ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ARFORMOTEROL IN PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

Dr. Meruva Sathish Kumar*,V.Vijayasree*, Muntaz begum*, D.Kavyanjali*, K.Harshitha Chowdary*

Department of pharmaceutical Analysis,MNR college of Pharmacy, Fasalwadi, Sangareddy District,Telangana.

Corresponding Author:Dr.MeruvaSathish Kumar

ABSTRACT

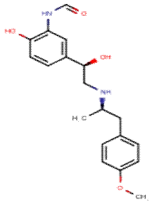
A simple, Precised, Accurate method was developed for the estimation of Arformoterol by RP-HPLC technique. Chromatographic conditions used are stationary phase Agilent C18 (150mm*4.6mm,5 m), Mobile phase 0.01N Potassium di hydrogen orthophosphate: Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1.0ml/min, detection wave length was 215.0 nm,column temperature was set to 30oC and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R2 value was found to be as 0. 999.Precision was found to be 0.5 for repeatability and 0.3 for intermediate precision. LOD and LOQ are 0.01µg/ml and 0.02µg/ml respectively. By using above method assay of marketed formulation was carried out 99.65% was present. Degradation studies of Arformoterol were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Arformoterol.

Key words: HPLC, Arformoterol, Method development. ICH Guidelines

INTRODUCTION

Arformoterol is a medication used for the treatment of chronic obstructive pulmonary disease. It is a beta-2 adrenergic agonist and a bronchodilator.It works by relaxing the smooth muscles in the airways to improve breathing.

IUPAC name of Arformoterol is N-{2-hydroxy-5-[(1R)-1-hydroxy-2-{[(2R)-1-(4methoxyphenyl) propanyl]amino} ethyl] phenyl}formamide



Structure of Arformoterol

MATERIALS AND METHODS

INSTRUMENTS USED

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Arformoterol solutions.
2.Sonicator (Ultrasonic sonicator)
3.PH meter (Thermo scientific)
4.Micro balance (Sartorius)
5.Vacuum filter pump

REAGENTS USED

Methanol HPLC Grade (RANKEM),Acetonitrile HPLC Grade (RANKEM),HPLC grade Water (RANKEM),Glacial Acetic acid

METHOD VALIDATION

PREPARATION OF STANDARD AND SAMPLE SOLUTIONS

Preparation of Standard stock solutions: Accurately weighed 1.5mg of Arformoterol transferred 50ml and volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes.Flasks were made up with diluents and labeled as Standard stock solution (30µg/ml of Arformoterol).
Preparation of Standard working solutions (100% solution): 1ml of Arformoterol from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.(3µg/ml of Arformoterol).
Preparation of Sample stock solutions: 1 vial of injection contains 2ml (equivalent to 15mcg of Arformoterol), take 10vials and transferred into a 25 mL volumetric flask, 5mL of diluent added and sonicated for 20 min, further the volume made up with diluent and filtered with 0.45um nylon filtluent and filtered by HPLC filters. (6µg/ml of Arformoterol).

Preparation of Sample working solutions (100% solution): 5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (3µg/ml of Arformoterol)

Preparation of buffer:

0.1%OPA Buffer: 1ml of Perchloric acid was diluted to 1000ml with HPLC grade water.

Buffer: 0.01N Potassium dihyrogen ortho phosphate

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water ad ded and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil.Orthophosphoric acid solution

PREPARATION OF DILUENT

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and buffer taken in the ratio of 50:50

VALIDATION PARAMETERS

SYSTEM SUITABILITY

The system suitability parameters were determined by preparing standard solutions of Arformoterol (10ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

SPECIFICITY STUDY OF THE DRUG

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

PRECISION :

Preparation of Sample stock solutions: 1 vial of injection contains 2ml (equivalent to 15mcg of Arformoterol), take 10vials and transferred into a 25 mL volumetric flask, 5mL of diluent added and sonicated for 20 min, further the volume made up with diluent and filtered with 0.45um nylon filtluent and filtered by HPLC filters. (6µg/ml of Arformoterol)

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (3µg/ml of Arformoterol)

LINEARITY

Preparation of Standard stock solutions: Accurately weighed 1.5mg of Arformoterol transferred 50ml and volumetric flasks, 3/4 Th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (30µg/ml of Arformoterol).

