihydrochloride Monohydrate

The limit of detection is at least 0.25 µg/mL.
Inject 5 µl of solution used for the limit of quantification.

Calculations

The quantity of the Active Ingredient is determined according to the sampling procedure. The assay of the Active Ingredient is calculated by comparing the peak area, applying the formulas:

Rinse

$$\frac{Ac * C}{As} = \text{ug/mL in wash}$$

Where

Ac: area in sample solution
As: area in standard solution
C: concentration solution standard (µg/mL)
Calculation µg/mL in product based on rinse

ug/mL product*V	= ppm Active ingredient
1000*Kg (prod)	

Where:

V: volume total solvent rinse (L)
Kg : Quantity in Kg of successive product
1000: Conversion Factor

Swab:

(Ac – Ab)*C x Vestr	=ug/cm^2 in swab
As x St	

Where

Ac: area in sample solution
Ab: area blank extracted with swab
As: area in standard solution
C: concentration standard solution(µg/ml)
Vestr: extraction solvent (ml)
St: sampled surface (cm^2)

Calculation ppm in product based on swab

ug/cm^2 product*S	=ppm Active ingredient
1000* kg(prod) *R	

Where,

S: total surface of employed plant (cm^2)
kg: Quantity in Kg of successive product
1000: conversion factor
R : recovery factor

CONCLUSIONS

The method developed for quantitative determination of Pramipexol Dihydrochloride Monohydrate residues in clean samples the method was completely validated showing satisfactory data for all method - validated parameters tested. The mobile phase composition of acetonitrile and 0.4% H3PO4 in water showed good separation and resolution. Satisfactory validation parameters such as linearity, recovery, precision LOD and LOQ were established by following ICH guidelines [16]. Therefore, the proposed analytical procedure could be useful for regular monitoring, pharma manufacturing labs and researchers.

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