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Novel simultaneous HPLC Method for Cleaning Validation of Four API Drugs

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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 0.7 mL/min was chosen. The column temperature was maintained at 30^{o}C and a detector wavelength of 220 nm was employed. The method was successfully validated by establishing System Suitability, Specificity, Linearity, Accuracy, Limit of Detection and Limit of quantification for Ziprasidone HCl, Perindopril Erbumine, Venlafaxine HCl and Imiquimod.
Keywords: HPLC, cleaning validation, Ziprasidone HCl, Perindopril Erbumine, Venlafaxine HCl, Imiquimod.

INTRODUCTION

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 [1]. 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 [2]. 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 [3, 4]. 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 (5, 6]. Consequently, 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. 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 [7-9]). 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 simultaneous HPLC method was developed, successfully quentification of Ziprasidone HCl, Perindopril Erbumine, Venlafaxine HCl, Imiquimod. As on date, there were no research articles for cleaning validation of Ziprasidone HCl, Perindopril Erbumine, Venlafaxine HCl, Imiquimod.

MATERIAL AND METHODS

Standards, reagents and samples

The analytical standard of Ziprasidone HCl , Perindopril Erbumine, Venlafaxine HCl and Imiquimod was obtained from Sigma Aldrich. The HPLC grade solvents i.e., Ortho phosphoric acid and acetonitrile were purchased from Rankem, New Delhi.

Experimental Conditions

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 30°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 0.7 mL/min with a detector wavelength at 220 nm. The retention time of Ziprasidone HCl, Perindopril Erbumine, Venlafaxine

HCl and Imiquimod were about 3.5, 4.1, 5.1 and 6.4 min. respectively .

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 (25%, 50%, 100%, 150% and 200%) 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 Ziprasidone HCl, Perindopril Erbumine, Venlafaxine HCl and Imiquimod, 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).

Weighed about 10mg of each product (Ziprasidone HCl, Perindopril Erbumine, Venlafaxine HCl and Imiquimod) into separate four 100ml volumetric flask, dissolve and diluted to the volume with methanol. Take 10ml of each solution from 100ml volumetric flask, transferred in to 100 ml volumetric flask and brought to volume with methanol. Separately, inject once 20µl of each solution.

Selectivity

10 ml of each solution was taken in a 100 ml volumetric flask and brought to volume to 100 ml with methanol. (This solution contains 10 ppm of each substance).

Injected six times 20µl of this solution.

Linearity

Procedure

The linearity was determined according to the ICH guidelines [10]. 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

Weighed about 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 brought to volume with methanol.

50% solution

Weighed about 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 brought to volume with methanol.

100% solution

Weighed about 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 brought to volume with methanol.

150% solution

Weighed about 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 brought to volume with methanol.

200% solution

Weighed about 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 brought to volume with methanol.

The linearity solutions were injected thrice and détails were given Table 1 and représentative chromatogram was showéd in Figure. 1.

Table-1: LINEARITY DETIALS

	AVERAGE AREAS OF			
Percent	Ziprasidone HCl	Perindopril Erbumine	Venlafaxine HCl	Imiqumod
25	**402853**	**50729**	**57022**	**195077**
50	**761112**	**99399**	**113195**	**387421**
100	**1529998**	**221021**	**202393**	**771512**
150	**2275270**	**337330**	**318479**	**1187160**
200	**2931822**	**445281**	**428384**	**1602450**

