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RP HPLC Method Development and Validation for Quantitative Estimation of Tiotropium Bromide and Oldaterol

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Abstract

A simple and selective LC method is described for the determination of Tiotropium Bromide and Olodaterol dosage forms. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of 50 volumes of acetonitrile and 50 volumes of Triethyl-amine buffer with detection of 225 nm. Linearity was observed in the range 30-70 µg /ml for Tiotropium Bromide (r2 =0.9987) and 40-80 µg/ml for Olodaterol (r2 =0.9977) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing % RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: RP-HPLC; Method Decvelopment; Tiotropium Bromide, Oldaterol; Pharmaceutical additives.

Introduction

The handling of the chromatography equipment seems complex to the analysts or researchers who have not encountered with it before but once they came to know the mechanism of its parts and its procedure they feel like it's a simple machine to handle. parts namely pump, detectors and injectors with different combinations yields a no of components based on its applications. Analyst should understand the vital role of each part like an an understanding of human anatomy towards your wellbeing and vitality with a knowledge on role of each organ. Once an analyst is familiar with the parts and its role it is easy to generate a data of highest reliability. An understanding of the function of each component on a conceptual basis will make you feel comfortable while handling HPLC system. The present module is intended to serve this very purpose and in simple terms you will appreciate the role of each part and its contribution to overall system efficiency. HPLC is a technique for separation, identification and quantification of components in a mixture. It is especially suitable for compounds with high molecular weights, thermally unstable and which are not easily volatilized can be separated, identified through HPLC technique [1].

Selectivity of HPLC-Method Development

HPLC method is used to analyze most of the drugs because of several advantages of this method like specific, accurate, precise, rapid, automatic and eliminates tedious extraction and isolation procedures [2]. Advantages are as follows:

1. Speed (analysis can be accomplished in 20 min or less).
2. Greater sensitivity (various detectors can be employed).
3. Improved resolution (wide variety of stationary phases).
4. Reliable columns (wide variety of stationary phases).
5. Ideal for substances of low volatility.
6. Easy sample recovery, handling and maintenance.
7. Easy programming of the numerous functions in each module.
8. Time programmable operation sequence, such as initiating operation of detector lamp and pump to obtain a stable base line and equilibrated column before the workday begins.
9. Excellent reproducibility of retention times.
10. An injection volume variable from 0.1 to 100 micro litres without any hardware modification.
11. The flexibility of data analysis.
12. Suitable to avoid any interference from impurity.
13. Suitable for preparative liquid chromatography on a much large scale.

The parameters that are affecting by the Changes in chromatographic conditions

1. Column efficiency (N)
2. Capacity factor (K')
3. Resolution factor (RS)
4. Retention Factor (Rf)
5. Retention time (Rt)
6. Relative retention (Rr)
7. Peak asymmetry factor (As)

Method development guides

1. Information on sample, define separation goals.
2. Need for special procedure, sample treatment.
3. Choose detector and detector settings.
4. Choose the method: preliminary run: estimate the best separation condition.
5. Optimize separation conditions.
6. Validate the method.

Parameters recommendations

1. Theoretical plates (N) = >2000
2. Tailing factor (T) = ≤ 2
3. Resolution (Rs) = >2 between peak of interest and the closest Eluting potential interference
4. Repeatability = RSD ≤ 2%
5. Capacity factor (k1) = >2.0

Method validation

Method validation is the process by which it is established, through laboratory studies, that the performance characteristics of the method meet the requirements for its intended purpose [3-5]. It is a part of the overall validation process that also includes software validation, instrument qualification and system suitability. Typical analytical characteristics used in method validation are highlighted below. Although all analytical procedures or methods used in a regulated laboratory must be validated, this chart focuses specifically on liquid chromatography. Typical analytical characteristics used in method validation, commonly referred to as the "Eight steps of method of validation".

