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Research Article

DRUG FORMULATIONS

Development and Validation of Stability-Indicating Impurity Profiling Method for Azelastine Hydrochloride and Fluticasone Propionate in Nasal Spray Product Using HPLC with a UV/PDA Detector

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Abstract

Background: Azelastine HCl (AZ) and fluticasone propionate (FL) nasal spray drug product is commonly used in the treatment of allergic rhinitis worldwide. To date, the impurity profiling of this product has not been reported.
Objective: The present study aimed to develop and validate a novel RP-HPLC stability-indicating analytical method for the estimation of impurities from AZ and FL nasal spray drug product.
Methods: A mixture of octane sulfonic acid sodium salt and trifluroacetic acid is used as a mobile phase A. Acetonitrile is used as a mobile phase B. Good separation was achieved on Baker bond phenyl hexyl, 250 × 4.6, 5 µm column at 1 mL/min flow rate in gradient elution mode. The chromatograms were monitored at 239 nm.
Results: The LOD and LOQ were found to be 0.006 and 0.019 µg/mL for AZ and 0.010 and 0.030 µg/mL for FL, respectively. The correlation coefficient for all the known impurities and principal analytes was 0.999 from LOQ level to 150% of standard concentration. The recovery for all the known impurities was found to be between 90 and 110%. In the stress study, 15% degradation was observed in basic conditions and 8.7% in acidic conditions. No significant degradation was observed in thermal and oxidative conditions.
Conclusion: An impurity profiling method for AZ and FL combination nasal spray product was successfully developed, validated, and demonstrated to be accurate, precise, specific, robust, and stability-indicating. The method can be routinely used for impurity testing of commercial batches in QC laboratories in the pharmaceutical industry.
Highlights: No impurity study has been reported for this combination product until now.

The efficacy and safety of any medicine is dependent on the purity of the drug product. Hence, impurity testing is the most critical part of any drug specification meant for registration with various drug regulatory bodies such as United States Food and Drug Administration (US FDA), United Kingdom Medicines and Healthcare products Regulatory Agency (UK MHRA) etc. The azelastine HCl (AZ) and fluticasone propionate (FL) nasal spray product is one of the most prescribed medications for the treatment of allergic rhinitis. The recommended dose is two sprays in each nostril twice daily (1). AZ acts as an antihistaminic and FL as a corticosteroid for the symptomatic relief from nasal allergy (2). The IUPAC name of AZ (Figure 1a) is (±)-1-(2H)-phthalazinone, 4-[(4-chlorophenyl)methyl]-2-(hexahydro-1-methyl-1H-azepin-4-yl)-, monohydrochloride. It is a white crystalline

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powder sparingly soluble in water, soluble in ethanol and methylene chloride (3). The known impurity of AZ (impurity E) is harmful if swallowed, toxic if it is absorbed through the skin, inhaled, and causes skin irritation. Hence strict control and monitoring of such impurities within the acceptance criterion is very important.

The International Union of Pure and Applied Chemistry (IUPAC) name of FL is [(6S, 8S, 9R, 10S, 11S, 13S, 14S, 16R, 17R)-6,9-difluoro-17-(fluoromethylsulfanylcarbonyl)-11-hydroxy-10,13, 16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]-phenanthren-17-yl] propionate (4). It has anti-inflammatory properties and, hence, is used in the treatment of asthma and allergic rhinitis in various nasal spray and dry powder inhaler dosage forms (5). The chemical structure of FL is shown in Figure 1b. AZ contains four known impurities viz. AZ impurity A, B, C, and E. FL contains 11 known impurities viz. FL impurity A, B, C, D, E, F, G, H, I, J, and K. As per the British Pharmacopeia (BP) 2020, FL impurities C, D, and G are classified under the specified impurities and the rest of the impurities are classified under unknown impurities. Hence, during method development we have monitored only FL impurities C, D, and G in the nasal spray product. We have proved the specificity for all the 15 known impurities of AZ and FL by individually injecting them. Although both active pharmaceutical ingredients (APIs) are official in the United States Pharmacopeia (USP), BP, and European Pharmacopeia (Ph. Eur.), the combination product is not official in any of the pharmacopoeias.

During an extensive literature review, we observed that few methods were reported for AZ estimation from nasal spray (5) and ophthalmic (6) dosage forms. Assay methods are reported for AZ and FL combination products by HPLC (7–8) and HPTLC (9). The impurity quantification methods for AZ from nasal spray (10–11) products are also reported. For FL, assay by HPTLC (12–13) Ultra Performance Liquid Chromatography (UPLC) (14), and HPLC (15) is reported for APIs, dry powder inhalation, and nasal spray dosage form. Impurity quantification for FL in combination with salmeterol xinafoate (15–17) and individual content of FL by UPLC (18) and combination with salmeterol xinafoate (19–22) is also reported. To the best of our knowledge, a method for the simultaneous estimation of organic impurities of AZ and FL in their combination nasal spray drug product is not reported. Hence, an attempt has been made to develop and validate a method as per International Conference on Harmonization (ICH) and USP guidelines (23) for the simultaneous quantitation of known and unknown impurities of AZ and FL from nasal spray drug product.

