

Analytical methods for determination of azelastine hydrochloride and fluticasone propionate in different matrices: A mini review

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ABSTRACT

Combined nasal medications are an efficient therapy for enhancing the symptoms of many respiratory disorders such as allergic rhinitis. The manifestations of rhinitis have been receiving more attention because of the potential for overlapping or misunderstanding between allergic rhinitis symptoms and those of coronavirus disease 2019 (COVID-19). Azelastine hydrochloride (AZH) and fluticasone propionate (FLU) are among the most effective drug combinations for elevating the symptoms of allergic rhinitis. To promote and make it easier for various researchers to get literature reviews for their studies on the drugs being mentioned, different analytical methods have been collected and discussed in this mini-review for the quantitative assay of AZH and FLU in their pharmaceutical formulation and different biological fluids. The assembled approaches include pharmacopeial, chromatographic (HPLC, CE, and TLC), spectroscopic (spectrophotometric and spectrofluorimetric), and electrochemical methods. In accordance with the findings of this study, AZH was determined either alone or with its genotoxic impurity, major metabolite, and alkaline degradant in different matrices using various analytical methods. Moreover, HPLC technique made it possible to analyze FLU along with its major metabolite in human plasma. In addition, the pharmaceutical formulation was the applicable matrix for the simultaneous analysis of AZH and FLU. The data provided in this study

can be used to successfully perform additional research for the quantitative analysis of AZH and FLU.

Keywords: Allergic rhinitis - Azelastine hydrochloride - COVID-19 - Fluticasone propionate – Respiratory diseases.

1. Introduction

Respiratory diseases are conditions affecting the airways of the lungs and other respiratory structures that have a considerable impact on individuals and societies. Communicable diseases, noncommunicable diseases, and lung cancer are the main three types of respiratory diseases. Communicable diseases include acute lower respiratory infections such as pneumonia and tuberculosis. Chronic obstructive pulmonary diseases (COPDs), cystic fibrosis, asthma, interstitial lung diseases, and allergic rhinitis are noncommunicable diseases (1).

Allergic rhinitis is a common illness accompanied by conjunctivitis and asthma. Sneezing, rhinorrhea, nasal discomfort, and congestion are all among the typical symptoms of the disease (2). These manifestations have been receiving more attention. This is because of the potential for overlapping or misunderstanding between allergic rhinitis symptoms and those of COVID-19 (3). Allergic rhinitis can be managed with different treatments. Nasal and oral antihistamines, intranasal glucocorticoids, allergen immunotherapy, and leukotriene-receptor antagonists are among the available therapies. Nasal sprays containing both antihistamine and corticosteroid could be utilized if antihistamine was ineffective when taken alone (2,4,5). Among the therapeutic combinations that are effective for elevating allergic rhinitis symptoms are azelastine hydrochloride (AZH) and fluticasone propionate (FLU).

AZH, with a chemical name of 4-(4-chlorobenzyl)-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl]phthalazin-1(2H)-one hydrochloride (Figure 1(a)) (6), is a histamine receptor antagonist that acts mainly on H₁-receptors. It also sounds to block the generation of inflammatory endogenous substances from the mast cells. AZH is utilized to elevate the symptoms of many allergies like conjunctivitis and allergic rhinitis (7). For these allergies, topical preparations of AZH have a rapid onset of action and more potency than oral ones. However, because topical preparations only affect local organs, they have to be administered

twice a day to provide a prolonged response (8). AZH is a white or nearly white crystalline powder that is sparingly soluble in water. It is soluble in anhydrous ethanol and in methylene chloride with a molecular weight of (418.4 g/mol). AZH has melting point, pKa, and log P values of (225°C), (8.88), and (3.81 - 4.04), respectively (6,9).

FLU, with a chemical name of 6 α ,9-Difluoro-17-[[[(fluoromethyl)sulphonyl] carbonyl]-11 β -hydroxy-16 α -methyl-3-oxoandrosta-1,4-dien-17 α -yl]propanoate (Figure 1 (b)) (6), is a primary glucocorticoid corticosteroid, powerful anti-inflammatory, and immunosuppressant via inhibiting the production of different cytokines. It acts on the lungs topically with no substantial systemic absorption at the standard doses. FLU is available in the form of inhalation powder to elevate the symptoms of asthma and COPDs. It is also available in nasal sprays to treat allergic rhinitis (7). FLU is a white or nearly white powder with a molecular weight of (500.6 g/mol). It is practically insoluble in water, sparingly soluble in methylene chloride, and slightly soluble in ethanol (96 %). The melting point of FLU is (261 – 273 °C), with pka and log p values of (13.61) and (3.69 - 3.72), respectively (6,10).