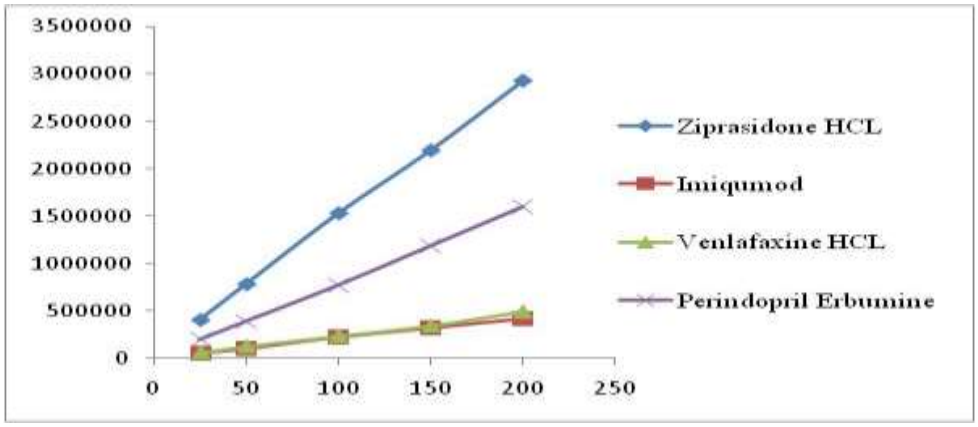

Fig-1: Representative chromatogram of linearity standard solution

Precision

Preparation of Precision Solution

Weighed about 100mg of each product in a 100ml volumetric flask and brought to volume with methanol. 1ml of this solution taken in a 100ml volumetric flask and brought to volume with methanol. The solutions were injected into a HPLC. The precision was determined on 6 different solutions having a concentration of 10µg/ml of each The Intermediate precision détails were given **Table 2.**

Table-2: PRECISON DETIALS

Injection	Weight (in mg)	Factor N	Area	Area N	Average ± S.D (% RSD)
ZIPRASIDONE HCl					
1	99.9	1.0010	1505585	1507092	1512601 ± 4944 (0.33)
2	100.2	0.9980	1516257	1513231	
3	100	1.0000	1517938	1517938	
4	99.7	1.0030	1513584	1518138	
5	100.2	0.9980	1515304	1512279	
6	99.9	1.0010	1505418	1506925	
PERINDOPRIL ERBUMINE					
1	100	1.0000	210619	210619	209838 ± 3333 (1.59)
2	99.9	1.0010	215784	216000	
3	99.9	1.0010	206935	207142	
4	99.8	1.0020	209177	209596	
5	99.9	1.0010	208525	208734	
6	100.2	0.9980	207352	206938	
VENLAFAXINE HCl					
1	100.1	0.9990	199286	199087	204077 ± 2898 (1.42)
2	105.4	0.9488	218921	207705	
3	100.1	0.9990	204363	204159	
4	100.0	1.0000	205267	205267	
5	99.9	1.0010	205037	205242	
6	99.6	1.0040	202188	203000	
IMIQUIMOD					
1	99.9	1.0010	770063	770834	774413 ± 8044 (1.04)
2	104.2	0.9597	823258	790075	
3	100	1.0000	774212	774212	
4	100.2	0.9980	773567	772023	
5	100	1.0000	772369	772369	
6	100	1.0000	766966	766966	

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:

- Transferred 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.

- 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 results.
- Extracted 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 3 and Table 4.**

Table-3: 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-4: 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

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 5 to Table 12.**

Table-5: ZIPRASIDONE HCl SWAB - GLASS LINED

	50%	100%	150%
Weight (mg)	49.9	100	149.9
Total dilution	100	100	100
µg/ mL	4.99	10.0	14.99
µg deposited	499	1000	1499

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	%Recovery	AV recovery%
50% A	4.99	691618	4.559	91.359	
100% A	10.00	1410846	9.300	92.996	93.215
150% A	14.99	2167055	14.284	95.291	
50% B	4.99	691072	4.555	91.287	
100% B	10.00	1417054	9.341	93.405	92.592
150% B	14.99	2116856	13.953	93.084	
50% C	4.99	706467	4.657	93.134	
100% C	10.00	1451465	9.567	95.673	95.573
150% C	14.99	2231135	14.707	97.913	
Mean recovery			93.79%		
RSD recovery			1.68%		