Experimental

Apparatus

(a) A Shimadzu LC 2010 C_{HT} HPLC with Lab Solution software and a UV detector was used. Wavelength selection, the selectivity study, and the method precision study was performed on a Waters HPLC with Empower 3 software connected to a2695 photo diode array (PDA) detector.

(b) A Mettler Toledo pH meter was used for pH adjustment of the mobile phase.

(c) Mettler Toledo (XO-26) and Sansui (HTR-220E) balances were used for weighing during the complete study.

(d) A Suntest XLS+ photo stability (Atlas) chamber was used for the photo-degradation study.

(e) A Thermo lab oven (TO00003252S) was used for the thermal degradation study.

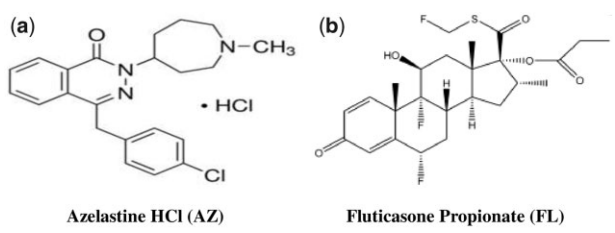

Figure 1. Chemical structure of azelastine hydrochloride and fluticasone propionate.

Study material

AZ (Ph.Eur grade) and FL In-house (IH) grade) was purchased from Aarti Drugs, Mumbai, India. These APIs were used for in-house working standard qualification against reference standards. The in-house qualified working standards for AZ (99.8% purity) and FL (99.6% purity) were used during the method development and validation study. The complete method development and validation study was carried out on In-house manufactured samples and placebo batches. The marketed formulation Dymista manufactured by Meda Pharmaceuticals Limited, Canada (137 mcg/50 mcg per spray of AZ and FL, respectively) was analyzed using this validated analytical method.

Reagents

HPLC grade Milli-Q water with conductivity less than 0.055 µS/cm at 25 °C was used during the development and validation. AR grade octane sulfonic acid sodium salt and trifluroacetic acid (TFA; Finar-India) and HPLC grade acetonitrile and methanol (Rankem-India) were used for mobile phase preparation.

Method Optimization

Method development trials were initiated by using 0.5% orthophosporic acid and acetonitrile (20:80) as a diluent and monobasic potassium phosphate buffer at different pH in various combinations of acetonitrile and methanol as a mobile phase. Inertsil ODS 250 × 4.6 mm, 5 µm; Hypersil BDS C18, 250 × 4.6 mm, 5 µm; Baker bond C18, 250 × 4.6 mm, 5 µm HPLC columns (JT Baker, USA) were used during the method development trials. Maximum peak resolution was obtained from 0.2 g/L 1-octane sulfonic acid sodium salt with 0.5 mL/L TFA in the mobile phase (24) and a Baker bond phenyl hexyl, 250 × 4.6 mm, 5 µ HPLC column. The gradient was optimized for better separation at 239 nm based on the optimum response of impurities and both the analytes.

Mobile phase preparation

1-Octane sulfonic acid sodium salt (0.2 g) was transferred into a glass bottle containing 1 L HPLC grade water. The mixture was sonicated and filtered through a 0.45 µ filter. TFA (0.5 mL) and 10 mL acetonitrile were added, mixed well, degassed, and used as mobile phase A. Acetonitrile with 0.02% TFA was used as mobile phase B. The gradient program reported in Table 1 was optimized for better separation and column efficiency.

Diluent

The diluent was 0.5% orthophosporic acid in water and acetonitrile in the ratio of 20:80.

Table 1. Gradient program

Time, min	Mobile phase A, %	Mobile phase B, %
0.1	78	22
30	52	48
60	52	48
90	30	70
110.0	30	70
110.1	78	22
120.0	78	22

Standard Preparation

(a) *Preparation of AZ standard stock solution.*—Accurately weighed 8 mg of AZ working standard was transferred to a 100 mL volumetric flask; about 70 mL diluent was added and the flask sonicated with intermittent shaking. The volume was made up to the mark with diluent.

(b) *Preparation of FL standard stock solution.*—Accurately weighed 6 mg of FL working standard was transferred to a 100 mL volumetric flask; about 70 mL diluent was added and the flask sonicated with intermittent shaking. The volume was made up to the mark with diluent.

