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Method Development and Validation of Olodaterol and Tiotropium by RP-HPLC Method

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A B S T R A C T

Reverse phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase, i.e., the sorbent. A schematic diagram showing the binding of a peptide or a protein to a reversed-phase surface. The literature review reveals the few UV and HPLC methods for the estimation of Olodaterol and tiotropium alone and in combination with other drugs. Few methods are also reported for estimation of both drugs from formulation .we intend to develop a RP-HPLC and UV methods by simultaneous determination with simple, rapid, greater sensitivity and faster elution.

Keywords: olodaterol, tiotropium, RP-HPLC

A R T I C L E I N F O

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1. Introduction

Analytical chemistry: Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of

clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- Qualitative analysis is the identification of elements, species and/or compounds present in sample.
- Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

Analytical techniques there are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various analytical techniques. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte (s) in a sample that can be measured. Atomic, molecular spectrometry and chromatography, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis.

Spectrometric techniques may involve either the emission or absorption of electromagnetic radiation over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels. The most important atomic and molecular spectrometric techniques and their principal applications are listed in Table.No.2. Chromatographic techniques provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called hyphenation, provides a powerful means of separating and identifying unknown compounds. Electrophoresis's another separation technique with similarities to chromatography that is particularly useful for this parathion of charged species. The principal separation techniques and their applications

2. Materials and Methods

2.1. Materials and methods

Olodaterol working standard, Tiotropium working standard, Potassium dihydrogenorthophosphate, Sodium perchlorate, Perchloric acid, Ortho phosphoric acid, Methanol, Acetontrile, Water, 0.45 µ m Nylon filter, 0.45µ m PVDF filter.

Analytical Method Development

Selection of chromatographic condition

Proper selection of the method depends up on the nature of the sample (ionic/ionisable/neutral molecule), its molecular weight and solubility. The drugs selected in the present study, were polar in nature. Thus reverse phase HPLC was selected for the initial separation because of its simplicity, suitability, ruggedness and its wider usage.

Initial separation condition

The mobile phase selected to elute the drug from the stationary phase was milliQ water and HPLC methanol, because of its favorable UV transmittance, low viscosity and low back pressure.

Trials

Optimized Method Preparation of mobile phase: Take 6.8 gm of KH2PO4 into 1000ml volumetric flask dissolved in HPLC graded water and adjust pH upto 3 with ortho phosphoric acid. From the above prepared buffer take 300 ml (30%) and 700ml Methanol (70%) HPLC were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: Mobile phase was used as Diluent.

Chromatographic conditions:

Flow rate : 0.8 ml per min

Column : Agilent C18 (4.6 x 150mm, 5µm)

