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Ultra Performance Liquid Chromatography Method for Fluticasone Propionate

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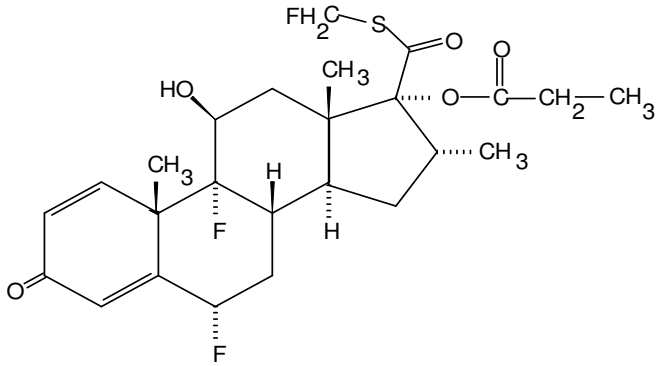
A simple, rapid, sensitive, gradient reverse phase ultra performance liquid chromatographic method involving ultraviolet detection (UPLC-UV) was developed for analysis of fluticasone propionate and it's impurities in nasal spray pharmaceutical formulation. It was carried out on a waters Acquity BEH C18 (1.7 µm, 100 mm × 2.1 mm) column using methanol:ammonium acetate:acetonitrile (50:35:15) as mobile phase A and methanol:acetonitrile (50:15) as mobile phase B at a flow rate of 0.250 mL/min and a 239 nm detection. Results showed that ultra performance liquid chromatography exhibited a rapid, sensitive and separation efficiency superior to that of existing conventional HPLC method.

Key Words: Ultra performance liquid chromatography, High performnace liquid chromatography, Fluticasone propionate.

INTRODUCTION

Fluticasone propionate is a synthetic corticosteroid having the chemical name S-(fluoromethyl)-6α, 9-difluoro-11β-17-dibydroxy-16α-methyl-3-oxaandrosta-1,4-diene-17β-carbothior,17-propionate. It is synthetic, tri fluorinated glucocorticoid with potent antiinflammatory activity.



Structure of fluticasone propionate (m.w. 506.22)

Various methods for determination of fluticasone propionate and its impurities in pharmaceutical formulations have been reported using C18 column, using different eluant. The most commonly used analytical column is C_{18}.

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.

Impurities of fluticasone propionate[1]:

Impurity A: 6α,9α-Difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carboxylic acid.

Impurity B: S-Fluoromethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3oxo-17α-acetyloxyandrosta-1, 4-diene-17β-carbothioate.

Impurity C: S-Methyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate.

Impurity D: S-Chloromethyl 6α, 9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1, 4-diene-17β-carbothioate.

Impurity E: S-Iodomethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1, 4-diene-17β-carbothioate.

Impurity F: *Bis*(6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1, 4-diene-17β-carbonyl) disulphide.

EXPERIMENTAL

Fluticasone propionate working standard, impurity A, B, C, D, E and F, and fluticasone propionate nasal spray (50 mg) were used for development. S.D. Fine Chemicals HPLC grade water was used throughout the experiment. HPLC gradient grade acetonitrile from J.T. Baker, HPLC grade methanol from Merck and ammonium dihydrogen orthophosphate and orthophosphoric acid of S.D. Fine Chemical was analytical grade. mdi 0.20 µm nylon filters were used for mobile phase filtration and mdi 0.2 µm syringe filters were used to filter samples.

UPLC analysis[2,3] was performed on waters acquity system. Equipped with TUV detector (Dual wavelength UV detector (CO5UPT 306 M), Binary Solvent Manager (CO5UPB 736 M), System Manager (CO5UPS 665 N)[4] and Column Manger. Separation was performed on reverse phase column[5] (Waters ACQUITY BEH C18, 1.7 µm, 100 mm × 2.1 mm) maintained at 35 ºC 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[6]: The mobile phase were prepared daily and filtered through a 0.2 µm nylon filters and degassed using a vaccum membrane degasser.

Preparation of mobile phase: Preparation of buffer solution, weighed accurately 1.15 g of ammonium dihydrogen orthophosphate into a 1000 mL volumetric flask. Dissolved in water with sonication and diluted up to the mark with water and mixed. pH of the buffer solution was adjusted to 3.50 with dilute orthophosphoric acid (1 in 10).