(c) *Preparation of mix standard solution.*—The mix standard solution was prepared by adding 5 mL AZ standard stock solution and 5 mL FL standard stock solution to a 100 mL volumetric flask and adjusting the volume with diluent.

(d) *Preparation of AZ impurity stock solution.*—Accurately weighed (about 2 mg each) AZ impurities A, B, C, and E were transferred separately to a 100 mL volumetric flask, dissolved by sonication, and diluted up to the mark with diluent.

(e) *Preparation of FL impurity stock solution.*—Accurately weighed (about 2 mg each) FL impurities A, B, C, D, E, F, G, H, and I were transferred separately to a 100 mL volumetric flask, dissolved by sonication, and diluted up to the mark with diluent.

(f) *Preparation of system suitability solution.*— Into a 50 mL volumetric flask, add 2 mL AZ standard stock solution, AZ impurities A, B, C, and E was transferred; 0.5 mL of FL standard stock solution and 1.5 mL of FL impurity A, B, C, D, E, F, G, H and I stock solution were transferred. The flask was made up to the mark with diluent, mixed well, and used as a system suitability solution.

Sample Preparation

Nasal spray solution equivalent to 8 mg of AZ (about 8 g sample) was transferred to a 20 mL volumetric flask. About 10 mL diluent was added, mixed well, and the flask sonicated for 20 min with intermittent shaking. The volume was made up to the mark with diluent and centrifuged at 5000 revolutions per minute (rpm) for 10 min. The supernatant was filtered through a 0.45 µ filter and used for HPLC analysis.

System Suitability Criteria

The relative retention times (RRTs) for all the known impurities are listed in Table 2. The similarity factor between standard-1 and standard-2 was determined (acceptance criterion 97–103%). The resolution between FL impurity A and AZ impurity C, FL impurity F and AZ impurity E (not less than 2.0), and FL and FL impurity C (not less than 3.0) is reported. The theoretical plates (not less than 2000) and tailing factor limit for standard-1 (not more than 2) was measured as a part of the system suitability study. The system suitability parameters are reported in Table 3.

Table 2. RRTs for known impurities

Serial Number	Analyte	RRT
1	AZ impurity A	About 0.215 with respect to AZ
2	AZ impurity B	About 0.287 with respect to AZ
3	AZ HCl	1.0
4	AZ impurity C	About 1.190 with respect to AZ
5	AZ impurity E	About 2.521 with respect to AZ
6	FL impurity A	About 0.548 with respect to FL
7	FL impurity B	About 0.602 with respect to FL
8	FL impurity C	About 0.836 with respect to FL
9	FL impurity D	About 0.911 with respect to FL
10	FL	1.0
11	FL impurity E	About 1.077 with respect to FL
12	FL impurity F	About 1.166 with respect to FL
13	FL impurity G	About 2.521 with respect to FL
14	FL impurity H	About 1.589 with respect to FL
15	FL impurity I	About 1.686 with respect to FL

Table 3. System suitability results

Sr. No.	System suitability parameters	AZ	FL
1	Theoretical plates	121399	54935
2	Tailing factor	1.22	1.10
3	Similarity Factor	99.6	99.7
	Resolution between co-eluting peaks	Result	Limit
1	FL impurity A and AZ impurity C	2.1	Not less than 2.0
2	FL and FL impurity C	3.9	Not less than 3.0
3	FL impurity A and AZ impurity C	2.3	Not less than 2.0

Analytical Method Validation

Analytical method validation for the developed method was carried out as per ICH guidelines (23) for parameters including accuracy, precision, selectivity, linearity, LOD, LOQ, and range.

Accuracy

The accuracy of the method was estimated in terms of its percent recovery for all the known impurities and principal analytes by spiking them in placebos at LOQ level, and 50, 100, and 150% of specification level. The spiked samples were then analyzed in triplicate and the % recovery of individual impurities calculated. The limit of % recovery of individual impurities was set 90 to 110% at 50 to 150% levels, and 85 to 115% at LOQ level.

Precision

In the precision study, standard solution-1 and standard solution-2 were injected and the similarity factor was calculated for both the analytes.

Method Precision and Intermediate Precision

The study was performed on six samples for the estimation of known impurities. The RSDs, %, of individual known impurities, single maximum impurity, and total impurities percentages were calculated. In the tested sample, both known and unknown impurities were found to be below the LOD. Hence, the study was carried out by spiking all the known impurities at the 100% level in the tested sample in six replicate preparations.