Table-6: ZIPRASIDONE HCl RINSE – GLASS LINED

	50%	**100%**	**150%**
Weight (mg)	49.9	100	149.9
Total dilution	100	100	100
µg/ mL	4.99	10.0	14.99
µg deposited	499	1000	1499

Sample No.	**Added (µg/ml)**	**Peak area**	**Found (µg/ml)**	**%Recovery**	**AV recovery%**
50% A	4.99	749880	4.943	99.055	
100% A	10.00	1537274	10.133	101.329	99.542
150% A	14.99	2234188	14.727	98.243	
50% B	4.99	749095	4.938	98.951	
100% B	10.00	1526679	10.063	100.631	99.270
150% B	14.99	2233832	14.724	98.228	
50% C	4.99	752912	4.963	99.256	
100% C	10.00	1539222	10.146	101.458	99.349
150% C	14.99	2217884	14.619	97.331	
Mean recovery			99.39%		
RSD recovery			0.14%		

Table-7: PERINDOPRIL ERBUMINE SWAB - GLASS LINED

	50%	**100%**	**150%**
Weight (mg)	49.7	99.8	150
Total dilution	100	100	100
µg/ mL	4.97	9.98	15.0
µg deposited	497	998	1500

Sample No.	**Added (µg/ml)**	**Peak area**	**Found (µg/ml)**	**%Recovery**	**AV recovery%**
50% A	4.97	90666	4.254	85.592	
100% A	9.98	197707	9.276	92.948	91.754
150% A	15.0	309225	14.508	96.723	
50% B	4.97	91890	4.311	86.748	
100% B	9.98	199674	9.368	93.872	91.963
150% B	15.0	304571	14.290	95.267	
50% C	4.97	93196	4.373	87.453	
100% C	9.98	204743	9.606	96.256	94.088
150% C	15.0	315294	14.793	98.556	
Mean recovery			92.60%		
RSD recovery			1.40%		

Table-8: PERINDOPRIL ERBUMINE RINSE – GLASS LINED

	50%	**100%**	**150%**
Weight (mg)	49.7	99.8	150
Total dilution	100	100	100
µg/ mL	4.97	9.98	15.0
µg deposited	497	998	1500

Sample No.	**Added (µg/ml)**	**Peak area**	**Found (µg/ml)**	**%Recovery**	**AV recovery%**
50% A	4.97	99306	4.659	93.749	
100% A	9.98	216249	10.146	101.665	99.694
150% A	15.0	331429	15.550	103.668	
50% B	4.97	97599	4.579	92.137	
100% B	9.98	214720	10.074	100.946	98.860
150% B	15.0	330877	15.524	103.496	
50% C	4.97	98405	4.617	92.341	
100% C	9.98	215369	10.105	101.251	99.145
150% C	15.0	332209	15.587	103.843	
Mean recovery			99.23%		
RSD recovery			0.43%		

Table-9: VENLAFAXINE HCL SWAB - GLASS LINED

	50%	**100%**	**150%**
Weight (mg)	50	100.1	149.7
Total dilution	100	100	100
µg/ mL	5.00	10.01	14.97
µg deposited	500	1001	1497

Sample No.	**Added (µg/ml)**	**Peak area**	**Found (µg/ml)**	**%Recovery**	**AV recovery%**
50% A	5.00	94174	4.655	93.102	
100% A	10.01	187288	9.258	92.486	94.591
150% A	14.97	297744	14.718	98.184	
50% B	5.00	95719	4.731	94.630	
100% B	10.01	180991	8.947	89.376	92.293
150% B	14.97	281641	13.922	92.874	
50% C	5.00	97810	4.835	96.891	
100% C	10.01	189163	9.351	93.599	96.555
150% C	14.97	300754	14.867	99.177	
Mean recovery			94.48 %		
RSD recovery			2.26		