1. Accuracy
2. Precision
3. Specificity
4. Limit of Detection
5. Limit of Quantification
6. Linearity and Range
7. Ruggedness
8. Robustness

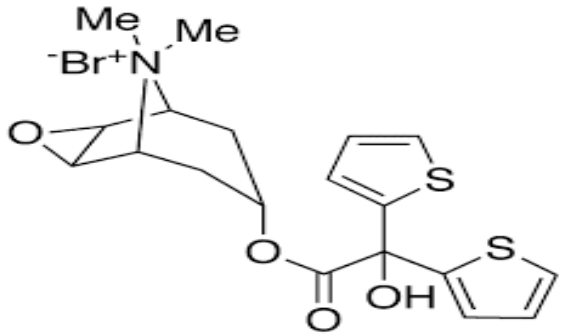
Drug Profile

Tiotropium Bromide

Description: Tiotropium is a long-acting, 24 h, anticholinergic bronchodilator used in the management of chronic obstructive pulmonary disease (COPD) [6,7].

Category: Bronchodilator Agents; Parasympatholytics.

Structure:



IUPAC Name:

(1R,2R,4S,5S,7R)-7-{[2-hydroxy-2,2-bis(thiophen-2-yl)acetyl]oxy}-9,9-dimethyl-3-oxa-9- azatricyclononan-9-ium

Chemical formula: C19H22NO4S2

Molecular weight: 392.512

Mechanism of action: It shoes its mechanism of action by producing a bronchodilatory effect through its selective action on M_3 muscarinic receptors located in the airways. Tiotropium is also referred to as an antimuscarinic or anticholinergic agent.

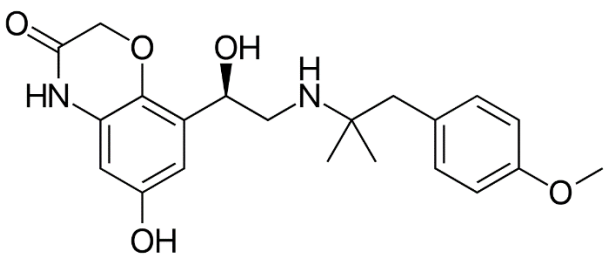
Indication: Used in the management of chronic obstructive pulmonary disease (COPD).

Olodaterol

Description: Olodaterol is a novel, long-acting beta2-adrenergic agonist (LABA)

Category: Drugs for Obstructive Airway Diseases Respiratory System

Structure:



IUPAC Name:

6-hydroxy-8-[(1R)-1-hydroxy-2-{[1-(4-methoxyphenyl)-2-methylpropan-2-yl]amino}ethyl]-3,4-dihydro-2H-1,4-benzoxazin-3-one

Chemical formula: C21H26N2O5

Molecular weight: 386.448

Mechanism of action: Olodaterol is a long-acting beta2-adrenergic agonist (LABA) that exerts its pharmacological effect by binding and activating beta2-drenergic receptors located primarily in the lungs. Beta2-adrenergic receptors are membrane-bound receptors that are normally activated by endogenous epinephrine whose signalling, via a downstream L-type calcium channel interaction, mediates smooth muscle relaxation and bronchodilation. Activation of the receptor stimulates an associated G protein which then activates adenylate cyclase, catalyzing the formation of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA). Elevation of these two molecules induces bronchodilation by relaxation of airway smooth muscles.

Indication: Olodaterol is indicated for use in chronic obstructive pulmonary disease (COPD), for the treatment of asthma.

Methods and Materials

The efficiency was more than 2000 Olodaterol and Tiotropium Bromide. Resolution between two peaks >1.5 [8-11].

Method development trials

Trial-1
Observation: Although the Efficiency was not satisfactory for Olodaterol. The peak response of Tiotropium Bromide was very less.
Trial-2
Observation: Efficiency of both the drugs was good. The run time is very more. The Peaks of Tiotropium Bromide and Olodaterol showed tailing.
Trial-3
Observation: Asymmetry factor for Olodaterol does not meet the system suitability requirements. The run time is 11 minutes.
Trial-4
Observation: Peak Asymmetry factor for Tiotropium Bromide and Diloxanide fuorate does not meet the system suitability requirements.
The run time is very more.
Trial-5
Observation: All the system suitability requirements were met. The peak Asymmetry factor was less than 2 for both Olodaterol and Tiotropium Bromide (Table 1).

Optimized chromatographic conditions

- Mobile phase=Triethylamine buffer+CAN
- pH = (-)
- Column=Inertsil ODS 3V column, C18 (150 x 4.6 ID) 5µm
- Flow rate=1.0 ml/min
- Column temperature= Room temperature (20-25^{o}C)
- Sample temperature= Room temperature (20-25^{o}C)
- Wavelength=225
- Injection volume=20 µL
- Run time=5 min
- Retention time=About 3.420 min for Tiotropium Bromide and 4.567 min for Olodaterol.