The intermediate precision study was performed by a different analyst on a different day on a different make of HPLC system with a different HPLC column lot. The RSD, %, for individual impurities of 12 samples from method precision and intermediate precision were determined as not more than (NMT) 15%.

Specificity

The specificity of the method was evaluated by the selectivity and forced degradation study. The individual solutions of all the known impurities and principal analytes were injected and examined for any interference from placebo and diluent at the retention time of each analyte.

Forced Degradation Study

This study was performed to evaluate the stability-indicating nature of the method. The sample and placebo were treated with acid, base, peroxide, light, and heat. The mass balance was determined by analyzing the % content by using validated assay method. The results are reported in Table 4.

LOD and LOQ

The LOD and LOQ values are calculated at S/Ns of 3 and 10, respectively. The LOD and LOQ determination was carried out at minimum four levels below the 50% level of the working concentration. For each level, the samples were injected in triplicate to calculate LOD and LOQ by the $10\sigma/S$ and $3\sigma/S$ methods as per ICH guidelines.

Linearity

The linearity study was conducted at seven different levels beginning from the LOQ level to 150% of the standard concentration. All the impurity solutions and standard solutions were spiked from LOQ to 150% and injected in triplicate. The correlation coefficient (r^2) for each individual impurity and the principal analyte were calculated (acceptance criterion of r^2 NLT (Not less than) 0.995).

Range

The range of the method was established for the lowest and the highest concentration at which the response was directly proportional to the analyte concentration during the linearity study with good precision and accuracy.

Robustness

The robustness of the method was proved by slightly varying the method parameters such as a change in column temperature ±5°C, wavelength ±3nm, flow rate ±0.1 mL, and organic composition in the mobile phase ±5%. The system suitability parameters were evaluated for each condition.

Solution Stability and Filter Validation

The solution stability of the sample solution, impurity standard solution, and working standard solution was monitored at room temperature. All the solutions were injected at different time intervals including initial and after 12, 24, and 48 h and evaluated for any significant variation in peak area. The filter validation study was carried out by injecting the filtered sample solution by discarding 0, 2, and 5 mL of the initial filtrate from various filters available in the market. The study was conducted on a 0.45 µ nylon filter, nylon prefilter, PVDF filter, PVDF prefilter, and PTFE filter. The sample chromatograms were evaluated for any unwanted peaks generated due to filter interference.

Results and Discussion

During the various method development trials, we found that the addition of 0.2 g/L 1-octane sulfonic acid sodium salt with 0.5 ml/L TFA in the mobile phase significantly improved the resolution between the FL impurities C and D, and AZ impurity E. The developed method was validated as per ICH (Q2 R1) guidelines for various parameters.

Accuracy

The % recovery of AZ was found to be 106.5, 109, 100.3, 99.8, and the % recovery of FL was found to be 96.9, 100.2,100, 101.8 at LOQ, 50, 100 and 150%, levels respectively. Also, the recovery of all the known impurities of AZ and FL was found to be within 90 to 110%. The data shows that the method has a good ability to accurately recover both analytes from the LOQ to 150% level.

Precision

The precision of the method was proved by calculating the RSD, %, of individual impurities spiked in six sample preparations during the method precision and intermediate precision study. The RSDs of each impurity and principal analyte from 12 samples (six from method precision and six from intermediate precision) were less than 15%.

Specificity

(a) *By selectivity*.—No interference was observed at the retention time of the each individual impurity and the principal analyte from the blank and placebo preparation. The peak purity was evaluated by using a PDA detector and found pure for all the impurity and analyte peaks. Typical chromatograms for blank, placebo, system suitability, and sample solution are shown in Figures 2, 3, 4, and 5 respectively.

Table 4. Forced degradation study

Sr. No.	Forced degradation condition	% Assay of AZ	% Assay of FL	% Degradation	Mass balance
1	Controlled sample	103.4	99.2	0.90	NA
2	0.5N methanolic HCl, 60 °C, 60 min	102.8	97	1.60	98.6
3	0.5N methanolic NaOH, 60 °C, 30 min	104.0	92.2	8.70	100.9
4	60°C, 72 h	102.5	99.80	1.03	100.8
5	1 mL 30% H_2O_2, 60 min at room temperature	102.4	97.6	2.33	99.9
6	Photolytic condition (1.2 million lux h)	102.6	96.1	5.22	101.3

(b) *By forced degradation study*.—The % degradation and mass balance values in acid, base, peroxide, photolytic, and thermal conditions are summarized in Table 4. During the forced degradation study AZ was found to be stable whereas FL showed significant degradation in alkali and light. No significant degradation was observed for acid, peroxide, and thermal conditions for both AZ and FL.

LOD and LOQ Determination

The LOD and LOQ values for all the impurities of AZ and FL along with the principal analyte are reported in Table 5.