The objective of this study is to cover various analytical methods for the quantitative assay of AZH and FLU, either individually or combined, regarding chromatographic, spectrophotometric, spectrofluorimetric, and electrochemical methods and to discuss any challenges or limitations associated with the reported methods.

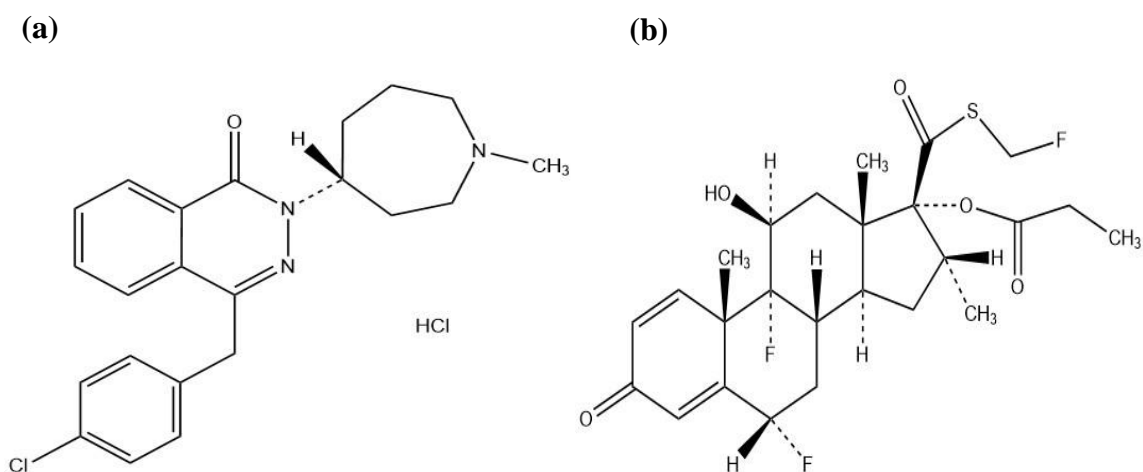


Figure 1: Chemical structures of (a) AZH, and (b) FLU (6).

2. Analytical techniques

2.1. Official and reported analytical methods for the analysis of AZH

2.1.1. Official analytical method

AZH is determined in British Pharmacopeia (BP) (6) and United States Pharmacopeia (USP) (11) by potentiometric titration via being dissolved in anhydrous formic acid and titrating against perchloric acid after the addition of acetic anhydride.

2.1.2. Reported analytical methods

a. Chromatographic methods

High-performance liquid chromatography

Different high-performance liquid chromatographic (HPLC) methods were reported for the determination of AZH in different matrices using various detectors as presented in (**Table 1**).

Table 1. Summary of HPLC methods published for determination of AZH:

Stationary phase	Mobile phase	Detector	Application	Ref.
Promosil C ₁₈ column (150 mm × 4.6 mm, 5 μm)	0.1 M Sodium dodecyl sulfate (SDS): 10% n-propanol: 1% n-octanol: 0.3% triethylamine (TEA) (pH 3.5)	UV at 210 nm	Bulk, and pharmaceutical formulations, Stability study	(12)
Cosmosil 5 C ₁₈ column (150 mm × 4.6 mm, 5 μm)	Acetonitrile (ACN): 0.04 M potassium dihydrogen phosphate (KH ₂ PO ₄) buffer (pH 3.5), (32: 68, v/v)	UV at 210 nm	Pharmaceutical formulations, Stability study	(13)