Table 1: Method development of five trials.

Chromatographic conditions	**Trail 1**	**Trail 2**	**Trail 3**	**Trail 4**	**Trail 5**	**Optimized**
Mobile phase	Methanol: CAN:Water	Methonol: CAN:PO_4 buffer	PO_4 buffer: CAN: MeOH: ODS	PO_4buffer: MeOH: ACN	TEA:ACN	TEA buffer:CAN
pH	5.0	4.5	4.0	4.5	3.5	
Ratio	50:10:40	0:30:20	30:30:40	30:50:20	50: 50	50:50
Columns	Analytical (Hyperchrom) ODS	Inertsil ODS 3V (250 × 4.6 × ~5µ)	Inertsil ODS 3V, (250 × 4.6 × 5µ)	Inertsil ODS, (250 × 4.6 × 5µ)	Inertsil ODS, (250 × 4.6 × 5µ)	Inertsil ODS 3V column, C_{18} (150 × 4.6 ID) 5µm
Wavelength	225 nm	225nm	225 nm	225 nm	225 nm	225 nm
Flow rate	1 ml/min	1 ml/min	1 ml/min	1 ml/min	1 ml/min	1 ml/min
Observations	-	-	-	-	-	+

Table 2: Chromatogram of Tiotropium Bromide and Olodaterol.

Tiotropium Bromide			**Olodaterol**	
	Standard area	Sample area	Standard area	Sample area
Injection 1	295.884	286.448	836.469	833.214
Injection 1	290.743	286.448	839.072	833.214
Injection 1	292.910	291.818	835.627	837.225
Injection 1	293.024	293.805	834.719	833.303
Injection 1	290.900	280.827	829.554	831.491
Average area	292.692	287.869	835.089	833.689
Standard deviation	3.1102		2.1174	
% RSD	1.9		1.2	
Assay (% purity)	99.8		99.6	

Assay

Preparation of mixed standard solution I

Weigh accurately 60 mg of Tiotropium Bromide and 50 mg of Olodaterol in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of Tiotropium Bromide and 50 µg/ml of Olodaterol is prepared by diluting 1ml to 10 ml with mobile phase. This solution is used for recording chromatogram.

Preparation of mixed standard solution II

(Each inhaler contains Olodaterol-6 mcg Tiotropium Bromide-9 mcg) in 10 ml of volumetric flask and dissolved in sufficient mobile phase. After that filtered the solution using 0.45 µ syringe filter and Sonicated for 5 min. Further dilutions are prepared in 5 replicates. From above stock solution 60 µg/ml of Tiotropium Bromide and 50 µg/ml of Olodaterol is prepared by diluting 1 ml to 10 ml with mobile phase. This solution is used for recording chromatogram.

Calculation

The amount of Olodaterol and Tiotropium Bromide present in the Formulation by using the formula given below, and results shown in above table

$$\% \text{ Assay} = \frac{AT \times WS \times DT \times P \times AW \times 100}{AS \times DS \times WT \times 100 \times LC}$$

Where,

AS: Average peak area due to standard preparation
AT: Peak area due to assay preparation
WS: Weight of Olodaterol/Tiotropium Bromide in mg
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation

Observation

The amount of Tiotropium Bromide and Olodaterol present in the taken dosage form was found to be 99.8% and 99.6% respectively (Table 2).

Validation Parameters

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system [12-14]. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated (Tables 3 and 4).

Acceptance criteria

1. The % RSD for the retention times of Tiotropium Bromide and Olodaterol Peaks from 6 replicate injections of each Standard solution should be not more than 2.0%
2. The % RSD for the peak area responses of Tiotropium Bromide and Lumacaftor peaks from 6 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Tiotropium Bromide and Olodaterol peaks is not less than 2000.
4. The Tailing factor (T) for the Tiotropium Bromide and Olodaterol peak is not more than 2.0.

Observation

The % RSD for the retention times and peak area of Tiotropium Bromide and Olodaterol were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

Table 3: Result of System Suitability of Tiotropium Bromide.