Mobile phase A: Mixed 50 volumes of methanol, 35 volumes of buffer solution and 15 volumes of acetonitrile, filtered and degassed.

Mobile phase B: Mixed 50 volumes of methanol and 15 volumes of acetonitrile, filtered and degassed.

Preparation of diluent: Equal volumes of acetonitrile and water, filtered and degassed.

Once the column was conditioned with the mobile phase the elution was monitored at wavelength 239 nm with step gradient programme.

Time (min)	Flow (mL/min)	M.P. A (%)	M.P. B (%)	Comment
Initial	0.250	100.0	0.0	Isocratic
10.0	0.250	100.0	0.0	Isocratic
15.0	0.250	20.0	80.0	Linear
16.0	0.250	20.0	80.0	Isocratic
18.0	0.250	100.0	0.0	Linear
20.0	0.250	100.0	0.0	Isocratic

M.P. A = Mobile phase A; M.P. B = Mobile phase B.

Separately 5 µL of previously filtered blank, resolution solution, placebo solution, standard solution and sample solution were injected.

Preparation of resolution solution

Impurity stock solution: 5 mg of each impurity (A, B, C, D and E) were weighed and transferred in separate 100 mL standard volumetric flask dissolved and diluted to volume with diluent and mixed.

Resolution solution: 12.5 mg of fluticasone propionate working standard was weighed in 50 mL standard volumetric flask and dissolved in 5 mL acetonitrile. 5 mL of each impurity stock solution was added to it and diluted to the volume with diluent and mixed.

Preparation of standard solution: 25 mg of fluticasone propionate working standard was weighed in 100 mL standard volumetric flask, 10 mL of acetonitrile was added to dissolved and diluted to volume with diluent and mixed. Further 5 mL of this solution was diluted to 50 mL with diluent and mixed. Further, again 5 mL of the above solution was diluted to 250 mL with diluent and mixed.

Preparation of placebo solution: 5 g of placebo was weighed in 10 mL standard volumetric flask. 3 mL of acetonitrile was added and sonicated for 10 min. The solution was allowed to attain room temperature and diluted to the volume with acetonitrile and mixed. The solution was filtered through syringe filter (mdi syringe filter 0.2 µm pore size).

Preparation of sample: 5 g of sample (equivalent to 2.5 mg of fluticasone propionate) was weighed in 10 mL standard volumetric flask. 3 mL of aetonitrile was added and sonicated for 10 min. The solution was allowed to attained room temperature and diluted to the volume with acetonitrile and mixed. The solution was filtered through syringe filter (mdi syringe filter 0.2 µm pore size).

RESULTS AND DISCUSSION

Existing gradient high performance liquid chromatography method: (HPLC analysis was performed on waters alliance system) equipped with PDA detector (Model 2996) separation was performed on a reversed-phase column[7-9] maintained at 40 °C by a temperature controlled module. The mobile phase A consisted of filtered and degassed a solution containing 0.05 % v/v of phosphoric acid and 3 % v/v of methanol in acetonitrile and mobile phase B consisted of filtered and degassed a solution containing 0.05 % v/v of phosphoric acid and 3 % v/v of methanol in water. The chromatography was monitored at 239 nm and the flow rate of 1 mL/min. Separately (50 µL) 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 time of 95 min with gradient programme listed below:

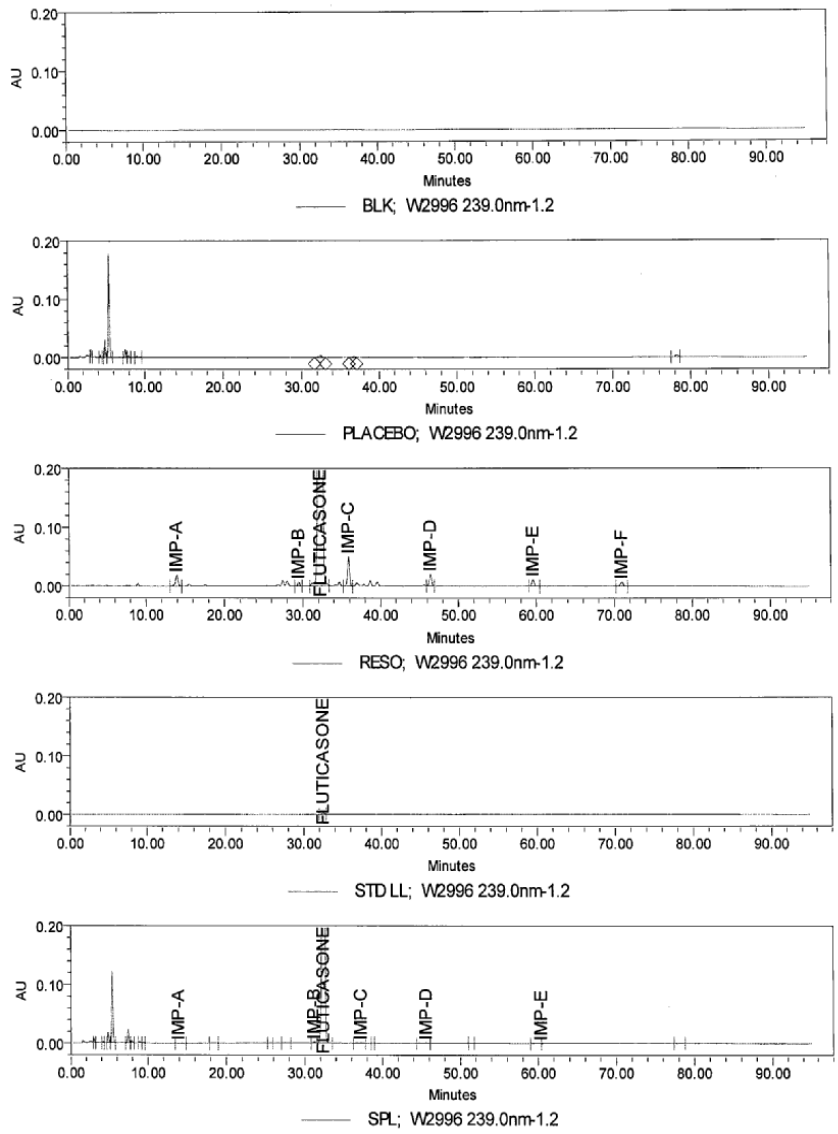
Time (min)	M.P. A (%)	M.P. B (%)	Comment
Initial	43	57	Isocratic
40	55	45	Isocratic
60	90	10	Linear
70	90	10	Linear
75	43	57	Isocratic
95	43	57	Isocratic

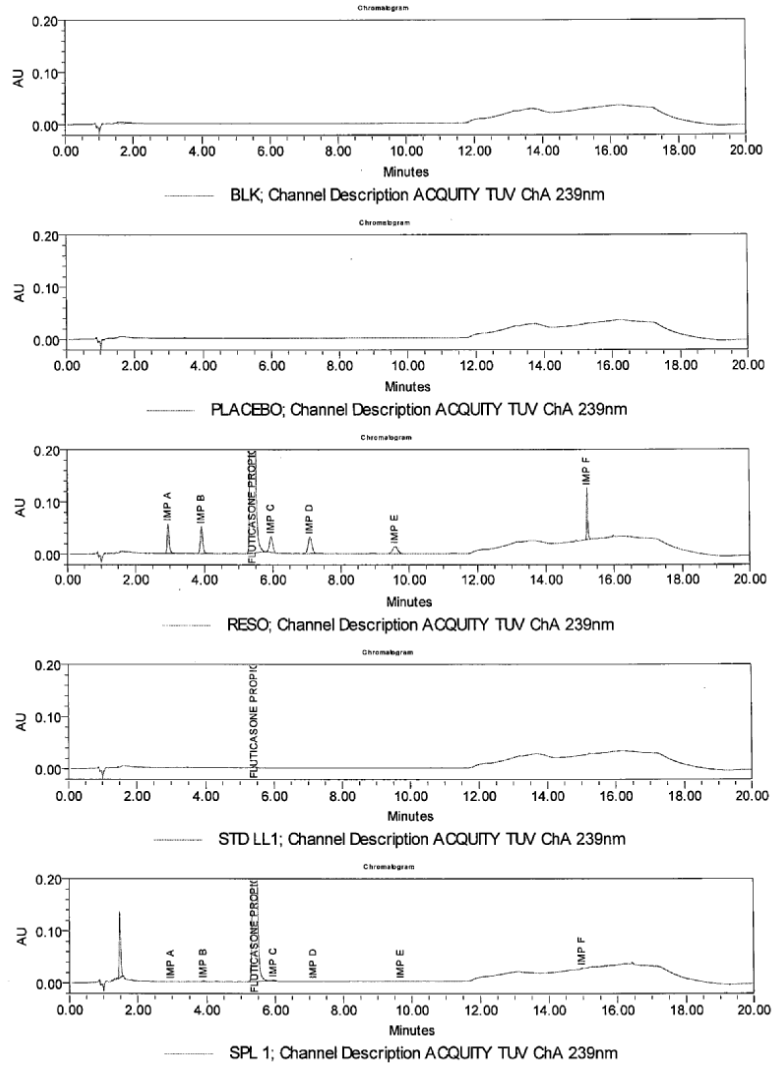
M.P. A = Mobile phase A; M.P. B = Mobile phase B.

