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Comparison of conventional HPLC with UPLC method for determination of albuterol sulfate and it's impurities in pharmaceutical formulation

P.N. DALVI, Z. FILMWALA, K. AVINASH and T BANE*

Analytical Development Laboratory, Cipla Ltd.
L. B. S. Marg. Vikhroli, Mumbai - 400 083 (India)

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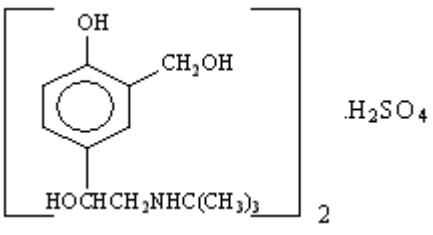
ABSTRACT

A simple, rapid, gradient reversed phase Ultra Performance Liquid Chromatographic method with ultraviolet detection (UPLC-UV) was developed for analysis of Albuterol sulfate and its impurities in Albuterol sulfate inhalation solution pharmaceutical formulation. Chromatography was carried out on a Waters Acquity BEH C_{18} (1.7µm, 100mm.x 2.1mm) column using 0.02 M Potassium Dihydrogen Orthophosphate as mobile phase A and Acetonitrile as mobile phase B. at a flow rate of 0.150mL/min and a 210 nm detection. Results from table number 3 and 4 showed that Ultra Performance Liquid Chromatography exhibited a rapid and separation efficiency superior to that of existing conventional HPLC method.

Key words : UPLC, HPLC, Albuterol Sulfate, Time, Cost.

INTRODUCTION

Albuterol sulfate, chemically known as (13-benzenedimethanol, a'- [(1,1-dimethylethyl) amino]-4-hydroxy, sulphate (2; 1) (salt), (+)- is a relatively selective beta2-adrenergic bronco dilator. Albuterol is official generic name in United States. The World Health Organisation recommended name for the drug is Salbutamol. It is bronochodilator used in treating asthma and other conditions reversible airway obstruction.



structure of Albuterol sulphate *M.*: 576.7

Various methods for determination of Albuterol sulfate and its impurities in pharmaceutical formulations have been reported using C8 column, using different eluant. The most commonly used analytical column is C_8.

The aim of the study is to develop a sensitive, faster method, which can reduce the time and the cost of analysis with better resolution of the impurities, and to compare it with existing HPLC method.

Degradation of albuterol sulphate

Impurity C: (1RS)-2-[(1,1-dimethylethyl) amino]-1-(4-hydroxy-3-methylphenyl) ethanol. Impurity D: [5-[(1RS)-2-[(1,1-dimetylethyl) amino]-1-hydroxyethyl]-2-hydroxybenzaldehyde.Impurity F: 1,1-[oxybis [methylene (4-hydroxy-1, 3-penylene)]] bis [2-[(1,1-dimetylethyl) amino] ethanol]. Impurity I: (1RS)-2-[(1,1-dimetylethyl) amino]-1-[3-(hydroxymethyl)-4-benzyloxyphenyl] ethanol.

EXPERIMENTAL

Material, chemicals and reagent

Albuterol sulfate working standard, Albuterol sulfate impurity C (EPCRS), Albuterol sulfate impurity D (EPCRS), Albuterol sulfate impurity F (EPCRS), Albuterol sulfate Impurity I (EPCRS), and Albuterol sulfate inhalation solution (1.25mg) were used for analysis. S.D. Fine Chemical's HPLC grade water was used throughout the experiment. All organic solvents used were of HPLC gradient grade and Potassium dihydrogen orthophosphate, orthophosphoric acid of analytical grade. mdi 0.20µm nylon filters were used for mobile phase filtration and mdi 0.20µm syringe filters were used to filter samples.

Equipment/Chromatographic systems

UPLC analysis was performed on Waters Acquity System. Equipped with TUV Detector (Dual wavelength UV detector (CO5UPT 306M), Binary Solvent Manager (CO5UPB 736M), System Manager (CO5UPS 665N) & Column Manger. Separation was performed on reverse phase column (Waters ACQUITY BEH C18, 1.7µm, 100mm x 2.1mm) maintained at 26.0°C by temperature control module (Column Oven Manager), Sonicator (Ultrasonic) was used to dissolve standard, impurities and samples in diluent. E-cord technology is provided to the system to accumulate total number of injections and to keep track of temperature and pressure changes during the development. Data were obtained and processed using Empower Software 1154(Waters corporation).