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (0.75µg/ml of Arformoterol)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and

made up to 10ml. (1.5µg/ml of Arformoterol)
75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (2.25µg/ml of Arformoterol)
100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (3µg/ml of Arformoterol)
125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (3.75µg/ml of Arformoterol)
150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (4.5µg/ml of Arformoterol)

ACCURACY

Preparation of Standard stock solutions: 1 vial of injection contains 2ml (equivalent to 15mcg of Arformoterol), take 10vials and transferred into a 25 mL volumetric flask, 5mL of diluent added and sonicated for 20 min, further the volume made up with diluent and filtered with 0.45 um nylon filtent and filtered by HPLC filters. (6µg/ml of Arformoterol). **Preparation of 50% Spiked Solution:** 2.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 7.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

ROBUSTNESS:

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation:

0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml Arformoterol, were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample preparation:

0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml of arformoterol, were transferred to 10ml volumetric flasks and made up with the same diluents

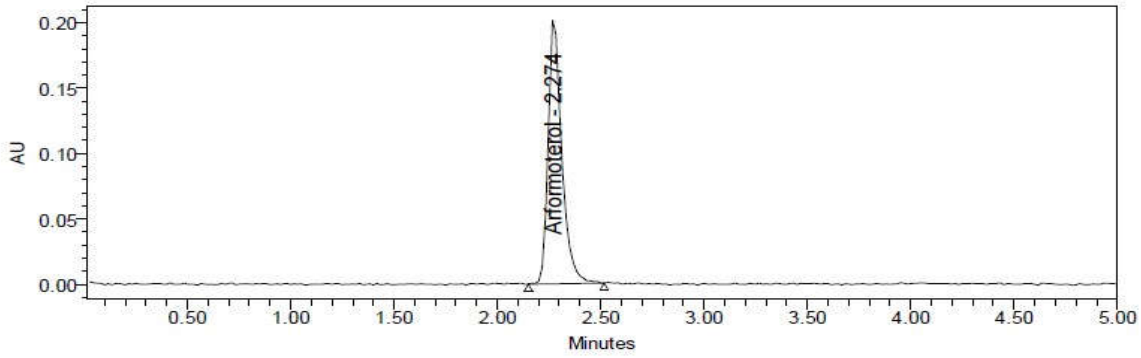
S no	**Arformoterol**		
Inj	RT(min)	USP Plate Count	Tailing
1	2.266	5957	5714
2	2.272	5720	5648
3	2.273	5532	6054
4	2.274	1.31	1.34
5	2.280	1.36	1.37
6	2.282	1.34	1.33

RESULTS AND DISCUSSIONS:

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

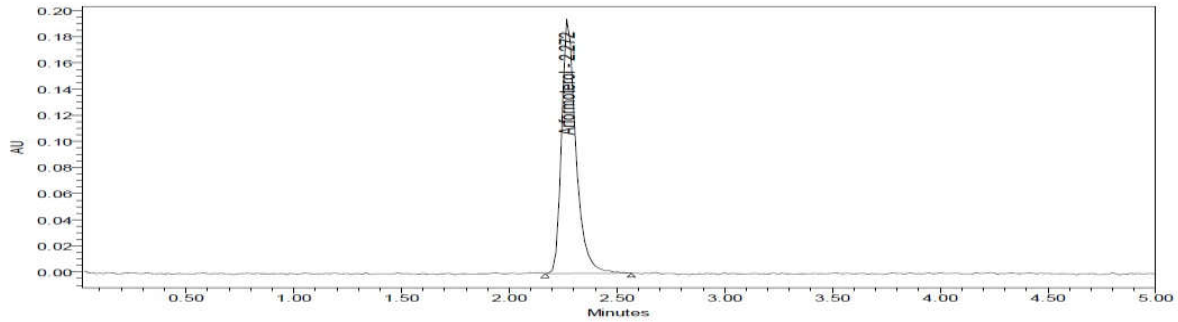
Table:1 System suitability parameters for arformoterol

Fig:1 System suitability chromatogram



Specificity

Fig:2 Optimised chromatogram



Linearity:

Arformoterol	
Conc (µg/mL)	**Peak area**
0	0
0.75	152973
1.5	302383
2.25	457286
3	605302
3.75	758104
4.5	891442

