DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUSE ESTIMATION OF DOXOFYLLINE AND ACEBROPHYLLINE IN THEIR COMBINE DOSAGE FORM

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ABSTRACT: *At today many type of drugs are available for respiratory infection. But to know its quality we need to study and test it .Doxofylline and Acebrophylline are the drug use for COPD problem. Doxofylline is a antitussive and bronchodilator effects, and acts as a phosphodiesterase inhibitor. It work by relaxing the muscles in the airway and widens airway. Acebrophylline is a mucolytic. It thin and loosen mucus making it easier to cough out. Together, they make breathing easier. To study we know that it was utilize HPTLC method for this combine form but not RP-HPLC method available for this combine dosage form. .Therefore, it was decided to develop RP-HPLC method for Doxofylline and Acebrophylline in combined dosage Form.*

Key Words: *Doxofylline, Acebrophylline, RP-HPLC, Validation, Stability, COPD.*

Introduction

Brief on Chronic Obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disease that causes obstructed airflow from the lungs. Symptoms include breathing difficulty, cough, mucus (sputum) production and wheezing

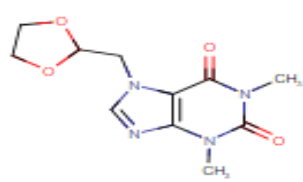
Emphysema and chronic bronchitis are the two most common conditions that contribute to COPD.

Chronic bronchitis is inflammation of the lining of the bronchial tubes, which carry air to and from the air sacs (alveoli) of the lungs. It's characterized by daily cough and mucus (sputum) production

Emphysema is a condition in which the alveoli at the end of the smallest air passages (bronchioles) of the lungs are destroyed as a result of damaging exposure to cigarette smoke and other irritating gases and particulate matter.

Drug Profile

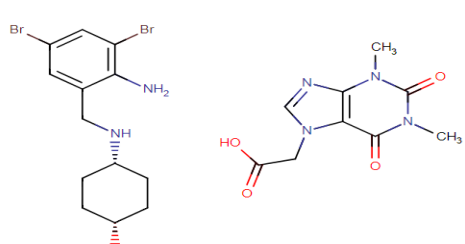
Doxofylline



Melting Point-144-145.5°

Mechnisam of action-The main mechanism of action of doxofylline is unclear. One of the mechanisms of action of is thought to arise from the inhibition of phosphodiesterase activity thus increasing the levels of cAMP and promoting smooth muscle relaxation

Acebrophylline



Mechanism of Action-It act by inhibiting intracellular phosphodiesterase associated with cAMP levels and facilitates bronchial muscles relaxation. Acebrophylline selectively inhibits phosphatidylcholine and

phospholipase A, TNF-alpha and leukotrienes. inhibition of such pro-inflammatory mediators can significantly reduce the airway inflammation and obstruction in chronic stages.

Weight Average-616.311mg

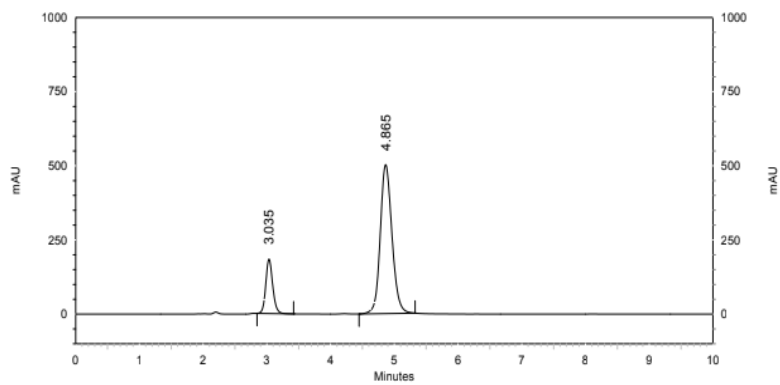
Melting Point-200-210ºC

Material and Reagent

Doxofylline was procured from Oasis laboratory .Acebrophylline was procured from Adavance laboratory Methanol and Acetronitrile are procured from Astron Chemical. Formulation used wasSpirodin-AB 400mg/100mg tablet (doxofylline 400mg/ acebrophylline100mg).HPLC used wasiUHPLC_3000PLUS. UV-Visible double beam spectrophotometer Shimadzu 1800 was used for allspectrophotometric measurements with UV probe. pH meter used from Analab.