Injection	Retention Time (min)	Peak area	Theoretical plates	Tailing factor (TF)
1	3.773	295.884	4667	1.111
2	3.733	290.743	4813	1.143
3	3.733	292.910	4813	1.176
4	3.770	293.024	4908	1.206
5	3.733	290.900	4813	1.176
Mean	3.748	292.692	-	-
SD	0.0049	55.704	-	-
%RSD	0.14	0.64	-	-

Table 4: Result of System Suitability of Olodaterol.

Injection	Retention Time (min)	Peak area	Theoretical plates	Tailing factor	Tailing factor (TF)
1	4.680	8815.579	2994	1.596	3.247
2	4.637	8708.391	2058	1.627	3.306
3	4.683	8510.447	2436	1.907	3.540
4	4.670	8553.080	2422	1.952	3.531
5	4.680	8815.579	2994	1.596	3.247
6	4.690	8708.391	2058	1.627	3.306
Mean	4.707	292.692	-	-	-
SD	0.064	55.704	-	-	-
% RSD	1.36	0.64	-	-	-

Linearity and range

Preparation of standard stock solution

Standard stock solutions of Tiotropium Bromide and Olodaterol (mg/ml) were prepared by dissolving 60 mg of Tiotropium Bromide and 50 mg of Olodaterol dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase (Tables 5 and 6) [13-20].

Acceptance criteria

The relationship between the concentration of Tiotropium Bromide and Olodaterol and area of Tiotropium Bromide and Olodaterol should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration *vs*. Area for standard preparations of Tiotropium Bromide and Lumacaftor is 0.998 and 0.997. The relationship between the concentration of Tiotropium Bromide and Olodaterol and area of Tiotropium Bromide and Olodaterol is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Figures 1 and 2).

Table 5: Linearity of Tiotropium Bromide.

S.No.	Conc (µg/ml)	Area
1	30	161.404
2	40	213.356
3	50	288.207
4	60	330.037
5	70	7541.702

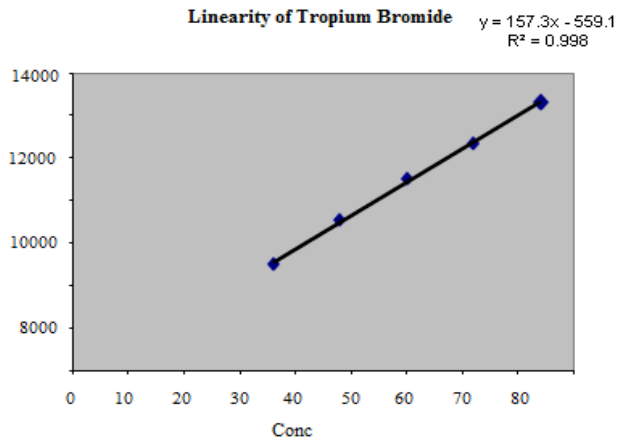


Figure 1: Linearity of Tiotropium Bromide.

Table 6: Linearity of Olodaterol.

S.No.	Conc (µg/ml)	Area
1	40	471.68
2	50	593.037
3	60	836.360
4	70	913.252
5	80	2763.590

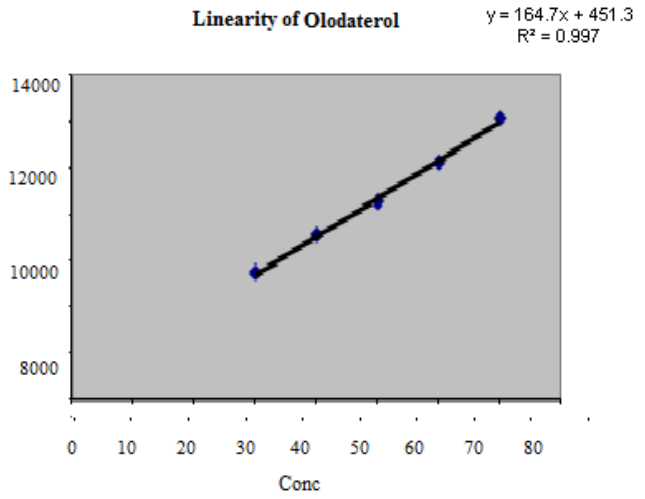


Figure 2: Linearity of Olodaterol.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. Recovery studies were carried out at three different levels 50%, 100%, 150% by addition of standard drug solution to pre-analyzed sample solution to check the accuracy of the method (Tables 7 and 8).

