RJC

RASĀYAN *J. Chem.*
Vol. 8 | No. 3 |316 - 320 | July - September | 2015
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR FAST, SENSITIVE AND SIMULTANEOUS DETERMINATION OF FEW (EIGHT) ACTIVE PHARMACEUTICAL INGREDIENT RESIDUES ON STAINLESS STEEL SURFACE IN MANUFACTURING PLANTS

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ABSTRACT

Prevention of cross contamination with active pharmaceutical ingredients is crucial and requires special attention in pharmaceutical industries. A High performance liquid chromatographic (HPLC-PDA) method was developed and validated for simultaneous quantification of Dopamine, Moxifloxacin, Etoricoxib, Carvedilol, Nebivolol, Olmesartan, Ezetimibe and Montelukast in cleaning samples. A new method is presented with which it is possible to verify cleaning process of any above mentioned API used for the production of various pharmaceuticals. The HPLC method was validated using an Symmetry C18 column with a particle size of 3.5micron (75 mm x 4.6 mm) with 0.075% ortho phosphoric acid in water as Mobile phase A and 0.075% ortho phosphoric acid in Acetonitrile as Mobile phase-B. Method development and method validation for cleaning control analysis are described. This rapid HPLC method is suitable for cleaning control assays within good manufacturing practices (GMP) of the pharmaceutical industry. The validated method was found to be simple, Specific, precise, linear, accurate, rugged and sensitive for demonstration of cleaning validation of all API residues on a stainless steel surface.

Keywords: HPLC-PDA, cleaning method validation, Active pharmaceutical ingredient, Good Manufacturing Practices.

INTRODUCTION

Pharmaceutical equipment has to be cleaned to prevent cross contamination with active pharmaceutical ingredients and requires special attention in pharmaceutical industries. The prime use of validation of cleaning method is to ensure that compliance with federal and other regulations. The most important factor to conduct such validation is to for identification and correction of potential problems previously unsuspected, which could compromise with safety, efficacy or quality of subsequent batches of drug substances produced with the same equipment. Liquid chromatography-Mass spectrometry [LC-MS] and Ultra performance liquid chromatography-Mass spectrometry [UPLC-MS] techniques applied in pharmaceutical cleaning methods have the advantage of improved sensitivity, selectivity and also applicable even the drug substance UV in active. Nowadays HPLC is the most common technique for cleaning validation[1-4].

The code of federal regulation (CFR) states that "Equipment and utensils shall be cleaned, maintained and sanitized at appropriate intervals to provide malfunctions and contamination that would alter the safety, identity, strength, quality or purity of the drug substance beyond the official or other established requirements" . According to the FDA, the limit should be based on the logical criteria involving the risk associated with the residues of the drug substances. Calculation of an acceptable residue limit and maximum allowable carry over for active pharmaceutical ingredients in production equipment should be based on therapeutic doses, toxilogical index and a general limit (10 ppm). According to Food and Drug Administration two different methods of sampling are generally admitted for performing a cleaning

control. The direct surface sampling, using the swabbing technique, and the indirect sampling based on the analysis of solution used for rinsing of equipment.[5-8]

EXPERIMENTAL[9-11]

Dopamine hydrochloride, Moxifloxacin hydrochloride, Etoricoxib, Carvedilol, Nebivolol hydrochloride, Olmesartan medoximil, Ezetimibe and Montelukast sodium standards were provided by Inogent laboratories private Limited, Hyderabad, India. Methanol HPLC grade (Merck, India), Acetonitrile HPLC grade (Merck, India), ortho phosphoric acid (Sigma Aldrich) and water HPLC grade were used. Waters alliance 2695 HPLC, PDA detector with Empower -2 software and Class-A volumetric flasks, class-A pipettes, beakers were used.