Fig:3 Calibration curve for arformoterol

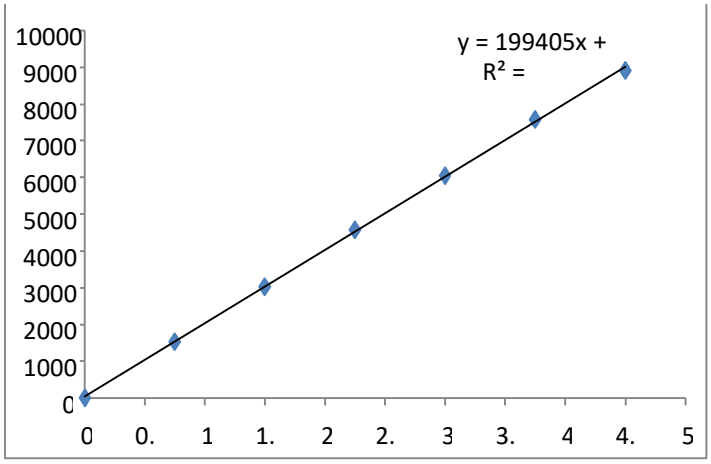


Fig:3 Calibration curve for arformoterol

Precision:

System Precision:

S. No	Area of Arformoterol
1.	606598
2.	601587
3.	602154
4.	599582
5.	603254
6.	606545
Mean	603287
S.D	2809.9
%RSD	0.5

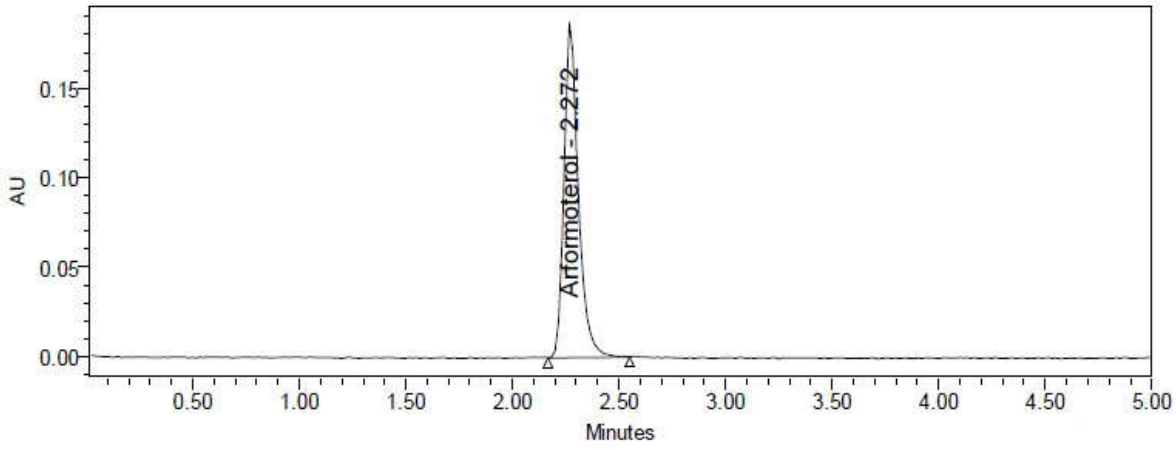
Table 2. System precision table of Arformoterol

Repeatability:

Table 3. Repeatability of arformoterol

S. No	Area of Arformoterol
1.	602326
2.	601628
3.	599962
4.	602562
5.	602985
6.	601283
Mean	601791
S.D	1089.6
%RSD	0.2

Fig 6.Repeatability chromatogram



Intermediate precision (Day_Day Precision):

Table 3

S.No	Area of arformoterol
1	**596254**
2	**599125**
3	**599216**

4	**601286**
5	**599125**
6	**601262**
Mean	**599378**
S.D	**1849.5**
%RSD	**0.3**

