Validation of ion pairing High-Performance Liquid Chromatography method for simultaneous quantification of Formoterol Fumarate, Beclomethasone Dipropionate and Glycopyrronium Bromide.

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Introduction

Formoterol Fumarate (FF) is a long acting beta-2-agonist (LABA) used in the management of Chronic Obstructive Pulmonary Diseases (COPD). It causes bronchodilatation through relaxation of the smooth muscle in the airway [1].
Glycopyrronium Bromide (GB) is a long acting muscarinic antagonist (LAMA), it is also used to threat COPD [2]. The combination of this two drugs is used for the treatment of airflow obstruction in patients [3].
Inhaled corticosteroids like Beclomethasone Dipropionate (BDP) are used to threat bronchial airways inflammation in patients suffering from COPD [4].
The combination of FF, BDP and GB is recommended for the treatment of COPD [5]. Trimbow (Chiesi®) is the first triple therapy inhaler developed which combines the three molecules. A method based on ion pairing High Performance Liquid Chromatography (HPLC) has been developed and validated according to the International Requirements for pharmaceuticals for Human Use (ICH) guidelines for the simultaneous determination of FF, GB and BDP.

Method

The method was adjusted from Parmar et al [6] and Zayed S et al [7].
Instrument Parameters :
The run were performed under the following conditions :
Column : 150 x 3 mm, Particle size 5µm, Reversed Phase C-18, **Flow rate** : 1.3 mL/min.
UV wavelength : 211 nm , **Temperature** : 35 ± 1 °C.
Mobile phase : Water adjust to pH 2,7 with orthophosphoric acid and Dodecyl Sulfate 0,02M / Methanol (35/65, v/v).
Injection volume : 20 µL.

Material and reagents

All the chemicals used were of analytical reagent HPLC grade. Standards substances of Formoterol Fumarate, Beclomethasone Dipropionate and Glycopyrronium Bromide were purchased from the European Pharmacopeia. Water, Acetonitrile, Methanol, and Orthophosphoric Acid (85%w/v) were purchased from Fisher Chemical. 10 mg of formoterol fumarate and 10 mg of glycopyrronium bromide were dissolved each into 10 mL of methanol and 150 mg of beclomethasone dipropionate were dissolved into 50 mL of methanol.

Sample Chromatogram

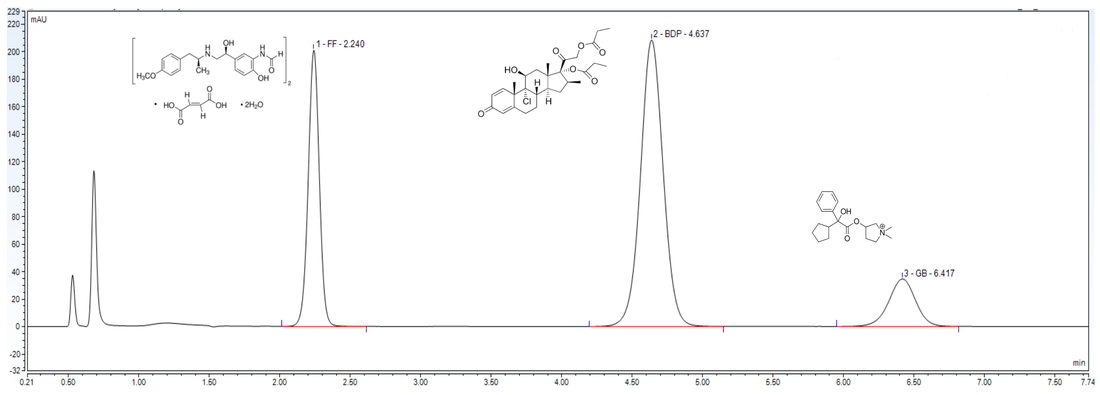

Figure 1 : An example of HPLC Chromatogram of mixture of Formoterol Fumarate (FF) (10µg/mL), Beclomethasone Dipropionate (BDP) (150µg/mL), and Glycopyrronium Bromide (GB) (20µg/mL).

Conclusion

The system suitability data permits to confirm the conformity of the method. The method can be used as rapid (time of experiment < 8 min) and sensitive, but it has to be adapted to acquire a better degree of robustness, specially with minor changes on the flow rates from the original method. The simultaneously quantification of Formoterol fumarate, Beclomethasone dipropionate and Glycopyrronium bromide will permit to study the in vitro performance of the inhalation chamber TipsHaler® (Laboratoire OptimHal, ProtecSom, France) with the Trimbow® inhaler.