Mobile phase and chromatographic analysis

The mobile phase were prepared daily and filtered through a 0.20 µm nylon filters and degassed using a vaccum membrane degasser.

Preparation of mobile phase

Mobile phase A

Weighed accurately 1.36gm of Potassium Dihydrogen Orthophosphate into a 500ml volumetric flask (0.02 M). Dissolved in water with sonication and diluted up to the mark with water and mixed. Filtered and degassed

Mobile phase B

Filtered and degassed Acetonitrile.

Preparation of diluen

Filtered and degassed distilled water.

Once the column was conditioned with the mobile phase the elution was monitored at wavelength 210 nm with step gradient programme. Separately 10µL of previously filtered blank, resolution solution; placebo solution, standard solution and sample solution were injected.

Preparation of resolution solution

Impurity stock solution

5.0mg of each impurity (impurity C, impuriy D, impurity F) were weighed and transferred in separate 50mL standard volumetric flask dissolved and diluted to volume with diluent and mixed. 5.0mg of impurity I was weighed and transferred in a separate 50mL standard volumetric flask dissolved in minimum amount of Acetonitrile and diluted to volume with diluent and mixed. Further diluted 5.0mL of each solution to 50mL with diluent.

Table 1: Gradient programme for UPLC method

Time (min)	Flow mL/min	% M.P. A	% M.P. B	Comment
Initial	0.150	90.0	10.0	Isocratic
4.00	0.150	78.0	22.0	Linear
5.00	0.150	75.0	25.0	Linear
6.00	0.150	50.0	50.0	Linear
8.00	0.150	90.0	10.0	Isocratic
9.00	0.150	90.0	10.0	Isocratic

Resolution solution

25.0mg of Albuterol sulfate working standard was weighed in 100mL standard volumetric flask and dissolved in 70mL diluent with the help of sonicator allow to attain room temperature 3.0mL of each impurity C, impurity D, impurity F, impurity I stock solution was added to it diluted to the volume with diluent and mixed.

Preparation of standard solution

40.0mg of Albuterol sulfate working standard was weighed in 100mL standard volumetric flask, 70mL of diluent was added to dissolve with the help of sonicator allowed it to attain room temperature and diluted to volume with diluent and mixed. Further 5.0mL of this solution was diluted to 50.0 mL with diluent and mixed. Further 2.0mL of the above solution was diluted to 100.0mL. with diluent and mixed. (0.5ppm i.e. 0.1% with respect to sample high load)

Table 2: Gradient programme for HPLC method

Time (min)	% M.P. A	% M.P. B	Comment
Initial	100.0	0.0	Isocratic
3.00	100.0	0.0	Linear
50.00	50.0	50.0	Linear
60.00	50.0	50.0	Linear
65.00	100.0	0.0	Isocratic
75.00	100.0	0.0	Isocratic

Table 3 : Precision study done by UPLC method

Impurity Name	SPL 1	SPL 2	SPL 3	SPL 4	SPL 5	SPL 6	Mean	% RSD
Imp C (%)	0.167	0.170	0.173	0.166	0.173	0.171	0.170	1.74
Imp D (%)	0.025	0.025	0.026	0.025	0.025	0.025	0.025	1.62
Imp F (%)	0.013	0.013	0.014	0.013	0.013	0.013	0.013	3.10
Imp I (%)	0.006	0.006	0.006	0.006	0.007	0.006	0.006	6.62
Single maximum unknown impurity (%)	0.133	0.137	0.138	0.133	0.136	0.136	0.136	1.53
Total impurity (%)	0.411	0.450	0.455	0.439	0.469	0.467	0.454	2.80

SPL = sample, RSD = relative standard deviation

Table 4 : Comparative results of HPLC and UPLC method

HPLC		UPLC	
Impurity name	**% Impurity**	**Impurity name**	**% Impurity**
Impurity C	0.20	Impurity C	0.17
Impurity D	0.03	Impurity D	0.03
Impurity F	0.02	Impurity F	0.01
Impurity I	0.01	Impurity I	0.01
Single maximum unknown impurity	0.16	Single maximum unknown impurity	0.14
Total impurity	0.49	Total impurity	0.45