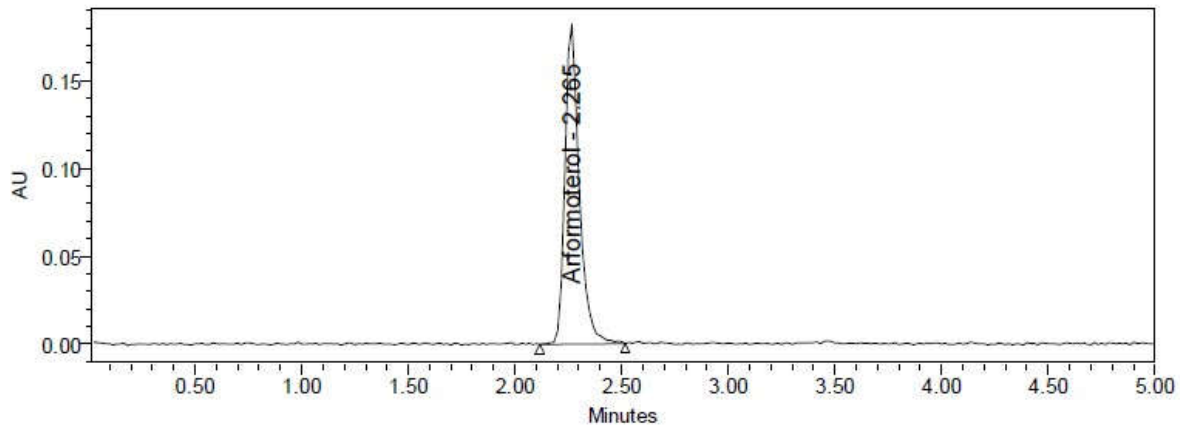


Fig:7 Intermediate precision chromatogram

Accuracy:

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	1.5	1.49	99.28	99.82%
	1.5	1.49	99.50	
	1.5	1.49	99.59	
100%	3	2.99	99.69	
	3	3.01	100.44	
	3	2.99	99.76	
150%	4.5	4.51	100.24	
	4.5	4.50	99.94	
	4.5	4.50	99.97	

Table 5. Accuracy table of Arformoterol

Sensitivity:

Table 6 Sensitivity table of Arformoterol

Molecule	LOD	LOQ
Arformoterol	0.01	0.02

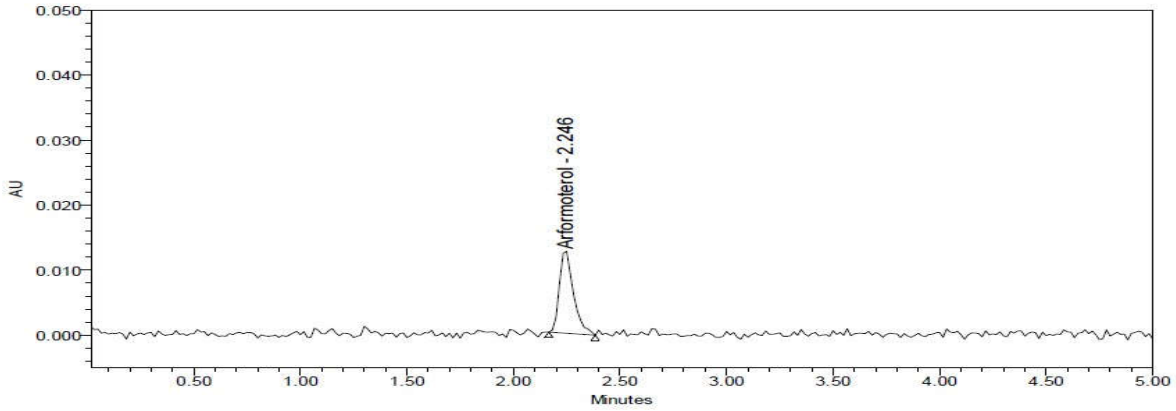


Fig:9 LOD Chromatogram of standard

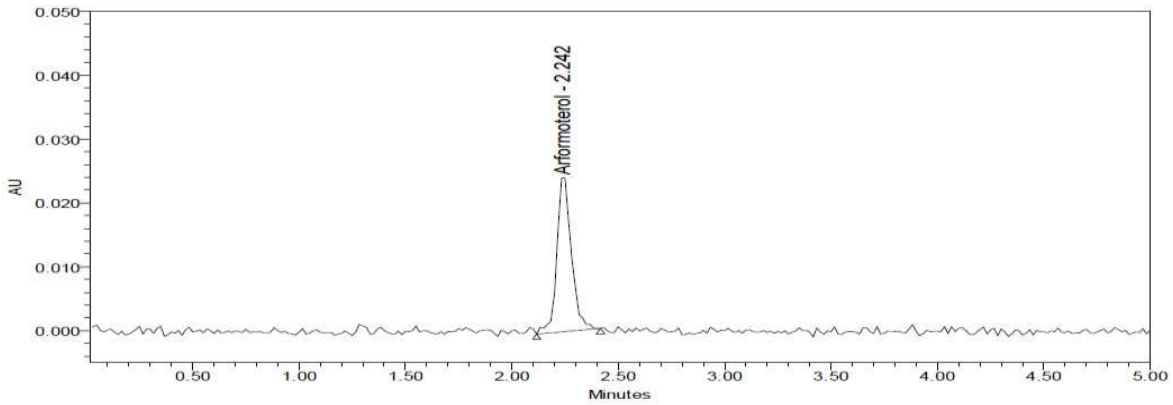


Fig:10 LOQ Chromatogram of standard

Robustness:

Table 7 Robustness data for Arformoterol

S.no	Condition	%RSD of Arformoterol
1	Flow rate (-) 0.9ml/min	0.1
2	Flow rate (+) 1.1ml/min	0.6
3	Mobile phase (-) 65B:35A	0.3
4	Mobile phase (+) 55B:45A	0.1
5	Temperature (-) 27°C	0.6
6	Temperature (+) 33°C	0.4

Fig:10 Temperature plus chromatogram of arformoterol

Assay: bearing the label claim Arformoterol 10mg. Assay was performed with the above formulation. Average % Assay for Arformoterol obtained was 99.65%.

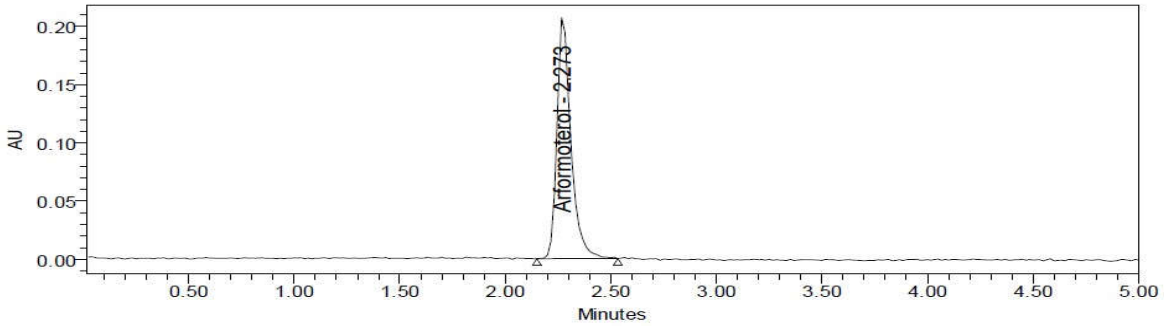


Fig:11 Chromatogram of working standard solution

S.no	Standard Area	Sample area	% Assay
1	606598	602326	99.74
2	601587	601628	99.63
3	602154	599962	99.35
4	599582	602562	99.78
5	603254	602985	99.85

6	606545	601283	99.57
Avg	603287	601791	99.65
Stdev	2809.9	1089.6	0.18
%RSD	0.5	0.2	0.2