Waters® Spherisorb CN column (150 mm × 4.6 mm, 5 μm)	KH ₂ PO ₄ buffer: ACN (50: 50, v/v)	UV at 290 nm	Pharmaceutical formulation, Stability study	(14)
XTerra® RP-C ₁₈ column (250 mm × 4.6 mm, 5 μm)	5 mM Ammonium acetate (pH 3.2): ACN (40: 60, v/v)	UV at 253 nm	Pharmaceutical formulations, Stability study	(15)
Chromasil ODS RP-C ₁₈ column (250 mm × 4.6 mm, 5 μm)	ACN: phosphate buffer (pH 3.1): (40: 60, v/v)	UV at 239 nm	Pharmaceutical formulation	(16)
Hypersil® Gold C ₁₈ column (50 mm × 2.1 mm, 1.9 μm)	ACN: 0.1% w/v sodium lauryl sulfate (55: 45, v/v) (pH 5)	UV at 215 nm	AZH and its genotoxic impurity, Pharmaceutical formulations	(17)
YMC Pack Pro C ₈ column (50 mm × 2 mm, 3 μm)	5 mM Ammonium acetate solution (pH 6.4): ACN (30: 70, v/v)	MS/MS m/z 382.2 → 112.2	Human plasma	(18)
Phenomenex Synergi Polar-RP column (50 mm × 2 mm, 4 μm)	ACN: 10 mM ammonium formate with 0.1% formic acid (45: 55, v/v)	MS/MS m/z at 382.2 → 112.1 for AZH and m/z at 368.1 → 98.1 for desmethylazela stine	AZH and its major metabolite, Human plasma	(19)

Capillary electrophoresis

Determination of AZH along with its genotoxic impurity was achieved by capillary electrophoretic (CE) method using fused silica capillaries, 40 mm phosphate buffer, 30 kV, and detection at 225 nm (17).

Thin layer chromatography

A thin layer chromatographic (TLC) method was reported for AZH assay in the presence of its alkaline degradant. The proposed approach involves the utilization of a mobile phase containing glacial acetic acid: water: ACN (1: 2: 8, v/v/v) (15).

AZH was also determined in the pharmaceutical formulation at 220 nm by stability indicating a high-performance thin layer chromatographic (HPTLC) approach (20).

In addition, an HPTLC method was reported for AZH quantification in bulk and nasal spray using precoated silica gel G 60 F₂₅₄ aluminum plates and MeOH: chloroform: toluene (2: 4: 5, v/v/v) as the mobile phase (21).

b. Spectroscopic methods

Spectrophotometric methods

Three spectrophotometric approaches namely; mean centering of ratio spectra (MCR), first derivative (¹D), and the first derivative of the ratio spectra (¹DD), were reported for the determination of AZH with the presence of its alkaline degradant in bulk powder and nasal spray (15).

Moreover, the difference in absorbance between AZH acidic and basic solution (measured at 228 nm) and the complex formed between the drug and Eosin Y (measured at 550 nm) were used for AZH assay in different pharmaceutical formulations (22).

Also, the colored complexes that were formed between the reaction of AZH with bromocresol green, methyl orange, and alizarin red S were utilized for the drug assay in pharmaceutical formulation (23).

Spectrofluorimetric methods

A stability study of AZH and its assay in pure form and pharmaceutical formulations was reported using different spectrofluorimetric methods (24,25).

c. Electrochemical methods

Ammonium Reineckate and Sodium Tetraphenyl-Borate polyvinyl chloride membranes were used as ion-selective electrodes for AZH quantification in pharmaceutical formulation and in plasma along with the drug alkaline degradant (26).

An ion-selective electrode composed of azelastine-tetrafluorophenyl borate (4%), dioctylphthalate plasticizer (64%), and polyvinyl chloride (32%) was also utilized for electrochemical determination of AZH in syrup and blood (27).

2.2. Official and reported analytical methods for the analysis of FLU

2.2.1. Official analytical method

FLU is determined in BP (6) and USP (11) by liquid chromatography using mobile phases containing ACN: 1.15 g/L solution of ammonium dihydrogen phosphate (pH 3.5): MeOH (15: 35: 50, v/v/v).

2.2.2. Reported analytical methods

a. Chromatographic methods

High-performance liquid chromatography

Different HPLC methods were reported for the determination of FLU in different matrices using various detectors as presented in **(Table 2)**.

Table 2. Summary of HPLC methods published for determination of FLU:

Stationary phase	Mobile phase	Detector	Application	Ref.