Linearity

The method was found to be linear from LOQ to 150% of standard concentration. From the linear regression curve, the correlation coefficient r^2 was found to be 0.998, 0.999, 0.999, 0.990, and 1 for AZ impurities A, B, C, E, and AZ, and more than 0.998 for FL impurities A to I and 0.999 for FL.

Range

The method was found to be linear from 0.2 to 6 μg/mL for both the analytes. All the known impurities were recovered within this concentration range.

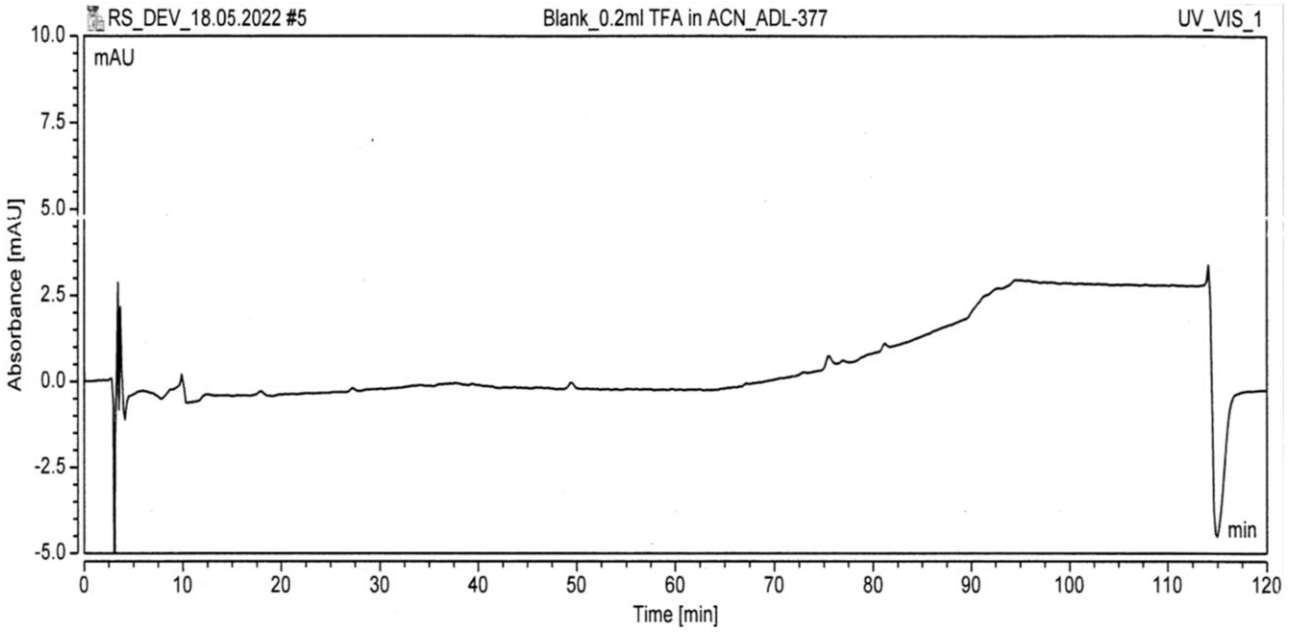

Figure 2. HPLC chromatogram of blank solution.