Method

Standard solutions of 250 µg/ml of Doxofylline and100 µg/ml Acebrophylline of were injected in Agilent (250 cm × 4.6 cm) 0.5µm column with20 µl micro-syringe. The chromatogram was run



for appropriate minutes with mobile phase Phosphate Buffer (pH 3.0): Acetonitrile (40:60). The detection was carried out at wavelength 274 nm. The chromatogram was stopped after separation achieved completely.

Method Validation

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Doxofylline and Acebrophylline it was found to be in range of given below.

Percentage level	Area	Conc.(µg/mL)	Recovered	Percentage Recovery
150_1	11396220	375	375.0002416	100.0000644
150_2	11396229	375	375.0005377	100.0001434
150_3	11396223	375	375.0003403	100.0000907
100_1	7597481	250	250.000283	100.0001132
100_2	7597478	250	250.0001843	100.0000737
100_3	7597484	250	250.0003817	100.0001527
50_1	3798740	125	125.0002586	100.0002069
50_2	3798735	125	125.0000941	100.0000753
50_3	3798744	125	125.0003902	100.0003122

Recovery data of Doxofylline

Percentage level	Area	Conc.(µg/mL)	Recovered	Percentage Recovery
150_1	1953011	150	150.0004211	100.0002807
150_2	1953023	150	150.0013428	100.0008952

150_3	1953019	150	150.0010355	100.0006904
100_1	1302012	100	100.0005376	100.0005376
100_2	1302006	100	100.0000768	100.0000768
100_3	1302009	100	100.0003072	100.0003072
50_1	651009	50	50.00034695	100.0006939
50_2	651006	50	50.00011653	100.0002331
50_3	651013	50	50.00065417	100.0013083

Recovery data of Acebrophylline

Linearity and Range

The linearity for Doxofylline and Acebrophylline were assessed by analysis of combined standard solution in range of 125-375 µg/mL,50-150 µg/mL respectively. Correlation co-efficient for calibration curve Doxofylline and Acebrophylline found to be near to 1.The regression line equation for Doxofylline and Acebrophyllin are as following:

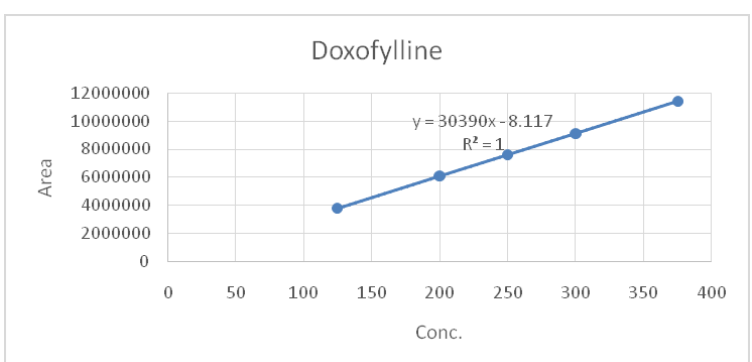
For Doxofylline: y = 30390x - 8.1172 and For Acebrophylline: y =13020x + 3.9655

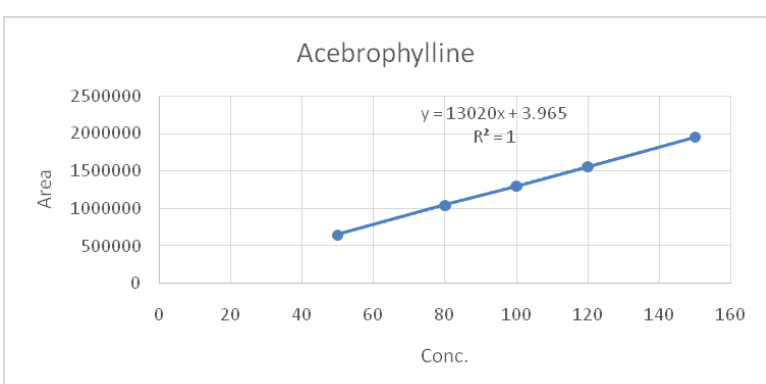
Sr no.	Conc.(µg/mL)	Area
1	125	3798733
2	200	6077975
3	250	7597474
4	300	9116966
5	375	11396214

Linearity data for Doxofylline

Sr no.	Conc.(µg/mL)	Area
1	50	651004
2	80	1041606
3	100	1302005
4	120	1562404
5	150	1953006

Linearity data for Acebrophylline





Precision

The data for repeatability of peak area measurement for Doxofylline(250 µg/mL) and Acebrophylline (100 µg/mL) based on six measurements of same solution of Doxofylline(250 µg/mL) and Acebrophylline (100 µg/mL). The % RSD for Doxofylline and Acebrophylline were found to be 0.01 and 1.11respectively.

