International Journal of General Medicine and Pharmacy (IJGMP)
Vol.1, Issue 1 Aug 2012 10-19

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

VALIDATION OF ASSAY METHOD OF INDAPAMIDE 1.5MG SUSTAINED RELEASE TABLET BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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ABSTRACT

The aim of this analytical method validation was to validate the assay of Indapamide1.5mg SR tablet with high performance liquid chromatography (HPLC). According to the International Conference on Harmonization (ICH) guidelines, Specificity, Linearity, Range, Accuracy of recovery, System Precision, Method Precision, Robustness were found within the recommended range (Not more than 2.0%). Considering product's inherent nature, many critical parameters specially stability of solution were focused and performed a thorough analysis in ambient system. It was observed that the reference and the testing solution of the product tended to be stable in ambient condition during HPLC analysis of assay. All other parameters were found within the recommended ranges and considering all primary data found from this analytical method validation report was prepared. This validation process was carried out both in ambient and in cool condition. It was noted that, as per method validation, assay of Indapamide is recommended to analyze within 18 hour if the sample temperature is ambient. During routine analysis of Indapamide 1.5 mg SR film-coated tablet, it is also observed that sample tends to show stability up to 24 hours if cooler is used.

KEYWORDS: Assay, HPLC, Indapamide, Method Validation.

INTRODUCTION

Indapamide is thiazide-like diuretic[1]. It is indicated for the treatment of hypertension, alone or in combination with other antihypertensive drugs. Its molecule contains both a polar sulfamoyl chlorobenzamide moiety and a lipid-soluble methylindoline moiety. It differs chemically from the thiazides in that it does not possess the thiazide ring system and contains only one sulfonamide group. The chemical name of Indapamide is 4-Chloro-N-(2-methyl-1-indolinyl)-3-Sulfamoylbenzamide. The compound is a weak acid, pKa=8.8, and is soluble in aqueous solutions of strong bases. The aim of this study is to validate the assay of Indapamide in Indapamide 1.5mg SR tablets. Throughout the whole validation process only B# E001 was considered as of validation batch which was supposed to be represented subsequent batches even subsequent strength if any. The drug stability test guidelines Q1A (R2) issued by International Conference on Harmonization (ICH) necessitate that the analytical

procedures for stability samples should be fully validated to ensure the stable formulation that indicates the stability[2,3,4,5]. Validation of assay is carried out to ensure that this is suitable for intended routine analysis, stability testing and give reliable data for regulatory approval. There are a lot of guidance on validation characteristics and considerations. Validation of assay method typically involves specificity, Linearity, Range, Accuracy or Recovery, System Precision, Method Precision, Robustness [6,7,8,9].

MATERIALS AND METHOD

For this method validation Specificity, Linearity, Range, Accuracy or Recovery, System Precision , Method Precision, robustness were carried out for assay.

Chemicals and Reagents used for validation:

Chemicals, reagents	Purity	Manufacturer
Methanol	HPLC grade	Merck, Germany
Perchloric Acid	Reagent grade	Merck, Germany
ortho-phosphoric acid	Reagent grade	Merck, Germany
Sodium hydroxide	Reagent grade	Merck, Germany
Potassium dihydrogen phosphate	Reagent Grade	Merck, Germany
Water	Mili Q	--

Reference Standards for Assay

Indapamide reference standard obtained from EP, France

HPLC Equipment and related parameters:

HPLC: Shimadju HPLC VP series with UV Detector (For Assay)

Column: Hypersil BDS C18 , 150mm x 4.6 mm, 5µ

Flow rate: 1.0 ml per minute

Run time: About 7 minutes (For Assay)

Detection: 254 nm

Mobile Phase: H_2O: Methanol (50:50), with 0.2 ml Perchloric in one liter of Mobile Phase.

Standard Solution Preparation: 15 mg reference standard was taken in 100 ml volumetric flask and diluted upto the mark with mobile phase.

Sample Preparation: 10 intact tablets were taken in 100 ml volumetric flask. 50 ml mobile phase was added and stirred with the magnetic stirrer. Then diluted to 100 ml with mobile phase and filtered 0.45 micron filter paper.

RESULTS AND DISCUSSIONS

Assay by HPLC

Precision (repeatability)

System Precision and Method Precision:

The System Precision was calculated from the results of 6 determinations of reference solution injected 6 times and the system precision was calculated from the results of 6 determinations using the test solutions prepared separately six times. The determinations were carried out by the same analyst, 6 times, immediately one after the other, under conditions as similar as possible.