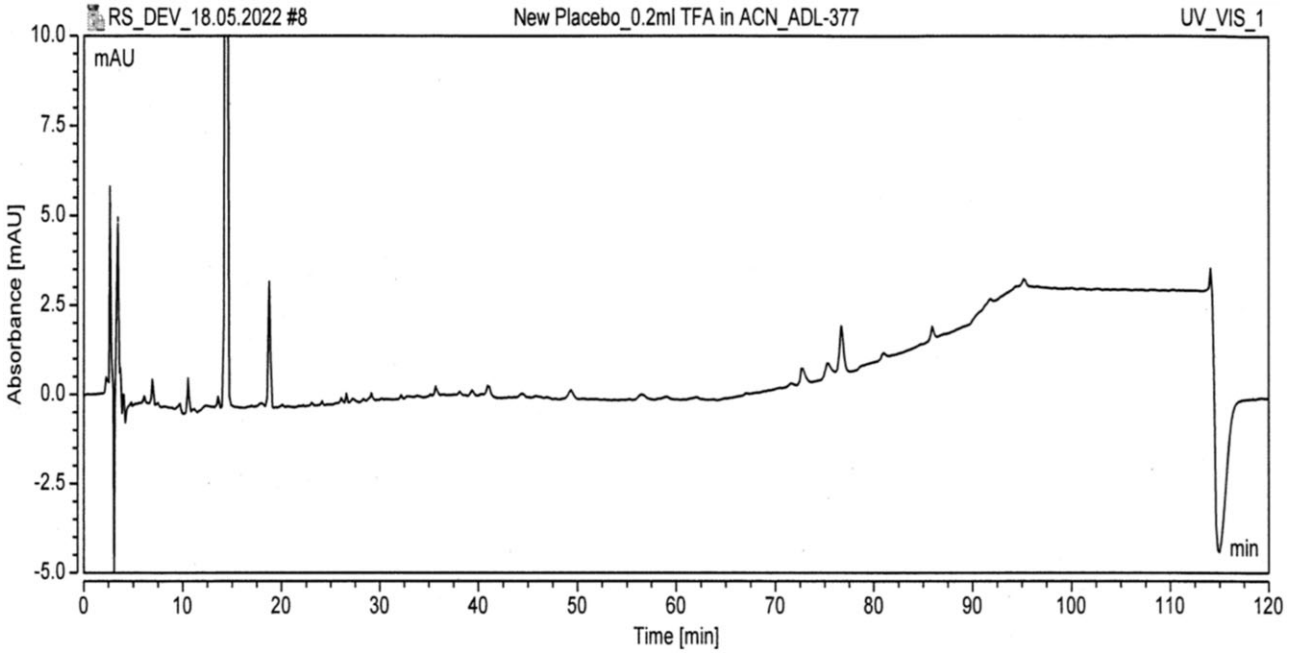

Figure 3. HPLC chromatogram of placebo solution.

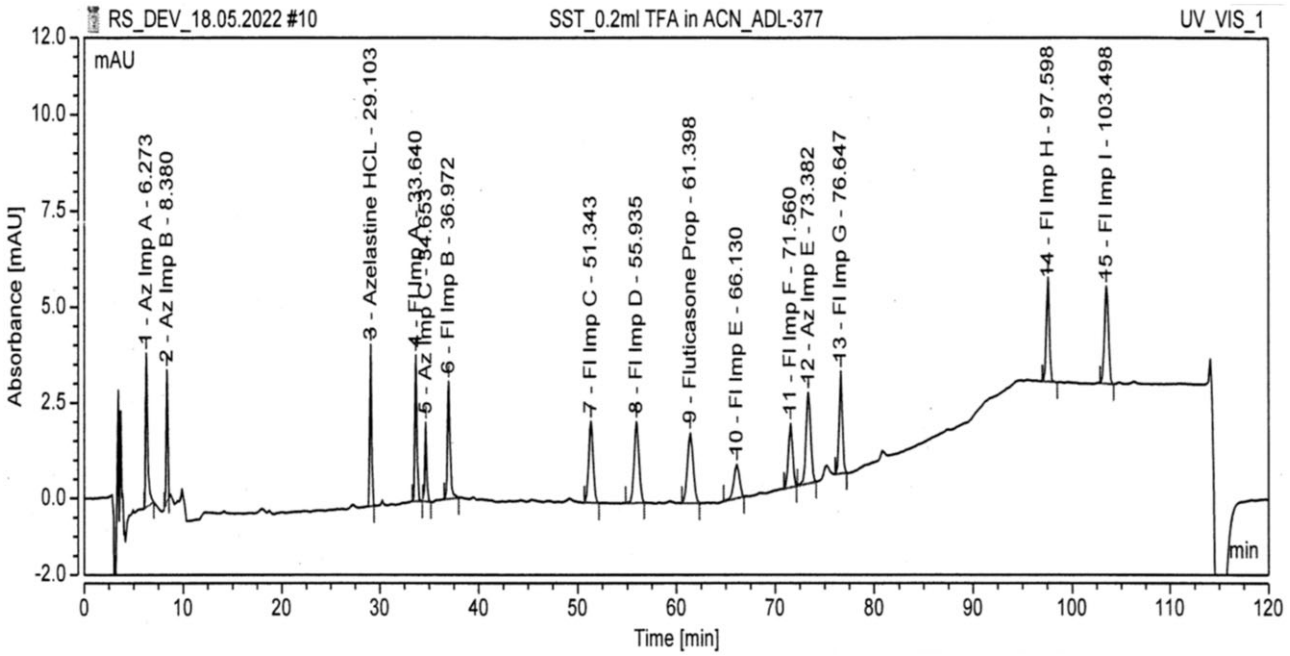

Figure 4. HPLC chromatogram of system suitability solution.