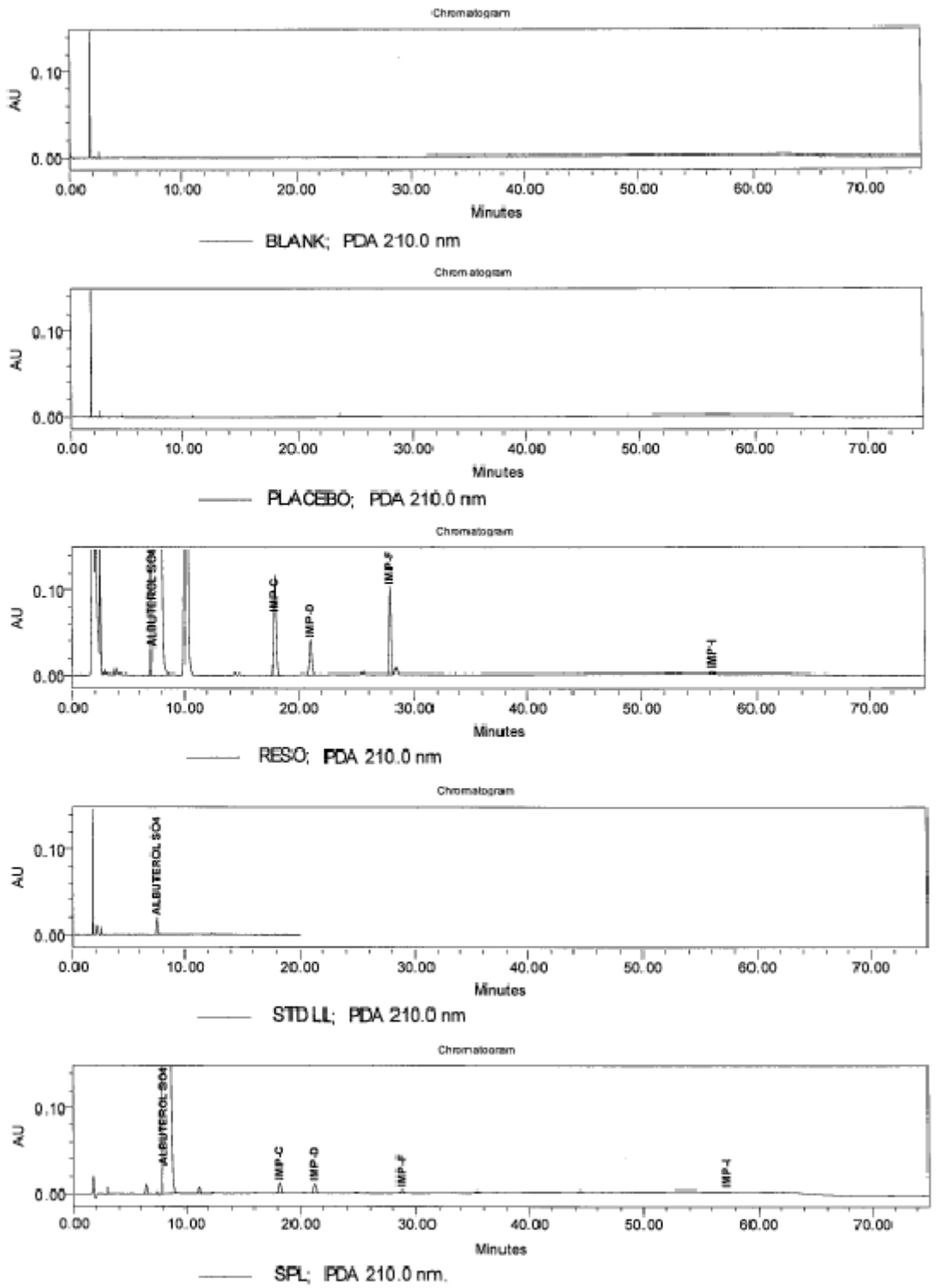
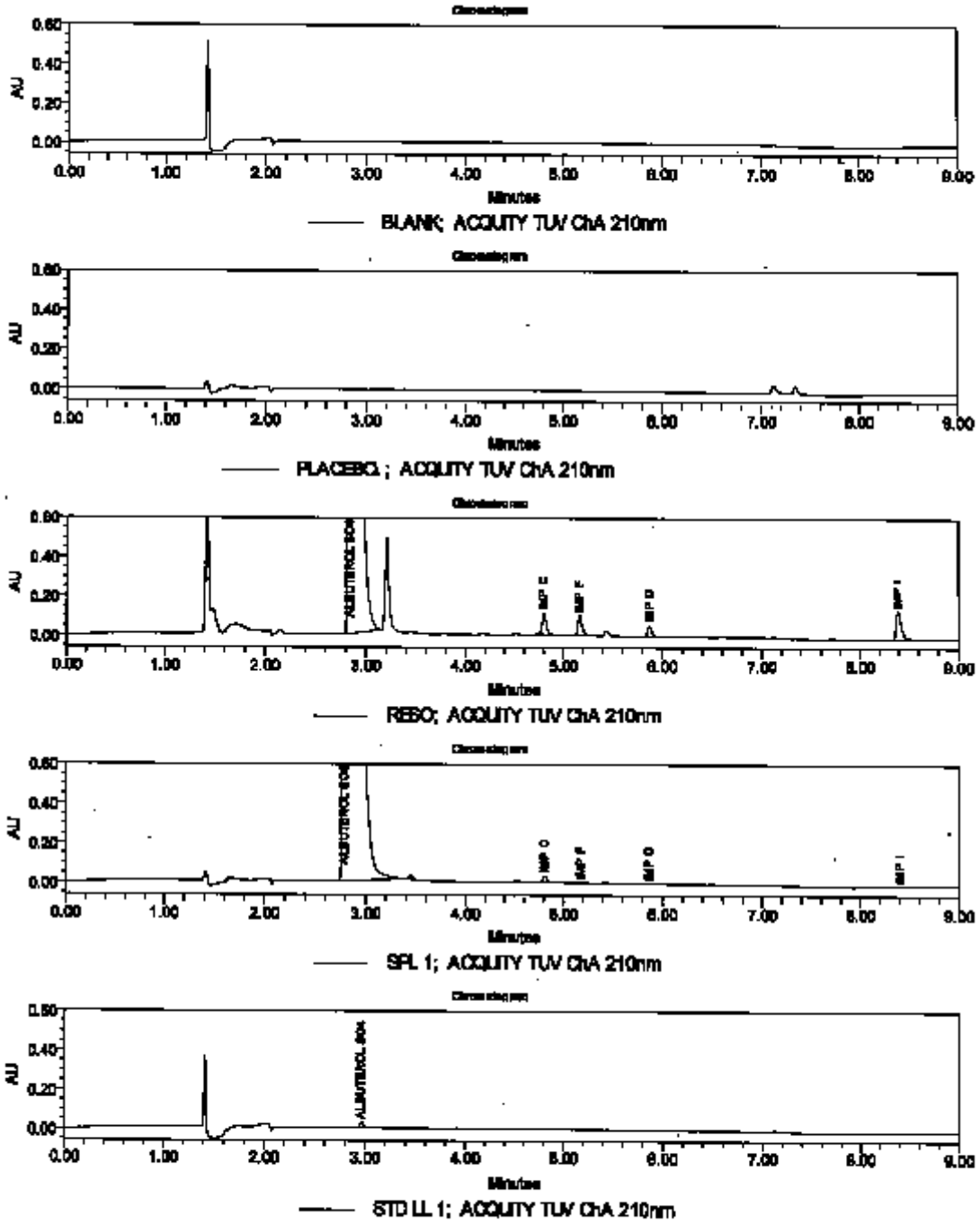