Chromatograms: Listed below are the chromatograms of HPLC conventional method.

To confirm the resolution chromatogram pattern each known impurity was spiked and injected individually and the elution pattern for fluticasone propionate and its impurities was confirmed blank, resolution, placebo and

sample were injected sequentially. It was confirmed that their was no interference of blank and placebo peak at the retention time of fluticasone propionate with known and unknown impurities. The relative standard deviation for retention time of five replicates of standard preparation was 0.09 % (Limit not more than: 1 %) and for area was 0.81 % (Limit not more than: 2 %). relative standard deviation of % impurity for six-sample preparation[10,11] is listed below (Limit: not more than: 10 %)

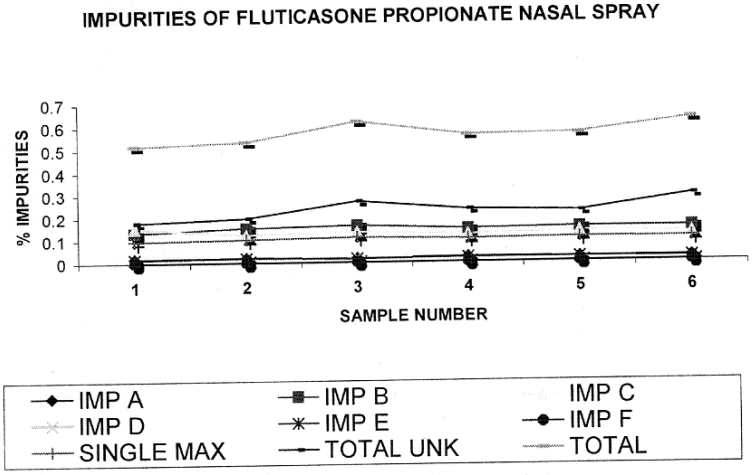




UPLC Chromatograms of fluticasone Propionate Nasal Spray Formulation

Impurity name	SPL 1 (%)	SPL 2 (%)	SPL 3 (%)	SPL 4 (%)	SPL 5 (%)	SPL 6 (%)	Mean (%)	RSD (%)
A	0.018	0.021	0.018	0.022	0.019	0.019	0.020	8.50
B	0.134	0.154	0.163	0.148	0.153	0.153	0.151	6.43
C	0.154	0.122	0.135	0.121	0.135	0.127	0.132	9.19
D	0.016	0.018	0.019	0.018	0.017	0.017	0.018	4.93
E	0.016	0.020	0.015	0.019	0.018	0.017	0.017	9.26
F	ND	ND	ND	ND	ND	ND	–	–
Single max unknown impurity	0.096	0.103	0.111	0.103	0.106	0.105	0.104	4.56
Total impurity	0.517	0.533	0.621	0.563	0.568	0.629	0.572	7.96

SPL = sample, RSD = relative standard deviation, ND = Not detected .



[IMP = impurity, SINGLE MAX = single maximum unknown impurity TOTAL UNK = total unknown]

Comparative results of HPLC and UPLC:

HPLC		UPLC	
Impurity name	Impurity (%)	Impurity name	Impurity (%)
Impurity A	0.03	Impurity A	0.02
Impurity B	0.14	Impurity B	0.15
Impurity C	0.14	Impurity C	0.13
Impurity D	0.02	Impurity D	0.02
Impurity E	ND	Impurity E	0.02
Impurity F	ND	Impurity F	ND
Single max unknown impurity	0.08	Single max unknown impurity	0.10
Total impurity	0.50	Total impurity	0.57

ND = Not detected

Conclusion

In present work, a rapid, sensitive, precise UPLC-UV method for quantitative determination of fluticasone propionate and it's impurities have been developed. 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 fluticasone propionate in formulation and raw material. The mobile phase consumption for existing HPLC method is 85 mL/run where as for UPLC it is 5 mL/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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