Table-10: VENLAFAXINE HCL RINSE – GLASS LINED

	50%	**100%**	**150%**
Weight (mg)	50	100.1	149.7
Total dilution	100	100	100
µg/ mL	5.00	10.01	14.97
µg deposited	500	1001	1497

Sample No.	**Added (µg/ml)**	**Peak area**	**Found (µg/ml)**	**%Recovery**	**AV recovery%**
50% A	5.00	104197	5.151	103.011	
100% A	10.01	197066	9.741	97.314	100.412
150% A	14.97	306012	15.126	100.911	
50% B	5.00	97000	4.795	95.896	
100% B	10.01	200798	9.926	99.157	100.058
150% B	14.97	318777	15.757	105.120	
50% C	5.00	106178	5.248	105.180	
100% C	10.01	202427	10.006	100.162	103.628
150% C	14.97	320058	15.821	105.542	
Mean recovery			101.37		
RSD recovery			1.94%		

Table-11: IMIQUIMOD SWAB - GLASS LINED

	50%	**100%**	**150%**
Weight (mg)	50	99.9	149.9
Total dilution	100	100	100
µg/ mL	5.00	9.99	14.99
µg deposited	500	999	1499

Sample No.	**Added (µg/ml)**	**Peak area**	**Found (µg/ml)**	**%Recovery**	**AV recovery%**
50% A	5.00	337693	4.384	87.674	
100% A	9.99	706943	9.177	91.862	91.602
150% A	14.99	1100108	14.281	95.269	
50% B	5.00	343041	4.453	89.062	
100% B	9.99	693975	9.009	90.177	90.671
150% B	14.99	1071310	13.907	92.775	
50% C	5.00	353066	4.583	91.482	
100% C	9.99	716185	9.297	92.784	93.432
150% C	14.99	1111102	14.424	96.029	
Mean recovery			91.90%		
RSD recovery			1.53%		

Table-12: IMIQUIMOD RINSE – GLASS LINED

	50%	100%	150%
Weight (mg)	50	99.9	149.9
Total dilution	100	100	100
µg/ mL	5.00	9.99	14.99
µg deposited	500	999	1499

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	%Recovery	AV recovery%
50% A	5.00	379976	4.933	98.652	
100% A	9.99	768116	9.971	99.811	100.254
150% A	14.99	1181296	15.335	102.300	
50% B	5.00	379062	4.921	98.414	
100% B	9.99	768699	9.979	99.887	100.109
150% B	14.99	1178118	15.293	102.025	
50% C	5.00	379639	4.928	98.367	
100% C	9.99	768500	9.976	99.562	100.019
150% C	14.99	1181673	15.340	102.128	
Mean recovery			100.13%		
RSD recovery			0.12%		

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 13 to Table 20.**

Table-13: ZIPRASIDONE HCl SWAB - STEEL

	50%	100%	150%
Weight (mg)	49.9	100	149.9
Total dilution	100	100	100
µg/ mL	4.99	10.00	14.99
µg deposited	499	1000	1499

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	% Recovery	AV recovery%
50% A	4.99	755621	4.981	99.813	
100% A	10.00	1414519	9.324	93.238	95.205
150% A	14.99	2105040	13.875	92.564	
50% B	4.99	654534	4.314	86.460	
100% B	10.00	1421995	9.373	93.731	93.172
150% B	14.99	2258773	14.889	99.324	
50% C	4.99	688061	4.535	90.889	
100% C	10.00	1406365	9.270	92.701	92.319
150% C	14.99	2123320	13.996	93.368	
Mean recovery			93.57%		
RSD recovery			1.59%		

Table-14 :ZIPRASIDONE HCl RINSE – STEEL

	50%	100%	150%
Weight (mg)	49.9	100	149.9
Total dilution	100	100	100
µg/ mL	4.99	10.00	14.99
µg deposited	499	1000	1499