Detector wavelength : 254nm

Column oven: Ambient

Injection volume: 10µl

Run time: 10 min

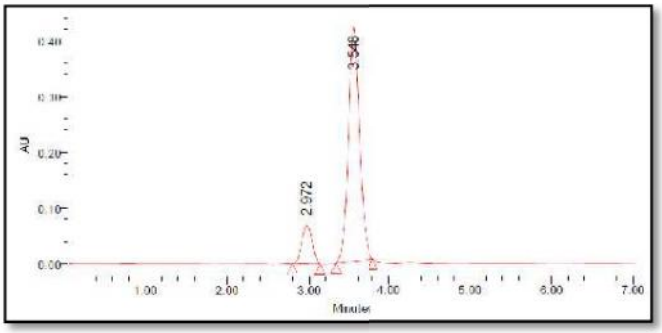

Figure 1: Standard Chromatogram for Optimized Method

3. Results and Discussion

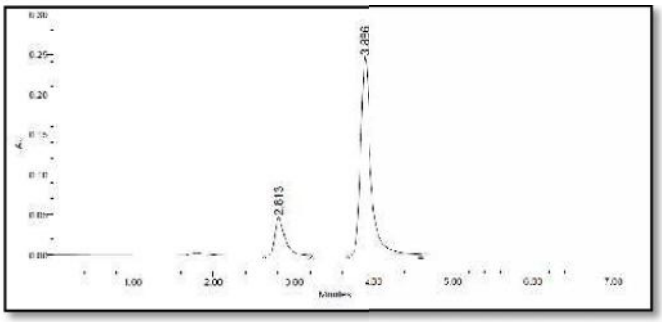

Figure 2: System Suitability

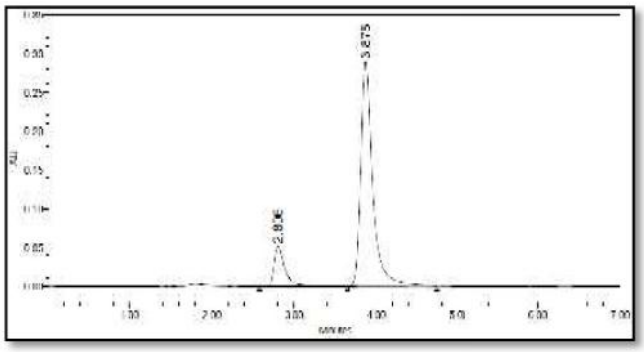

Figure 3: Chromatogram for Linearity level

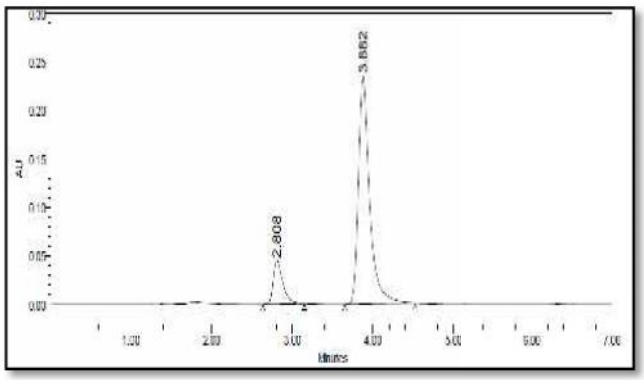

Figure 4: Precision

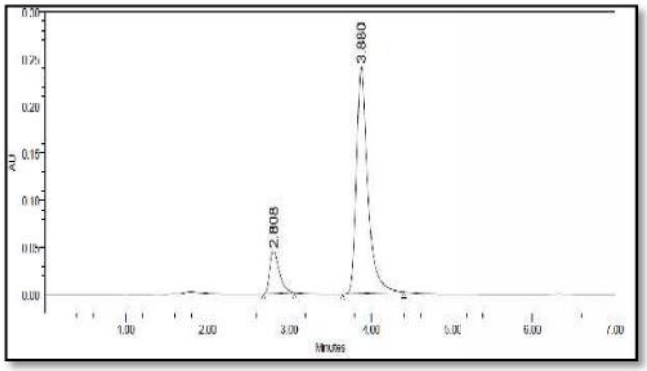

Figure 5: Chromatograms for Intermediate precision

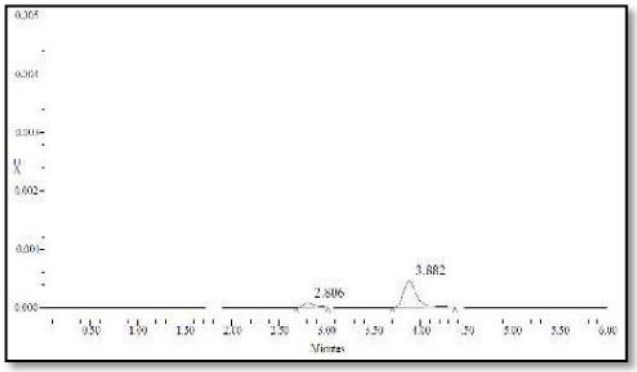

Figure 6: Chromatogram for Olodaterol and Tiotropium

Calculation of S/N Ratio for Olodaterol:
Average baseline noise obtained from Blank: 48 µV
Signal Obtained from LOD solution (0.15% of target assay concentration): 146µ V S/N = 146/48 = 3.041
Acceptance Criteria: S/N Ratio value shall be not more than 3 for LOD solution.
Calculation of S/N Ratio for Tiotropium:
Average Baseline Noise obtained from Blank: 48µ V
Signal Obtained from LOD solution (0.22% of target assay concentration): 148 µ V S/N = 148/48 = 3.08
Acceptance Criteria: S/N Ratio value shall be not more than 3 for LOD solution.