Parameters	System Precision	Method Precision
Number of determinations (n)	6	6
Individual results (areas):	3479251,3477495,3474208,3474976, 3475407,3475407	97.7, 98.3, 97.0, 99.3, 97.8, 97.5,
Upper confidence limit (P = 0.95)	3485337	102
Lower confidence limit (P =0.95)	3467416	95
Mean (area) :	3476377	97.7
Standard deviation	1866.34	0.78203
Relative Standard Deviation	0.05	0.8
Acceptance Criteria:	Coefficient of variation NMT 2.0%	Coefficient of variation NMT 2.0%

Results and Evaluation

The system and method were precise, as the coefficient of variation was less than 2.0%.

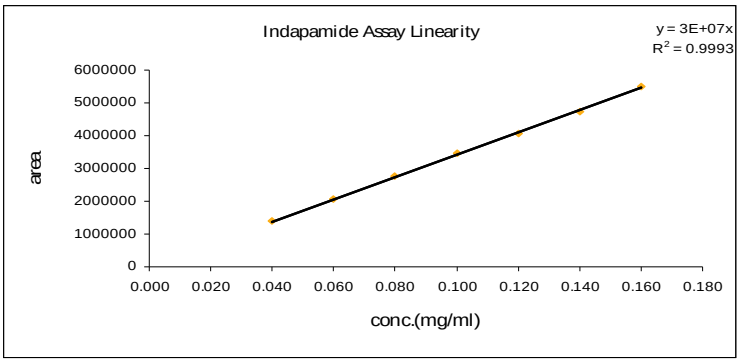
LINEARITY

Linearity with Standard Solution

The linearity of peak area (y) / concentration (x) of a dilution series was determined for standard solution in the range 40% – 160% (e.g. 40,60, 80,100, 120, 140,160)% of the nominal concentration

Concentration of Indapamide (x) of the Solution for measurement (mg/ml)	Area of Indapamide (y)
0.06	1413629
0.9	2070293
0.12	2763865
0.15	3452081
0.18	4057829
0.21	4746249
0.24	5495808
[x] = (0.150) mg/ml = 100 percent	y = area of Indapamide
Correlation Coefficient, r2	0.999
The method is sufficient linear as the correlation coefficient, r2 is ≥ 0.999	

Linearity graph for Indapamide assay



Acceptance Criteria: Correlation Coefficient, r2 is ≥ 0.999

Result and evaluation	
Correlation Coefficient, r2	0.9993
The method is sufficient linear as the correlation coefficient, r2 is ≥ 0.999	

RANGE

To determine the range of the method , 40% solution for 6 replica & 160% solution for 6 replica was injected and % RSD was calculated

Ranges	Readings	% RSD	Remarks
40%	1393020,1390451,1395959,1417629,1393170,1419930	0.95	complies
160%	5565337,5576221,5530372,5568645,5587258,5558477	0.35	complies

Acceptance Criteria

Method is suitable if coefficient of variation is less then 2.0% for each level.

Accuracy (recovery)

The quantity of excipients equivalent to 10 tablets was mixed with approx. 80,100,120 percent of the declared amount of active ingredient and analyzed according to the test procedure. The experiment was repeated thrice at each level.

Accuracy and analysis precision (Spiked Placebo Method)

Range relative to theoretical concentration	% Recovery			Mean value
	1st value	2nd value	3rd value	
80	103.1	99.4	101.9	102
100	97.5	97.1	99.7	98.1
120	99	99.4	99.2	99.2

Standard deviation	1.72
Mean Assay	99.6
Standard deviation	1.72
Number of determination	9
Minimum	97.1
Maximum	103.1
Upper confidence limit (P = 0.95)	111
Lower confidence limit (P = 0.95)	88

Acceptance Criteria: Coefficient of variation (n = 9) NMT 2.0%

Results and Evaluation

The confidence interval of the mean values found includes the corresponding theoretical values. The hypothesis that the test procedure provides accurate values is assured by this test (P = 0.95) Coefficient of variation (n = 9) NMT 2.0%

STABILITY OF THE SOLUTIONS

A reference and a sample solution was analyzed under ambient condition for 24 hours as similar as the condition mentioned as per test method.