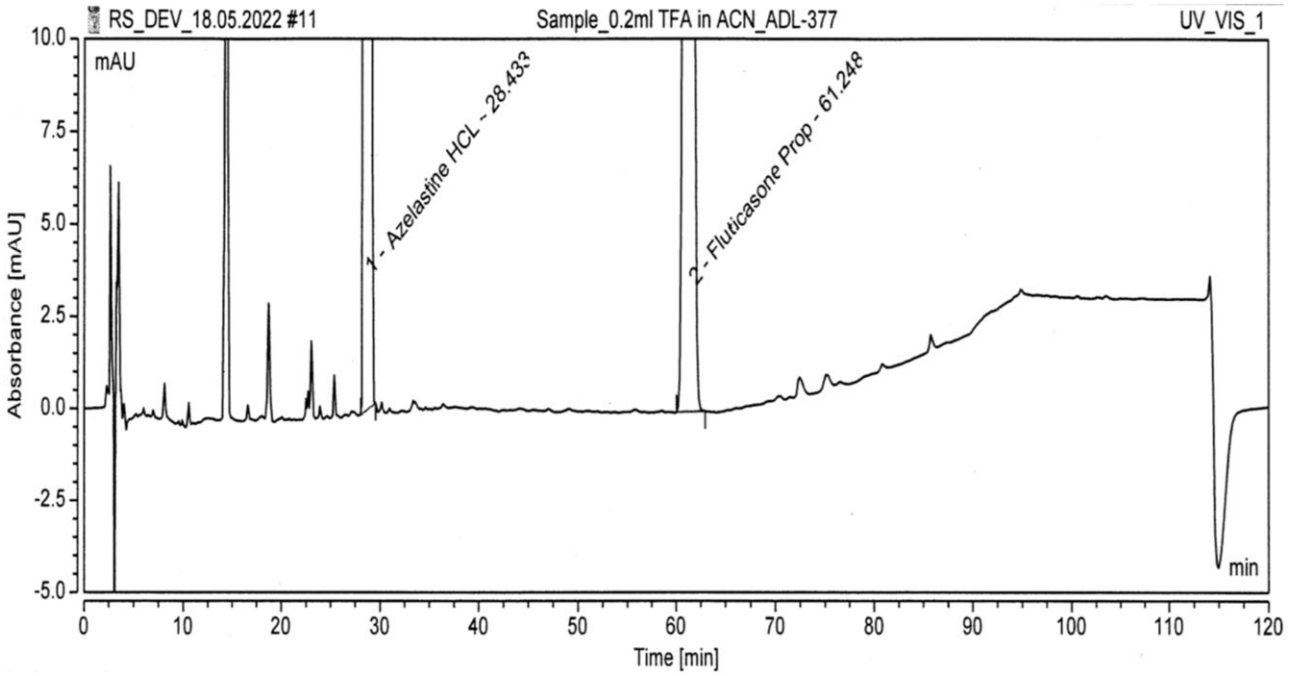

Figure 5. HPLC chromatogram of sample solution.

Robustness

No significant variation was observed in system suitability parameters during the robustness study. The relative retention time for all the impurities remained same. The resolution between FL impurity A and AZ impurity C, and FL impurity F and AZ impurity E, was more than 2.0 during the robustness study. The similarity factor between standard-1 and standard-2 was found to be within the limits.

Solution Stability

All the solutions were found to be stable for up to 48 h at room temperature. During the filter validation study, it was observed

Table 5. LOD and LOQ

Sr. No.	Impurity name	LOD, µg/mL	LOQ, µg/mL
1	AZ impurity A	0.442	1.339
2	AZ impurity B	0.998	3.023
3	AZ HCL	0.148	0.483
4	AZ impurity C	0.306	0.923
5	AZ impurity E	0.251	0.760
6	FL impurity A	0.334	1.013
7	FL impurity B	0.163	0.495
8	FL impurity C	0.090	0.276
9	FL impurity D	0.140	0.426
10	FL	0.159	0.483
11	FL impurity E	0.525	1.591
12	FL impurity F	0.722	2.188
13	FL impurity G	0.129	0.392
14	FL impurity H	0.481	1.458
15	FL impurity I	0.145	0.439

that none of the filters used showed any interference. The 0.45 µm nylon filer showed higher filtration efficiency.

Conclusions

A precise, accurate, and robust stability-indicating analytical method was developed for the impurity profiling of AZ and FL from a nasal spray drug product. The method is able to successfully quantitate four known impurities of AZ and seven known impurities of FL along with unknown degradants. To date, there is no reported method available for the simultaneous estimation of related substances for this combination product. The method is successfully validated as per ICH guidelines. This method can be successfully used during product development for stability studies and routine analysis of the commercial batches in the QC laboratory.

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Conflict of Interest

All authors declare no conflict of interest.