Lichrospher 100 RP-C ₁₈ column (125 mm × 4 mm, 5 μm)	Water: ACN (40: 60, v/v).	UV at 236 nm	Pharmaceutical formulation	(28)
Shim-pack CLC-ODS column (150 mm × 4.6 mm, 4 μm)	ACN: MeOH: 0.01 M phosphate buffer (pH 4), (35: 35: 30, v/v/v)	UV at 240 nm	Pharmaceutical formulations	(29)
ACE C ₁₈ column (50 mm × 4.6 mm, 5 μm)	ACN: 0.01% formic acid (48: 52, v/v)	MS/MS m/z at 501 → 293	Human plasma	(30)
Reprosil Gold 100 C ₁₈ column (100 mm × 2 mm, 2 μm)	1 mM Ammonium trifluoroacetate buffer: MeOH (10: 90, v/v)	MS/MS m/z 501 → 293.2	Human plasma	(31)
Acquity UPLC BEH C ₁₈ column (50 mm × 2.1 mm, 1.7 μm)	0.1% Ammonium hydroxide: MeOH, gradient elution	MS/MS m/z 501.3 → 293.2	Rat plasma	(32)
Acquity UPLC BEH C ₁₈ column (50 mm × 2.1 mm, 1.7 μm)	MeOH with ACN (50: 50, v/v): 2 mM ammonium trifluoroacetate (85: 15, v/v)	MS/MS m/z at 501.1 → 293.2 for FLU and m/z at 453.3 → 293.2 for fluticasone propionate- 17beta- carboxylic acid	FLU and its major metabolite, Human plasma	(33)

Capillary electrophoresis

A micellar electrokinetic chromatographic approach with stability study was reported for FLU assay in nasal spray formulation using fused silica capillary and mobile phase containing 25

mM borate and 25 mM SDS solution. The applied voltage was 20 kV and the detection wavelength was 238 nm (34).

Thin layer chromatography

An HPTLC approach with stability study was reported for FLU assay at 239 nm in capsule dosage form. The developed method using ethyl acetate: toluene (3: 7, v/v) as the mobile phase (35).

b. Spectroscopic method

Spectrophotometric method

Direct spectrophotometry was reported for FLU assay by measuring the absorbance at λ_{\max} 236 nm (36).

2.3. Reported analytical methods for the simultaneous analysis of AZH and FLU

a. Chromatographic methods

High-performance liquid chromatography

Different HPLC methods were reported for the simultaneous determination of AZH and FLU in a pharmaceutical formulation as presented in (**Table 3**).

Table 3. Summary of HPLC methods published for simultaneous determination of AZH and FLU:

Stationary phase	Mobile phase	Detector	Application	Ref.
Altima C ₁₈ column (150 mm × 4.6 mm, 5 μm)	MeOH: ACN: KH ₂ PO ₄ buffer (pH 4), (5: 33: 62, v/v/v)	UV at 235 nm	Pharmaceutical formulation	(37)

Inertsil ODS C ₁₈ column (250 mm × 4.6 mm, 5 μm)	100% ACN and 20.35 mM phosphate buffer (pH 3), gradient elution	UV at 236 nm	Pharmaceutical formulation, Stability study	(38)
C ₁₈ column	10% butanol: 0.2 M SDS: 0.3% TEA (pH 6)	UV at 220 nm	Bulk and pharmaceutical formulation, In the presence of additives	(39)
Waters® Spherisorb CN column (250 mm × 4.6 mm, 5 μm)	ACN: 50 mM KH ₂ PO ₄ buffer (45: 55, v/v)	UV at 215 nm	Pharmaceutical formulation, In the presence of additives	(40)

Thin layer chromatography

A stability-indicating HPTLC approach was developed for the concurrent assay of AZH and FLU in pharmaceutical dosage form, applying the design of the experiment approach and using a mobile phase consisting of MeOH: chloroform: toluene (2: 4: 5, v/v/v) (41).

b. Spectroscopic methods

Spectrophotometric methods

Simultaneous quantification of AZH and FLU was performed in the presence of pharmaceutical formulation preservatives using different spectrophotometric methods; in which direct measurement at 290 nm was used for AZH quantification. While, MCR, ratio subtraction coupled with ratio difference approach, and derivative of double divisor of ratio spectra were reported for FLU assay (42).