Doxofylline				
Sr No.	Conc (µg/ml)	Area	Avg. ± S.D (n=6)	% R.S.D
1.	250	7597929 7596761 7598094 7597389 7597251 7596957	7597397±526.9362	0.01

Acebrophylline				
Sr No.	Conc (µg/ml)	Area	Avg. ± S.D (n=6)	% R.S.D
1.	150	2120036 2085341 2114905 2099954 2152204 2097746	2111698±23442.75	1.11

Robustness

The %RSD should Be less than 2%.

SR NO.	Area at Mobile phase(-5)	Area at Mobile phase(+5)	Area at pH (-0.2)	Area at pH (+0.2)
1.	7596745	7597648	7597632	7595864
2.	7596896	7598456	7597745	7594896
3.	7598869	7698466	7596856	7595864
%RSD	0.02	0.78	0.01	0.01

Robustness data of Doxofylline

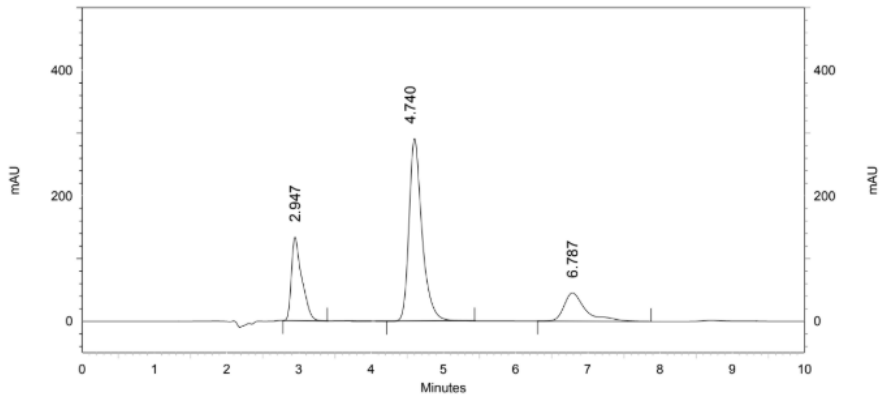
SR NO.	Area at Mobile phase(-5)	Area at Mobile phase(+5)	Area at pH (-0.2)	Area at pH (+0.2)
1.	1302851	1304537	1303654	1300846
2.	1302555	1304936	1304454	1300896
3.	1302745	1305122	1304154	1300625
%RSD	0.01	0.02	0.03	0.01

Robustness data of Acebrophylline

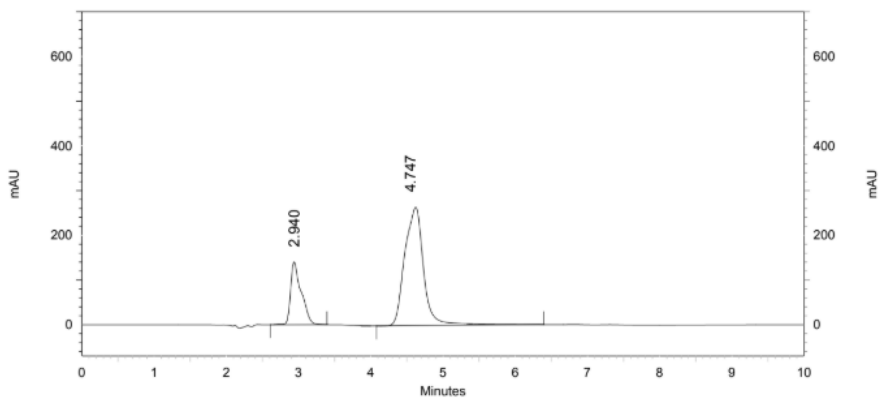
Stability Study

Take a 1 ml of stock solution and put it in different condition. Then neutralize it and make up with diluent. Then inject in HPLC. And study degradation of product by calculation. There are following data of degradation.