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	% Recovery	AV recovery%
50% A	4.99	748517	4.934	98.875	
100% A	10.00	1523243	10.040	100.405	98.721
150% A	14.99	2203248	14.523	96.883	
50% B	4.99	745571	4.914	98.486	
100% B	10.00	1521839	10.031	100.312	98.471
150% B	14.99	2197170	14.483	96.615	
50% C	4.99	743622	4.902	98.228	
100% C	10.00	1517329	10.001	100.015	98.577
150% C	14.99	2216983	14.613	97.487	
Mean recovery			98.59%		
RSD recovery			0.13%		

Table-15: PERINDOPRIL ERBUMINE SWAB - STEEL

	50%	100%	150%
Weight (mg)	49.7	99.8	150
Total dilution	100	100	100
µg/ mL	4.97	9.98	15.0
µg deposited	497	998	1500

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	%Recovery	AV recovery%
50% A	4.97	100191	4.701	94.584	
100% A	9.98	199846	9.377	93.953	95.438
150% A	15.00	312587	14.666	97.775	
50% B	4.97	84975	3.987	80.220	
100% B	9.98	203900	9.567	95.859	90.409
150% B	15.00	304192	14.272	95.149	
50% C	4.97	90143	4.229	90.889	
100% C	9.98	200585	9.411	92.701	92.774
150% C	15.00	316261	14.839	93.368	
Mean recovery			92.87%		
RSD recovery			2.71%		

Table-16: PERINDOPRIL ERBUMINE RINSE – STEEL

	50%	100%	150%
Weight (mg)	49.7	99.8	150
Total dilution	100	100	100
µg/ mL	4.97	9.98	15.00
µg deposited	497	998	1500

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	%Recovery	AV recovery%
50% A	4.97	98204	4.608	92.709	
100% A	9.98	212452	9.968	99.880	98.599
150% A	15.00	329955	15.481	103.207	
50% B	4.97	97779	4.588	92.307	
100% B	9.98	215193	10.097	101.168	99.030
150% B	15.00	331253	15.542	103.613	
50% C	4.97	97722	4.585	92.254	
100% C	9.98	212845	9.986	100.065	98.389
150% C	15.00	328807	15.427	102.848	
Mean recovery			98.67%		
RSD recovery			0.33%		

Table-17: VENLAFAXINE HCL SWAB - STEEL

	50%	**100%**	**150%**
Weight (mg)	50	100.1	149.7
Total dilution	100	100	100
µg/ mL	5.00	10.01	14.97
µg deposited	500	1001	1497

Sample No.	**Added (µg/ml)**	**Peak area**	**Found (µg/ml)**	**%Recovery**	**AV recovery%**
50% A	5.00	106713	5.275	105.499	
100% A	10.01	180356	8.915	89.063	97.573
150% A	14.97	297666	14.714	98.158	
50% B	5.00	83423	4.124	82.474	
100% B	10.01	189133	9.349	93.397	90.679
150% B	14.97	291624	14.415	96.166	
50% C	5.00	96583	4.774	95.484	
100% C	10.01	186856	9.236	92.273	94.295
150% C	14.97	288476	14.260	95.128	
Mean recovery			94.18%		
RSD recovery			3.66%		

Table-18: VENLAFAXINE HCL RINSE – STEEL

	50%	**100%**	**150%**
Weight (mg)	50	100.1	149.7
Total dilution	100	100	100
µg/ mL	5.00	10.01	14.97
µg deposited	500	1001	1497

Sample No.	**Added (µg/ml)**	**Peak area**	**Found (µg/ml)**	**%Recovery**	**AV recovery%**
50% A	5.00	105743	5.227	104.540	
100% A	10.01	196188	9.698	96.881	102.197
150% A	14.97	318934	15.765	105.172	
50% B	5.00	105950	5.237	104.744	
100% B	10.01	195314	9.655	96.449	100.883
150% B	14.97	307663	15.208	101.455	
50% C	5.00	105894	5.234	104.689	
100% C	10.01	198701	9.822	98.122	101.703
150% C	14.97	310218	15.334	102.298	
Mean recovery			101.59%		
RSD recovery			0.65%		