Table 8 Data of arformoterol

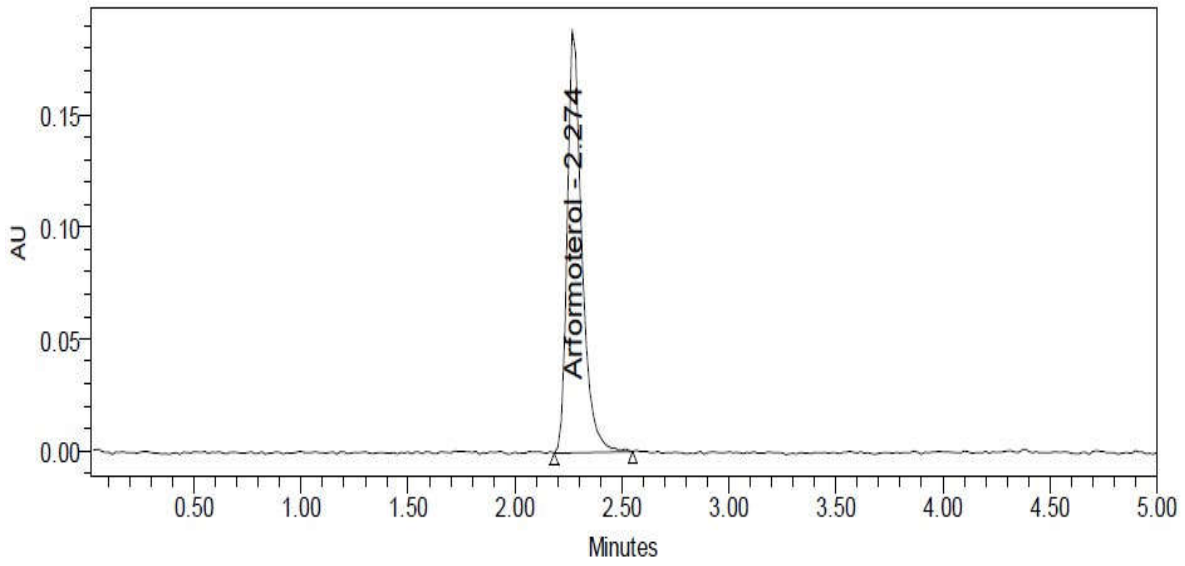


Fig 12 Chromatogram of working sample solution

SUMMARY AND CONCLUSION

Parameters		**Arformoterol**	**LIMIT**
Linearity Range(µg/ml)		0.75-4.5µg/ml	R< 1
Regressioncoefficient		0.999	
Slope(m)		199405	
Intercept(c)		3837	
Regression equation (Y=mx+c)		y = 199405x + 3837	
Assay (% mean assay)		99.65%	90-110%
Specificity		Specific	No interference of any peak
System precision %RSD		0.5	NMT 2.0%
Method precision %RSD		0.	NMT 2.0%
Accuracy%recovery		99.82%	98-102%
LOD		0.01	NMT 3
LOQ		0.02	NMT 10
Robustness	**FM**	0.1	%RSD NMT 2.0
	FP	0.6	
	MM	0.3	
	MP	0.1	
	TM	0.6	
	TP	0.4	

Conclusion

A simple, sensitive, specific and precise RP-HPLC method for the pharmaceutical dose estimation of Arformoterol. Retention time of Arformoterol was found to be 2.276min. %RSD of the Arformoterol were and found to be 0.5. %RSD of Method precision of Arformoterol was found to be 0.2. %Recovery was obtained as 99.62% for Arformoterol. LOD, LOQ values obtained from regression equation of Arformoterol were 0.01, 0.02. Regression equation of Arformoterol is y = 199405x + 3837. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

REFERENCES

1. B.k Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication , Meerut, (2007)
2. Lindholm.J, Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis, pg , 13-14, (2004).
3. Rashmin, An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences, Vol.2, Issue 2, Pg 191-196 (2012).
4. Malvia R, Bansal V , Pal O.P and Sharma P.K. A Review of High Performance Liquid Chromatography. Journal of Global Pharma technology (2010)
5. Douglas A Skoog, F. James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg 725-760.
6. Dr.S. Ravi Shankar, Text book of Pharmaceutical analysis, Fourth edition, Pg 13.113.2
7. David G.Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed., Pg 221-232.
8. Remingtonn's The Sciences and Practise of Pharmacy, 20th Edition (2000)
9. Connors Ka. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc; Delhi, 3rd Ed, Pg 373-421, (1994)
10. Gurdeep R.Chatwal , Sham K .Anand, Instrumental Methods of Chemical Analysis , Pg 2.566-2.638 (2007)
11. Nasal.A, Siluk.D, and Kaliszan.R. Chromatographic Retention Parameters in Medicinal Chemistry and Pharmacology, Pubmed, Vol.10, Issue 5 Pg no-381-426, March (2003)
12. Ashok Kumar, Lalith Kishore, navpreet Kaur , Anroop Nair. Method Development and Validation for Pharmaceutical Analysis. International Pharmaceutica Sciencia, Vol 2, Issue 3, Jul-Sep (2012)
13. Kaushal.C, Srivatsava.B, A Process of Method Development: A Chromatographic Approach. J Chem Pharm Res, Vol.2, Issue 2, 519-545, (2010)
14. Green JM. A Practicle guide to analytical method validation, Anal Chem (1996) 305A309A
15. ICH, Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA , Geneva , (1996).

16. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2 (2010) 16571658.
17. British Pharmacopoeia, The British Pharmacopoeial Commission, the stationary office, UK, London, 1408-1409 2 (2011).
18. https://go.drugbank.com/drugs/DB01274
19. Samuel O Akapo, Muhammad Asif. Validation of a RP-HPLC method for the assay of formoterol and its related substances in formoterol fumarate dihydrate drug substance, J Pharm Biomed Anal. 2003 Dec 4;33(5):935-45. doi: 10.1016/s0731-7085(03)00425-4.
20. Shyamala Mudavath (2021), Validated RP-HPLC method for simultaneous estimation of aclidinium and formoterol in bulk drug and dosage form, The P.harma Innovation Journal 2020; 9(4): 938-943.