Linearity

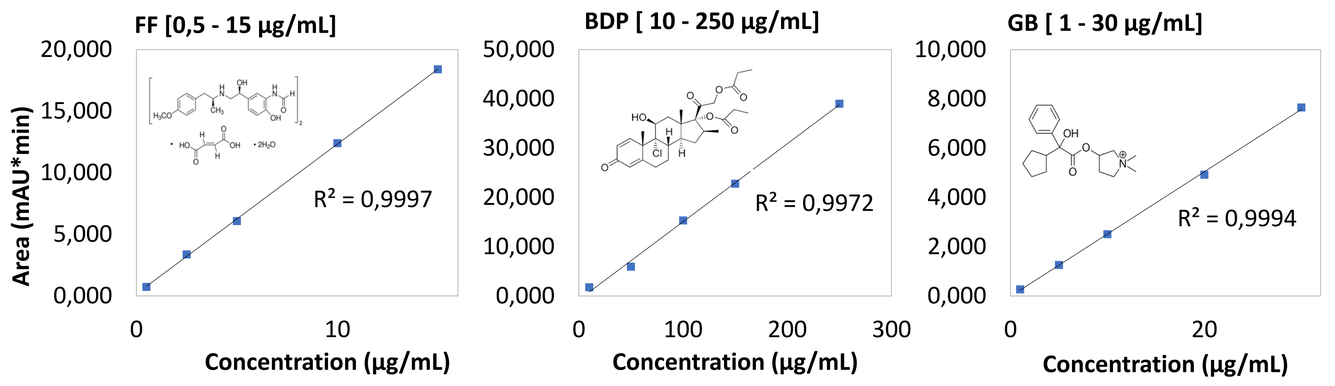

Figure 2 : Linearity plots for Formoterol Fumarate (FF), Beclomethasone Dipropionate (BDP) and Glycopyrronium Bromide (GB).

System Suitability

	FF	BDP	GB	Acceptance criteria [8]
Efficiency (N)	3985	4083	5623	> 2000
Capacity factor (K)	3,48	8,27	11,83	> 2
Resolution (Rs)	11,11		5,52	> 1,5
Tailing Factor	1,00	1,03	0,97	< 2
Relative Standard Deviation (RSD) (n=3) (%)	0,32	0,36	0,54	< 1

Table 1 : System suitability data obtained on the chromatogram (figure 1).

Validation of analytical procedure

Linearity (Figure 2), Limit of quantification and limit of detection, robustness, intra-day and inter-day precision (table 2) were determined for all of the standards. Inter-day precision was measured by testing the solutions on a different day. Robustness was tested by conducting measurements with minor changes on the method; the tests were performed with variation of temperature (± 5 °C) and flow rate (± 0,1 mL/min) from the original method.

		FF	BDP	GB
Limit of Quantification (µg/mL)		0.270	0.446	0.394
Limit of Detection (µg/mL)		0.081	0.134	0.118
Robustness (RSD) (%)	Higher Temperature (+5°C)	0.93	0.92	2.65
	Lower Temperature (-5°C)	0.46	0.12	1.15
	Higher Flow rate (+0,1mL/min)	6.81	5.04	5.44
	Lower Flow rate (-0,1mL/min)	7.50	6.78	6.63
Intra-day Precision (RSD) (%) FF, BDP, GB	2.5µg/mL; 50µg/mL; 5 µg/mL	1.1	0.6	0.7
	3.75µg/mL; 75µg/mL; 7.5µg/mL	0.4	1.1	0.9
	5µg/mL; 100µg/mL; 10µg/mL	0.3	0.3	0.7
	7.5µg/mL; 120µg/mL; 15µg/mL	0.7	0.4	0.7
	10µg/mL; 150µg/mL; 20µg/mL	0.2	0.4	0.4
Inter-day Precision (RSD) (%) FF, BDP, GB	2.5µg/mL; 50µg/mL; 5 µg/mL	3.7	4.0	2.1
	3.75µg/mL; 75µg/mL; 7.5µg/mL	3.5	3.0	1.2
	5µg/mL; 100µg/mL; 10µg/mL	5.6	2.9	0.9
	7.5µg/mL; 120µg/mL; 15µg/mL	1.6	3.0	1.6
	10µg/mL; 150µg/mL; 20µg/mL	5.0	2.1	0.6

Table 2 : Limit of Quantification, Limit of Detection, Robustness, Intra and Inter-day precision of the method.

Although the method has a high degree of robustness when levels of temperature are adjusted, it is not as good when slightly different flow rates are used. The percentage of RSD values were found to be lower to 2% for the intra day precision. The percentage of RSD values are higher for the inter day precision.

References

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