¹D spectrophotometry was also reported for concurrent quantification of AZH and FLU in their combined pharmaceutical dosage form by measuring the amplitudes at 288.19 and 237.16 nm for AZH and FLU, respectively (43).

In addition, the respective zero crossing point of the first derivative spectra was reported for AZH and FLU assay in pharmaceutical formulation (44).

Moreover, eco-friendly spectrophotometric approaches were reported for AZH and FLU simultaneous quantification in their pharmaceutical formulation by applying absorbance subtraction, amplitude modulation, and chemometric methods (45).

3. Result and discussion

The physicochemical attributes of both drugs were considered when establishing the analytical methods. The form (neutral/ionic) of the components, the degree of ionization (pKa) of the drugs (8.88 for AZH and 13.61 for FLU), the nature of the silica beds, and the trials made at different pH values, where the best resolution can be obtained, should be taken into consideration when selecting the mobile phase pH. The most reported pH values of the mobile phases for the quantitative analysis of AZH and FLU were between (3 to 6.4) (12,13,15–18,29,37–39). The pH should be \pm two pH units above or below the pKa of the drugs for reproducibility. Furthermore, extreme pH values, either extremely high or very low, should be avoided to extend the life of the column (46). Moreover, most of the reported methods (14,17,18,20,21,28,29,31,33,35,41) utilized mobile phases with a higher percentage of organic modifier with respect to the log p values of the drugs (3.81 - 4.04 for AZH and 3.81 - 4.04 for FLU). In addition, the most preferable wavelengths for estimation of AZH and FLU will be 290 nm (λ_{\max} of AZH) and 236 nm (λ_{\max} of FLU), respectively which was addressed by some of the reported methods (14,28,29,34–36,42).

Challenges or limitations associated with the reviewed methods

The gathered analytical methods were reviewed for any challenges or limitations associated with them during their development to compile and analyze as much data as possible about the reported analytical methodologies for AZH and FLU. The reported HPLC-MS/MS method (19) for concurrent estimation of AZH and its major metabolite, desmethylazelastine, showed abnormal signal loss for desmethylazelastine while developing the method. It was attributed to the strong adsorption affinity of the metabolite to the glass tubes. Excellent results were obtained when plastic tubes were substituted for glass ones. Moreover, the water

insolubility of FLU in the reported HPLC-UV method (28) for FLU assay in inhalation particles on many matrices has been overcome by using Cyclodextrins. They were utilized to improve this restriction because they were able to form inclusion complexes with the guest drug molecule and boost the bioavailability as well as the stability of the medications. Besides, the addition of ammonium hydroxide before the liquid-liquid extraction for HPLC-MS/MS determination of FLU in human plasma maximized the recovery of the drug (85–90%) and reduced the effect of the matrix (30). In addition, simultaneous UPLC-MS/MS analysis of FLU either alone (31) or with its major metabolite (33) in human plasma at pg/mL levels was challenging particularly with the extremely low concentrations and the significant influence from the endogenous plasma components. This was overcome by employing a solid phase extraction (SPE) on Oasis MAX cartridges. Also, the reported UPLC-MS/MS method (32) for analysis of FLU in rat plasma had poor analyte recovery (28%) with plasma samples in comparison to the used aqueous standard solutions (>95%) during the SPE process. Plasma-protein binding was the suggested reason for this decrease in the analyte recovery. The extraction process was enhanced to more than 95%, by using 0.04 M ammoniacal zinc sulphate before the SPE process. It was demonstrated that using 0.04 M ammoniacal zinc sulphate to disrupt the protein binding was more than four times more efficient than using the aqueous base alone.

4. Conclusion

Different analytical methods have been summarized in this article for the quantitative assay of AZH and FLU either in pharmaceutical formulations or in biological fluids. In light of the findings of this study, the chromatographic methods were the most applied ones, which were followed by spectroscopic and electrochemical approaches. Some of the provided methods were carried out in accordance with green chemistry and evaluated using different green assessment metrics.

- **Conflict of Interest**

All authors confirm that there are no conflicts of interest to declare.

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