Table-19: IMIQUIMOD SWAB - STEEL

	50%	**100%**	**150%**
Weight (mg)	50	99.9	149.9
Total dilution	100	100	100
µg/ mL	5.00	9.99	14.99
µg deposited	500	999	1499

Sample No.	**Added (µg/ml)**	**Peak area**	**Found (µg/ml)**	**%Recovery**	**AV recovery%**
50% A	5.00	374294	4.859	97.176	
100% A	9.99	703162	9.128	91.371	94.326
150% A	14.99	1090446	14.155	94.432	
50% B	5.00	329193	4.273	85.467	
100% B	9.99	718975	9.333	93.426	90.701
150% B	14.99	1076347	13.972	93.211	

50% C	5.00	342655	4.448	88.962	92.481
100% C	9.99	707044	9.178	91.875	
150% C	14.99	1115531	14.481	96.605	
Mean recovery			92.50%		
RSD recovery			1.96%		

Table-20: IMIQUIMOD RINSE – STEEL

	50%	100%	150%
Weight (mg)	50	99.9	149.9
Total dilution	100	100	100
µg/ mL	5.00	9.99	14.99
µg deposited	500	999	1499

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery%	AV recovery%
50% A	5.00	372312	4.833	96.662	
100% A	9.99	763385	9.910	99.196	99.288
150% A	14.99	1177900	15.291	102.006	
50% B	5.00	376363	4.886	97.713	
100% B	9.99	780103	10.127	101.369	100.082
150% B	14.99	1168187	15.165	101.165	
50% C	5.00	376189	4.883	97.668	
100% C	9.99	758105	9.841	98.510	99.197
150% C	14.99	1171043	15.202	101.412	
Mean recovery:			99.52%		
RSD recovery:			0.49%		

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 21 and Table 22 and représentative LOQ chromatogram was showed in Figure 2.**

Table-21: LOQ DETAILS

	Injections						**Average**	**S.D**	**% RSD**
	1	**2**	**3**	**4**	**5**	**6**			
ZIPRASIDONE HCl									
Area	150290	150834	149702	149976	149918	150282	150167	397	0.26
PPM	0.987	0.991	0.983	0.985	0.985	0.987	0.986	0.003	0.26
% Recovery	98.73	99.09	98.34	98.52	98.48	98.72	98.65	0.26	0.26
PERINDOPRIL ERBUMINE									
Area	20286	20475	20454	20569	20480	20589	20475.5	108	0.53
PPM	0.951	0.959	0.959	0.964	0.96	0.965	0.96	0.005	0.53
% Recovery	95.06	95.95	95.85	96.39	95.97	96.48	95.95	0.5	0.5
VENLAFAXINE HCl									
Area	20270	20554	20524	20586	20447	20518	20483	114	0.56
PPM	0.992	1.006	1.004	1.007	1.000	1.004	1.0022	0.005	0.56
% Recovery	99.17	100.56	100.42	100.72	100.04	100.39	100.22	0.56	0.56
IMIQUIMOD									
Area	72731	72743	72684	72737	72397	72397	72615	170	0.23
PPM	0.944	0.944	0.943	0.944	0.939	0.939	0.9421	0.002	0.23
% Recovery	94.36	94.38	94.3	94.37	93.93	93.93	94.21	0.22	0.23

Table-22: LOD DETAILS

Sample	Area	Found (ppm)	Recovery %
Ziprasidone HCl	**37557**	**0.247**	**98.69**

Perindopril Erbumine	**5059**	**0.237**	**94.83**
Venlafaxine HCl	**5014**	**0.245**	**98.13**
Imiquimod	**19850**	**0.258**	**103.02**