Area Variation of Indapamide Under Ambient Condition		
Time[h]	**Deviation of Standard**	**Deviation of Sample**
Zero hour	0	0
6 th hour	-0.02	-0.03
12th hour	-1	-1.77
18th hour	1.21	-0.25
24th hour	-1.67	-7.32

Stability of the reference solution and sample solution

Acceptance Criteria: % difference NMT 2.0%

RESULTS

The reference solution was found stable up to 24 hours at ambient condition and sample solution was found stable up to 18 hours (around 20 to 25 ºC) as the difference was found NMT 2.0%

SPECIFICITY

Blank, placebo, standard and test solution were prepared and injected. In the chromatogram the placebo solution and the diluting media has no influence at RT of Indapamide.

Sample Information	Active ingredients	Peak to be expected at about (min)	observation	Remarks
Blank	Indapamide	5	NPA	Method is specific/selective
Placebo	Indapamide	5	NPA	
Standard	Indapamide	5	RPA	
Test sample	Indapamide	5.13	RPA	

NPA = No Peak Appeared

RPA = Respective Peak Appeared

Results and Evaluation

The method was found specific for the determination of Indapamide.

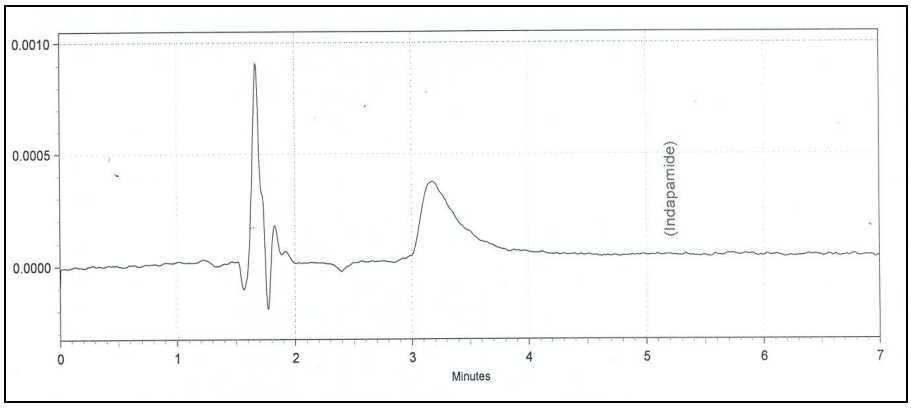


Fig: 1. Blank Chromatogram of Indapamide Assay

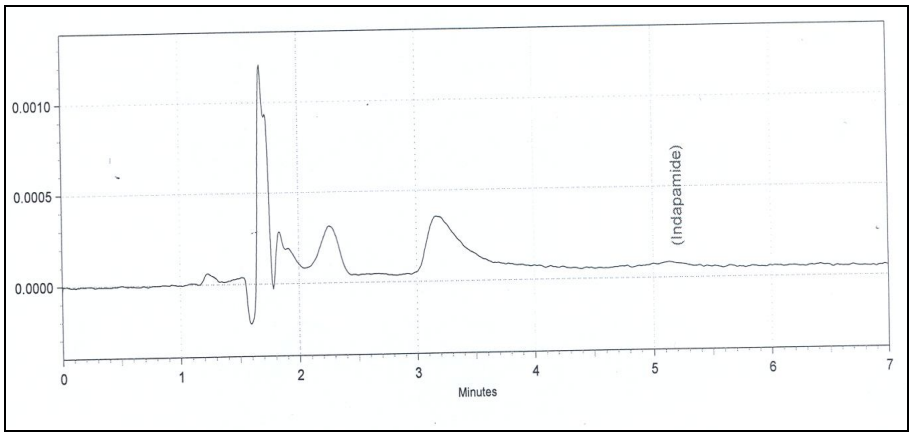


Fig: 2. Placebo Chromatogram of Indapamide Assay

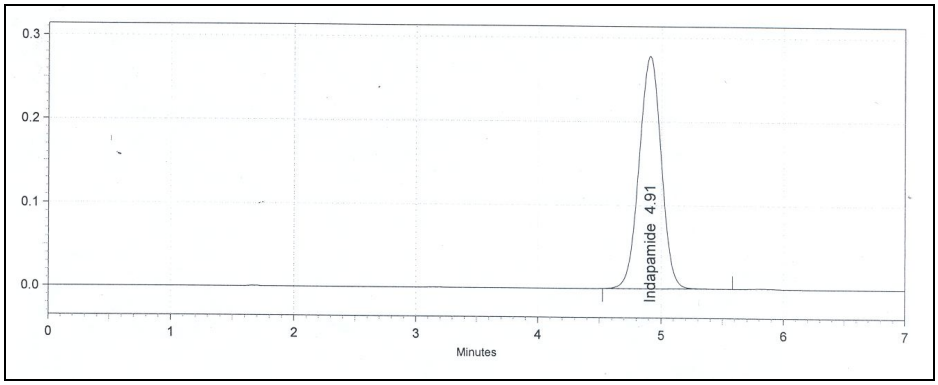


Fig: 3. Standard Solution of Indapamide Assay

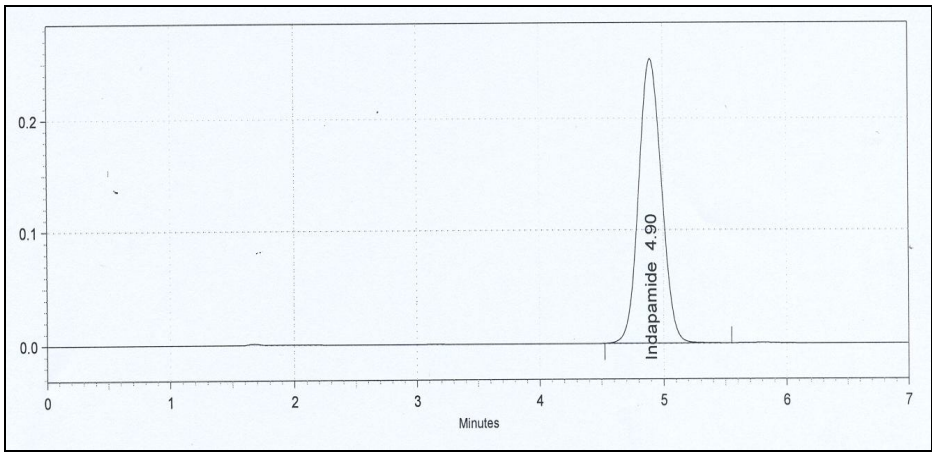


Fig: 4. Sample Solution of Indapamide Assay

Robustness

Condition applied for Standard	RT(min)	Assymetry & peak shape	Remarks
FR@0.9ml/min	5.71	1.03	acceptable
FR@1.0ml/min	5.17	1.02	acceptable
FR@1.1ml/min	4.72	1.06	acceptable

Condition applied for Sample	RT(min)	Assymetry & peak shape	Remarks
FR@0.9ml/min	5.77	1.07	acceptable
FR@1.0ml/min	5.17	1.03	acceptable
FR@1.1ml/min	4.76	1.07	acceptable

Acceptance Criteria

Tailing factor should be within (0.85-1.5)

Peak should be reasonable shaped in compare to initial.

Result: complies

CONCLUSIONS