Acceptance criteria

The % recovery of Tiotropium Bromide and Olodaterol should lie between 98% and 102%.

Observation

The % mean recovery of Tiotropium Bromide and Olodaterol is 100.93% and108.93% respectively.

Precision

Method precision

Prepared sample preparations of Olodaterol and Tiotropium Bromide as per test method and injected 6 times in to the column (Tables 9 and 10).

Acceptance criteria

The % RSD of Assay preparations of Olodaterol and Tiotropium Bromide should be not more than 2.0%.

Observation

Test results for Olodaterol and Tiotropium Bromide are showing that the % RSD of Assay results are within limits.

Limit of detection

LOD =3.3σ
S=(3.3)*(2.1174)/164.7
=0.0424µg/ml (Lumacaftor)
=(3.3)* (4.1102)/157.3
=0.0862µg/ml (Ivacaftor)

Where,
σ = the standard deviation of the response
S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation

The LOD for this method was found to be 0.0862 µg/ml and 0.0424 µg/ml for Olodaterol.

Limit of quantification

LOQ=10σS
=(10)*(2.1174)/164.7
=0.128µg/ml(Lumacaftor)
=(10)* (4.1102)/157.3
=0.261µg/ml(Ivacaftor)

Where,
σ = the standard deviation of the response
S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation

The LOQ for this method was found to be 0.261 µg/ml for Tiotropium Bromide and 0.128 µg/ml for Lumacaftor

Table 7: Recovery results for Tiotropium Bromide.

Recovery level	Accuracy of Tiotropium Bromide					Average % Recovery
	Amount Taken (mcg/ml)	Area	Average area	Amount recovered	%Recovery	
50%	50	5789.751	5789.898	54.03	102.18	100.93%
	50	5790.192				
	50	5789.751				
100%	60	7070.222	7071.38	32.99	98.99	
	60	7073.715				
	60	7070.222				
150%	70	7242.895	7258.66	101.62	101.62	
	70	7290.219				
	70	7242.895				

Table 8: Recovery results for Olodaterol.

Recovery level	Accuracy of Olodaterol					Average % Recovery
	Amount Taken (mcg/ml)	Area	Average area	Amount recovered	%Recovery	
50%	80	2099.428	2100.890	19.594	126.70	108.93
	80	2103.816				
	80	2099.428				
100%	96	2602.209	2590.043	33.15	98.999	
	96	2565.673				
	96	2602.249				
150%	112	2642.187	2645.416	101.11	101.11	
	112	2651.875				
	112	2642.187				

Table 9: Precision of Tiotropium Bromide.

S.NO	Rt	Area
1	3.717	286.770
2	3.717	287.146
3	3.733	283.647
4	3.727	285.277
5	3.733	281.675
6	3.733	5740.309
Avg	3.726667	
SD	0.000784	
%RSD	0.0053	

Table 10: Precision of Oladaterol.

S.NO	Rt	Area
1	2.673	811.336
2	2.673	810.062
3	2.687	811.956
4	2.683	810.151
5	2.687	806.248
6	2.203	2058.026
Avg	3.726667	
SD	0.000784	
%RSD	0.0053	

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision (Table 11).

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

Observation

From the observation it was found that the system suitability parameters were within
limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts (Table 12).

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Observation

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Table 11: Robustness of Tiotropium Bromide and Olodaterol.

Parameter	Tiotropium Bromide		Olodaterol	
Flow rate				
0.8 ml/min	4.660	1.171	3.363	1.333
1.2 ml/min	3.490	1.167	2.597	1.391
Wave length				
223 nm	3.490	1.167	2.597	1.391
227nm	3.707	1.219	2.203	1.409

Table 12: Results for Ruggedness.

Tiotropium Bromide	%Assay	Olodaterol	%Assay
Analyst 01	99.92%	Analyst 01	98.64%
Anaylst 02	98.36%	Anaylst 02	99.60%

Conclusion

From the above experimental results and parameters, it was concluded that, this newly developed method for the simultaneous estimation Tiotropium Bromide and Olodaterol was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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