References

1. Lalitha, A., Mamatha, K.R., & Puttamadaiah, G.M. (2019) *Natl. J. Physiol. Pharm. Pharmacol.* **9**, 5, 1–360 doi: 10.5455/njppp.2019.9.0204820022019
2. Gill, C.H., Kulkarni, P.N., Nipate, A.S., Jadhav, C.K., Chate, A.V., & Dodake-Supekar, A.M. (2018) *J. Drug Deliv. Ther.* **8**, 236–240 doi: https://doi.org/10.22270/jddt.v8i6-s.2222
3. British pharmacopeia (BP). (2020) The British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare Products Regulatory Agency (MHRA), TSO (The Stationery Office), London, PP 236–237
4. British pharmacopeia (BP). (2020) The British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare Products Regulatory Agency (MHRA), TSO (The Stationery Office), London, PP 1099–1101
5. Patel, S., & Pasha, T.Y. (2018) *Asian J. Pharm. Clin. Res.* **11**, 8, 248–251 doi: http://dx.doi.org/10.22159/ajpcr.2018.v11i8.25506
6. Thangabalan, B., & Palanirajan, V. (2012) *Int. J. Pharm. Sci. Rev. Res.* **17**, 2, n°, 62–64
7. Hanan, A.M., Sally, S., El- Mosallamy, N.Y.H., & Badr, A.E. (2016) *Spectrochim. Acta* **160**, 50–57 doi:https://doi.org/10.1016/j.saa.2016.02.010
8. Rao, K.L.N., Reddy, K.P., Babu, K.S., Raju, K.S., Rao, K.V., & Shaikh, J.V. (2010) *Int. J. Res. Pharm. Sci.1* **4**, 473–480
9. Patel, K.G., Patel, S.G., Shah, A.P., Tandel, D.B., Tejal, R., & Gandhi, T.R. (2019) *IJPER.* **54**, 155–165 doi: 10.5530/ijper.54.1.19
10. Da Costa, L.M., De Leite, H.O., Kassab, N.M., & Singh, A. (2019) *Int. J. Anal. Chem.* **2019**, 1 doi:https://doi.org/10.1155/2019/9489723
11. El-Shaheny, R.N., & Yamada, K. (2014) *Anal. Sci.* **30**, 691–697 doi: https://doi.org/10.2116/analsci.30.691
12. Kasaye, L., Hymete, A., & Mohamed, A.-M.I. (2010) *Saudi Pharm. J.* **18**, 153–159 doi:10.1016/j.jsps.2010.05.001
13. Thorat, J., Rao, J.R., & Dhale, C. (2018) *J. Emerg. Technol. Innov. Res.* **5**, 8, 503–514
14. Rasheed, A., & Osman, A. (2017) *World J. Pharm. Life Sci.* **3**, 5, 168–172
15. Sá Couto, A., Cardoso, D.E. & Cabral-Marques, H.M. (2014) *Sci. Pharm.* **82**, 787–797.
16. Mamillapalli, S.P., Koyya, S., Subbaiah, B.V., & Annapurna, N. (2021) *Asian J. Chem.* **33**, 867–872 doi: https://doi.org/10.14233/ajchem.2021.23101
17. Çayir, A., Çilen, H., Sakızcı, C.D., & Türkyılmaz, A. (2017) Chromatography Today, May/June 14–19
18. Rao, M.R.P., Thota, R.K., Senthi, M., Mogadati, P., & Arutla, S. (2020) *Int. J. Pharm. Pharm. Sci. Sci.* **12**, 11, 80–87 doi:https://doi.org/10.22159/ijpps.2020v12i11.38750
19. Shammout, M.J., Yousef, H.B., Abu-Shandi, K.H., Alomari, M.I., Hasan, M.R., Al-Othman, A.O., & Manasreh, W.A. (2021) *Int. J. App. Pharm.* **13**, 4, 204–210 doi:https://doi.org/10.22159/ijap.2021v13i4.41678
20. Samir, A., Salem, H., & Abdelkawy, M. (2012) *Pharmaceut. Anal. Acta.* **3**, 8, 1–7 doi: 10.4172/2153-2435.1000180
21. Murnane, D., Martin, G.P., & Marriott, C. (2006) *J. Pharm. Biomed. Anal.* **40**, 1149–1154 Doi:10.1016/j.jpba.2005.09.028
22. Duran, A., Dogan, H.N., & Ulgen, M. (2014) *Drug Metab. Lett.* **8**, 31–35 doi:10.2174/1872312808011409291611 36
23. International Conference on Harmonization (ICH) (2005) Validation of analytical procedure text and methodology, Q2 (R1). https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf
24. Fayyad, M.K., Misha, A.K., & Yousef Al-Musaimi, O.I. (2010) *J. Anal. Bioanal. Tech.* **1**, 103 doi: 10.4172/2155-9872.1000103