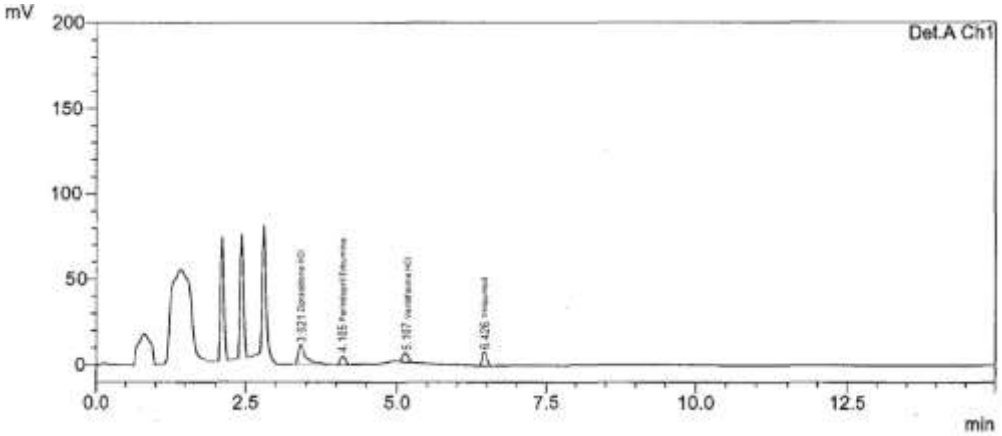

Fig-2 : LOQ Level chromatogram of Pramipexol Dihydrochloride Monohydrate

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

$$\frac{\text{ug/mL product} * V}{1000 * Kg\ (prod)} = \text{ppm Active ingredient}$$

Where:

V: volume total solvent rinse (L)

Kg : Quantity in Kg of successive product

1000: Conversion Factor

Swab:

$$\frac{(Ac - Ab) * C \times Vestr}{As \times St} = \text{ug/cm}^2 \text{ in swab}$$

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 (ICH,Q2B, 1996). Therefore, the proposed analytical procedure could be useful for regular monitoring, pharma manufacturing labs and researchers.

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REFERENCES

1. Johnson JD, Van Buskirk GE. Analytical method validation. J. Validation Technol. 1998;2:88-105.
2. Akhtar MS, Verma SK. Establishing a cleaning method validation programme of solid dosage form of a finished drug product. Int J Pharm Qual Assur. 2016;7:29-34.
3. Patel N, Jansari S, Arvadiya A, Panchal K, Desai H. Development and validation of cleaning procedure of mixing equipment used for manufacturing ceftriaxone and Sulbactam injection tablet by using total organic carbon. IOSR J Pharm. 2012 Sep;2:46-50.
4. Murthy DN, Chitra K. A Review Article on cleaning validation. International journal of pharmaceutical sciences and research. 2013 Sep 1;4(9):3317.
5. Venugopal S. Designing of Cleaning Validation Program for Activepharmaceutical Ingredients. World Journal of Pharmaceutical Research, 2014; (3): 3819-3844
6. Kathiresan K, Sreenu VS, Moorthi C, Bharath Reddy Gade BK, Yellamula Prathyusha MR. Cleaning validation of acetaminophen tablets. Rasayan J. Chem. 2010;3(3):503-6.
7. S. Kumar, International Research Journal of Pharmaceutical Sciences, 2012, 3, 17-19
8. Ghosh A, Dey S. Overview of cleaning validation in pharmaceutical industry. International Journal of Pharmaceutical Quality Assurance. 2010;2(2):26-30.
9. Prabu SL, Suriyaprakash TN. Cleaning Validation and its importance in Pharmaceutical Industry. Pharma times. 2010;42(07):21-4.
10. ICH M. Q2B validation of analytical procedures: methodology. Inproceeding of the International conference on Harmonization, Geneva, Switzerland 1996.