Symmetry C18 column with dimensions of 75 x4.6mm. 3.5µ with flow rate of 1.0 $mL.min^{-1}$ with PDA detector is used. Samples are prepared in methanol with an injection volume of 5µL having gradient elution mode, with column temperature of 50°C

Analysis was performed on a chromatographic system waters 2695 HPLC system equipped with an auto injector with PDA detector and Quaternary pump. The output signal was monitored and processes using Empower-2 software (Waters). The programme is as Time / %B is as 0.0/10, 1.0/10, 3.0/30, 4.0/30, 6.0/50, 7.0/50, 8.0/90, 13.0/90 and 14.0/10. Standard stock solution was prepared of each standard having the concentration of 10 $\mu g.mL^{-1}$. The standard stock solution was subsequently diluted with diluent to furnish calibration curve (Linearity) in the range of 2 - 15 $\mu g.mL^{-1}$

Theory and Calculations

The developed chromatographic method was validated for specificity, Linearity, accuracy, precision and limit of detection and limit of quantification.

Specificity was evaluating each API retention time versus all API's spiked and checked the peak purity for each API in spiked chromatogram.

Precision was evaluated by carrying out six independent preparations 10µg/mL of standard containing all API'S. The percentage of R.S.D. of six preparations obtained was calculated. Linearity was studied over a small drug concentration from 5-15 µg/mL. Calculated the peak area versus concentration data was performed by correlation coefficient obtained for the regress

The accuracy of the method was evaluated in triplicate at three concentration levels, *i.e*. 80, 100 and 120% concentration. At each concentration, three sets were prepared and injected in triplicate. The percentage of recovery was calculated at each level.

The limit of detection and limit of quantification of each API was determined by using standard deviation of the response and slope of the calibration curve method.

Solution of known concentration of API is applied on the stainless steel plate of thickness 10 cm^2 and allow the plate to air dry. Swab the plate with cotton swab and dissolve in the diluent, sonicate the cotton swab with diluent for 3-5 minutes to dissolve completely and make up the solution with diluent to get the standard solution concentration. Mix the solution proper and analyze as per the method above. Calculate the recovery factor by using the below formula-

$$\%\text{Recovery} = \frac{\text{Area of sample solution (swab) x standard dilution x100}}{\text{Area of standard solution (respective API) x sample dilution}}$$

RESULTS AND DISCUSSION

Spectroscopic method for the determination of the Dopamine hydrochloride, Moxifloxacin hydrochloride, Etoricoxib, Carvedilol, Nebivolol hydrochloride, Olmesartan medoximil, Ezetimibe and Montelukast sodium API'S in single method was developed and validated by determining the specificity, Linearity, precision, accuracy, Limit of detection and limit of quantification. Detection wavelength was selected for analysis by injecting the standard solution in PDA. The absorption maxima for each API are shown in Table-1

Table-1: Absorption maxima for all API'S

S.No.	Name of the API	UV maxima(nm)
1	Dopamine	280
2	Moxifloxacin	295
3	Etoricoxib	230
4	Carvedilol	240
5	Nebivolol	280
6	Olmesartan	205
7	Ezetimibe	230
8	Montelukast	254

There is no interference of any API with another proved that the method is specific. The specificity results are given in the Table-2.

Table-2: specificity results

S.No.	Name of the API	Individual retention time(min)	Retention time in spiked(min)	Peak purity
1	Dopamine	0.69	0.70	Pass
2	Moxifloxacin	3.80	3.82	Pass
3	Ezetimibe	4.13	4.15	Pass
4	Carvedilol	4.86	4.85	Pass
5	Nebivolol	5.51	5.50	Pass
6	Olmesartan	5.70	5.79	Pass
7	Etoricoxib	8.57	8.57	Pass
8	Montelukast	9.69	9.71	Pass

The R.S.D. for six preparations of each API was within 2% which confirms the good precision of the developed analytical method. The results are shown in Table-3.