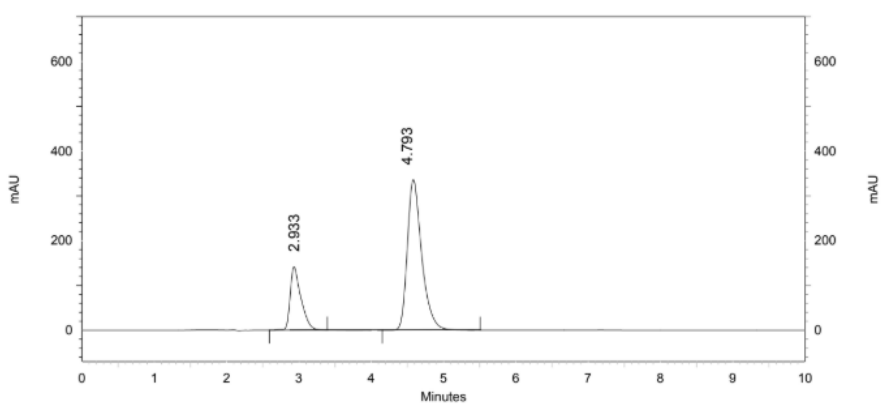
1. Acid Degradation



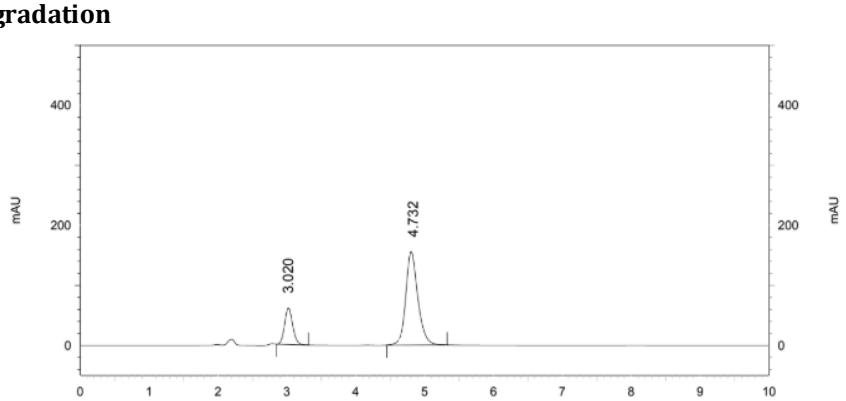
2. Base degradation



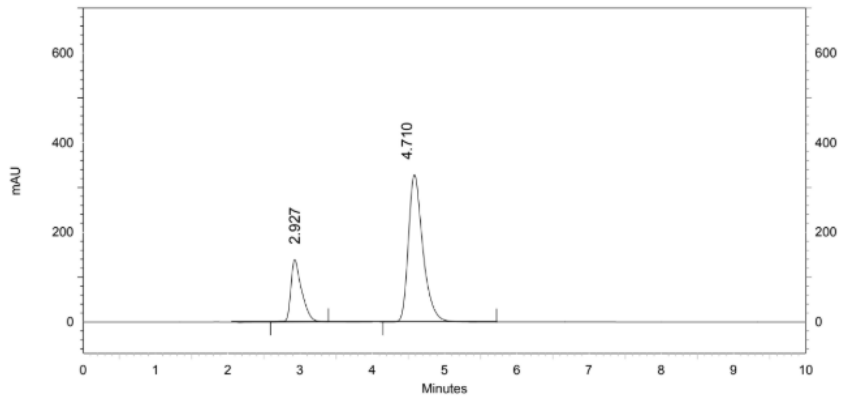
3.Thermal degradation



4. Oxidative degradation



5. Photo degradation



Conclusion

The aim of the present study is to develop sensitive, simple, rapid, economical, precise andaccurate derivative spectrophotometric method to determine Doxofylline and Acebrophylline in marketed tablet formulation. For the analysis of drugs in multicomponent mixtures, derivative spectrophotometry analytical technique is frequently used in today's scenario. Thevalidated developed RP-HPLC methods used for simultaneous estimation of Doxofylline and Acebrophylline were found simple, specific, accurate, rapid, precise, economical andreliable for contemporary analysis of drugs in tablet. The purpose of proposed project wassatisfied as the developed methods were meeting all the validation parameters requirementsof the regulatory guidelines. In the absence of official monograph these validated methodscan be used for determination of Doxofylline and Acebrophylline

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