Fig. 1: Chromatograms of HPLC conventional method



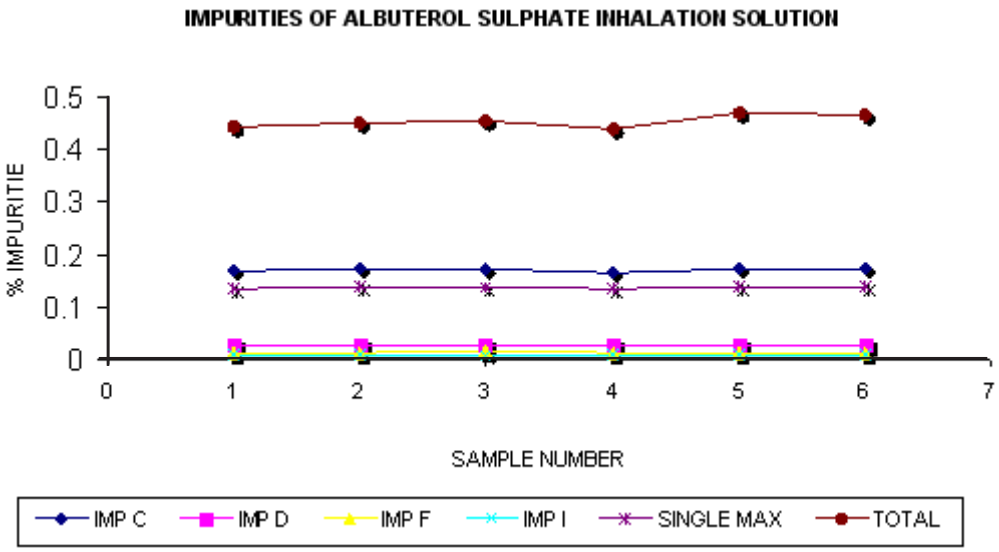
UPLC chromatograms of Albuterol Sulphate Inhalation Solution Formulation