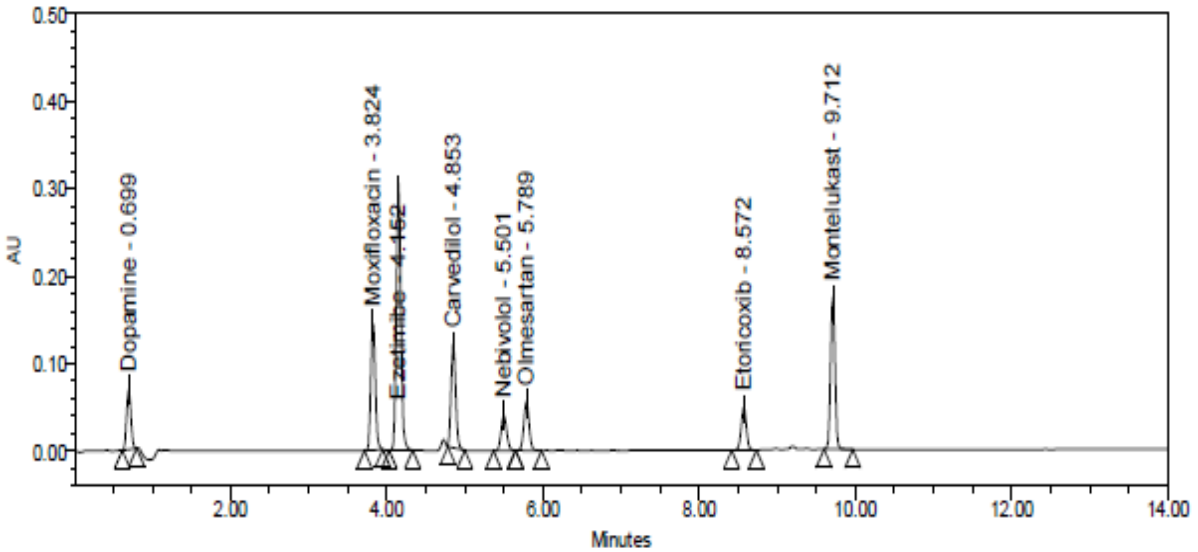

Fig.-1: Spiked chromatogram of all API'S

Table-3: Precision results

S.No.	Name of the API	Precision results (%RSD)(n=6)
1	Dopamine	1.2
2	Moxifloxacin	0.8
3	Etoricoxib	1.1
4	Carvedilol	0.8
5	Nebivolol	0.8
6	Olmesartan	0.4
7	Ezetimibe	0.5
8	Montelukast	0.6

Linear calibration plot was obtained for each API over the calibration ranges tested, i.e., 2-15 µg/mL and the correlation coefficient obtained for each API r>0.99 showed excellent linear relationship between concentration versus absorbance. Results of Linearity are shown in Table-4.

Table-4: Linearity results

S.No.	Name of the API	Correlation coefficient (r)	Slope	Y-Intercept
1	Dopamine	0.999	11857.186	-6123.804
2	Moxifloxacin	0.999	26960.146	-8383.47826
3	Etoricoxib	0.999	8170.941	-1349.087
4	Carvedilol	0.999	30699.246	-6265.717
5	Nebivolol	0.999	3350.181	-412.0435
6	Olmesartan	0.998	5083.872	-1111.957
7	Ezetimibe	0.999	23124.143	-5129.022
8	Montelukast	0.999	7228.827	-281.0217

The percentage recovery of each API is given in Table-5.

Table-5: Accuracy Data

S.No.	Name of the API	%Recovery		
		80% level	100 % level	120% level
1	Dopamine	99.3	99.4	99.1
2	Moxifloxacin	99.8	99.5	99.2
3	Etoricoxib	99.4	99.8	99.4
4	Carvedilol	99.8	100.5	99.6
5	Nebivolol	99.5	100.8	100.5
6	Olmesartan	99.8	100.1	100.8
7	Ezetimibe	99.4	100.2	100.2
8	Montelukast	99.8	100.1	100.1

The limit of detection and limit of quantification of each API of each API were shown in the Table-6.

Table-6: Limit of detection and Limit of quantification results

S.No.	Name of the API	Limit of detection	Limit of quantification
1	Dopamine	0.9	2.6
2	Moxifloxacin	0.7	2.1
3	Etoricoxib	1.1	3.3
4	Carvedilol	0.8	2.3
5	Nebivolol	0.9	2.6
6	Olmesartan	1.0	3.0
7	Ezetimibe	0.8	2.4
8	Montelukast	0.8	2.5

CONCLUSION

The proposed method is simple, rapid, sensitive, quantitative and economic. Hence this method can be used for routine analysis for any API listed above.

ACKNOWLEDGEMENTS

Authors thanks to Inogent Laboratories private limited for the providing lab facility and chemicals, Reagents.

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[RJC-1292/2015]