A single batch was selected as representative to validate the assay by High Performance Liquid Chromatography. Considering product's inherent nature, many critical parameters specially stability of solution has been focused and performed a thorough analysis in ambient system. It has been observed that the reference and the testing solution of the product tend to be stable in ambient condition during HPLC analysis of assay. It is noted that, as per method validation, assay of Indapamide is recommended to analyze within 18 hour if the sample temperature is ambient. During routine analysis of Indapamide 1.5 mg SR film-coated tablet, it is also observed that sample tends to show stability up to 24 hours if cooler is used. Moreover, since the Indapamide 1.5 mg film-coated tablet contains very trace amount of active ingredient and due to use of HPMC as sustained release agent and opadry as the coating agent into the formulation, in some cases it has been observed that pulverization of tablets into finely powder was too critical task which leads poor homogeneous sample representation for different analyst at different time. To avoid this critical situation, an alternative sample preparation technique was followed (10 intact tablets into 100 ml dissolved with the help of magnetic stirring up to disintegrate) and acceptable level of method ruggedness was experienced. Additionally, filter effect on assay sample preparation has also been studied and suitable result was found with significant findings. In that case, filtering the sample with 0.45 micron is suggested if there is no centrifuge. Filter may be avoided if sample liquor is centrifuged at 3000 RPM for 5 min. So this analytical method validation for assay of indapamide can be used for quality control, stability testing and regulatory approval.

ACKNOWLEDGEMENT

The authors would like to thank Centre for Excellence of the University of Dhaka, Novartis (Bangladesh) Limited, Department of Pharmaceutical Technology, University of Dhaka for giving technical supports.

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