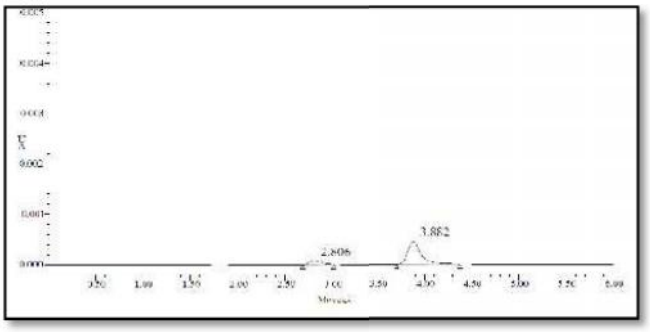

Figure 7: Chromatogram for Olodaterol and Tiotropium

Calculation of S/N Ratio for Olodaterol:
Average Baseline Noise obtained from Blank: 48 µ V
Signal Obtained from LOQ solution (0.05% of target assay concentration): 470µ V S/N = 470/48= 9.79
Acceptance Criteria: S/N Ratio value shall be not more than 10 for LOQ solution.
Calculation of S/N Ratio for Tiotropium:
Average Baseline Noise obtained from Blank : 48 µ V
Signal Obtained from LOQ solution (0.06% of target assay concentration): 498µ V S/N = 498/48 = 10.37
Acceptance Criteria: S/N Ratio value shall be not more than 10 for LOQ solution.

4. Conclusion

High performance liquid chromatography and spectroscopy are at present one of the most sophisticated tool of the analysis. The estimation of Olodaterol and Tiotropium was done by RP-HPLC and UV Methods. The mobile phase was optimized with consists of Phosphate buffer pH 3: Methanol mixed in the ratio of 30:70 % v/ v. Agilent C18 column C18 (4.6 x150mm, 5µm, Make: XTerra) or equivalent chemically bonded to porous silica particles was used as stationary phase.. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. The linearity range of Olodaterol and Tiotropium were found to be from 75-375 µg/ml and 10-50µg/ml respectively. Linear regression coefficient was not more than 0.999. The maximum absorbance is found to be at 254 nm. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102%of Olodaterol and Tiotropium. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision

Table1: Linearity of Olodaterol

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	839286
2	II	40 ppm	1067774
3	III	60 ppm	1246474
4	IV	80 ppm	1439994
5	V	100 ppm	1639065
Correlation Coefficient			0.99816

Table 2: Linearity of Tiotropium

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	626221
2	II	15 ppm	778750
3	III	20 ppm	931447
4	IV	25 ppm	1070162
5	V	30 ppm	1196060
Correlation Coefficient			0.99816

Table 3: Repeatability of Olodaterol

Injection No	Peak Area	R_t
1	1247256	2.808
2	1248579	2.807
3	1243273	2.804
4	1243262	2.806
5	1249574	2.805
Avg	1246389	
S	2965.62	
% RSD	0.23793	

Table 4: Repeatability of Tiotropium

Injection No	Peak Area	R_t
1	9	3.880
2	9	3.882
3	9	3.881
4	9	3.878
5	9	3.882
Avg	929	
SD	48	
% RSD	0	

Table 5: Intermediate Precision Olodaterol

Injection No	Peak Area	R_t
1	1231404	2.808
2	1233196	2.806
3	1231008	2.805
4	1238575	2.807
5	1232407	2.804
Mean	1233318	

SD	3061.06	
%RSD	0.2481	

Table 6: Intermediate Precision Tiotropium

Injection No	Peak Area	R_t
1	912412	3.882
2	913062	3.880
3	909642	3.801
4	916881	3.882
5	914005	3.880
Mean	913200.4	
SD	2621.886	
%RSD	0.287	

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