Fig. 2: Chromatograms of UPLC method

Table 5 : Comparison of HPLC and UPLC method

	HPLC		UPLC	
1	Injection Volume : 50 µl		Injection Volume : 10 µl	
2	Flow rate: About 1.0ml/minutes		Flow rate: About 0.150 ml/minutes	
3	Run time = 75 minutes		Run time = 9 minutes	
4	Name of Impurity	RRT	Name of Impurity	RRT
	Impurity C	2.66	Impurity C	1.72
	Impurity D	3.11	Impurity D	1.95
	Impurity F	3.87	Impurity F	1.86
	Impurity I	8.08	Impurity I	3.01
	Single maximum unknown Impurity	1.59	Single maximum unknown Impurity	1.23

RRT = Relative Retention Time



Fg. 3 : Graphical representation of % Impurities by UPLC method

Preparation of placebo solution

Placebo solution was filtered through syringe filter. (mdi syringe filter 0.20 um pore size) and injected as such.

Preparation of sample

Sample as such (416.67ppm) (1.25mg/ 3mL of Albuterol sulfate equivalent to Albuterol) was filtered through syringe Filter. (mdi syringe filter 0.20 um pore size) and injected as such. 416.67ppm

RESULTS AND DISCUSSION

Existing gradient High Performance Liquid Chromatography method

(HPLC analysis was performed on Waters Alliance System) equipped with (Photodiode Array detector (Model 2996). Separation was performed on a reversed-phase column (Inertsil C8, 5µm, 4.6mm x 150mm) maintained at 25.0° C by a temperature-controlled module. The mobile phase A consisted of filtered and degassed mixture of 5 volumes of Acetonitrile with 95 volumes of 0.02 M Potassium dihydrogen orthophosphate the pH of the solution is about 4.70. And mobile phase B consisted of filtered and degassed mixture of 40 volumes of Acetonitrile and 60 volumes of 0.02 M Potassium dihydrogen orthophosphate the pH of the solution is about 5.40. The chromatography was monitored at 210nm and the flow rate of 1.00mL/min. separately (50µL) of blank, resolution solution; placebo solution and standard solution and sample solution were injected and monitored. A well-separated resolution chromatogram was achieved with a run tine of 75.0 mins with gradient programme listed below.

To confirm the resolution chromatogram pattern each known impurity was spiked and injected individually and the elution pattern for Albuterol sulfate and it's impurities was confirmed blank, resolution, placebo and sample were injected sequentially and it was confirmed that there was no interference of blank and placebo peak at the retention time of Albuterol sulfate, and it's known and unknown impurities. The % known, unknown and total impurities were calculated with respect to Albuterol sulfate standard solution The relative standard deviation for retention time of five replicates of standard preparation was 0.22 % (Limit not more than: 1.0%) and for area was 0.90 % (Limit not more than: 2.0%). relative standard deviation of % impurity for six-sample preparation is listed below (Limit: not more than: 10.0%). Limits for % impurities are as per ICH guidelines 1994.

CONCLUSION

We developed rapid, sensitive, precise UPLC-UV method for quantitative determination of Albuterol sulfate and it's impurities. Thus the proposed method could overcome the existing HPLC method in terms of cost (reagents and HPLC grade solvents, instrument life, power supply) and the most important is analysis time. This method can also be used for assay determination of Albuterol sulfate in formulation and raw material. The mobile phase consumption for existing HPLC method is 75mL/run where as for UPLC method it is 1.35mL/run. So the total amount of mobile phase solvent consumption during analysis is reduced which is not negligible for environment viewpoint.

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