RJC

**RASĀYAN** *J. Chem.*
Vol. 16 | No. 1 |76-84| January - March | 2023
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

# SIMULTANEOUS REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC) ESTIMATION OF AZELASTINE HYDROCHLORIDE, FLUTICASONE PROPIONATE, AND PHENYL ETHYL ALCOHOL IN DYMISTA (MEDA) NASAL SPRAY

**A. K. Gupta[1,2], P. K. Singour[2] and S. K. Jain[1,✉]**

[1]Department of Pharmacy, Guru Ghasidas Vishwavidyalaya (A Central University), Bilaspur-495009, (Chhattisgarh) India

[2]Department of Pharmaceutical Chemistry, VNS Group of Institutions-Faculty of Pharmacy, Bhopal-462044, (Madhya Pradesh) India

✉Corresponding Author: sanmatijain72@yahoo.co.in

**ABSTRACT**

This work reported a reverse phase-high performance liquid chromatography method for quantitative estimation of Azelastine Hydrochloride, Fluticasone Propionate, and Phenylethyl Alcohol in Nasal Spray formulation. The assay involved an isocratic elution of these components by using Intersil Octadecylsilane (C18) column (25 cm x 4.6 mm x 5 µm) using mobile phase composition of Buffer: Solvent Mixture (40:60 %v/v) and pH adjusted to 6.5 with dilute orthophosphoric acid. The flow rate was 1.4 $mLmin^{-1}$ and the analytes are monitored at 254 nm. Separation was completed within 20 minutes. Calibration curves were linear with correlation coefficient more than 0.99 over a concentration range of 50%, 75%, 100%, 125%, and 150% for Azelastine Hydrochloride, Fluticasone Propionate and Phenylethyl Alcohol. The method was proven to be accurate between 50 to 150% with 98 to 102 % recovery of the actives from a spiked placebo. The method was shown to be precise yielding acceptable results for the system reproducibility and method repeatability. All the validation parameters were within the acceptance range according to International Conference on Harmonization norms. The developed method is simple and rapid which could be applied for routine estimation of the formulation.

**Keywords:** Azelastine Hydrochloride, Fluticasone Propionate, Seasonal Allergic Rhinitis, RP-HPLC, Nasal Spray, DYMISTA.

RASĀYAN*J. Chem.*, Vol. 16, No.1, 2023

## INTRODUCTION

Seasonal allergic rhinitis (SAR) occurs when a sensitized individual is exposed to airborne allergens (usually a tree, grass, or weed pollen) that trigger inflammation of the upper airways.[1] Azelastine Hydrochloride (AZH) and Fluticasone Propionate (FP) are present in DYMISTA to treat Allergic Rhinitis (AR). It is more effective for a two-week therapy period, despite symptom, seriousness or time of year.[2,3] A combination of Intranasal Antihistamine (INAH) and Intranasal Corticosteroid (INCS) shows a synergistic effect in decreasing AR symptoms with a dissimilar mechanism of action. DYMISTA also provides more rapid and better recovery in rhinal and eyes symptoms than AZH or FP monotherapy.[4,5] DYMISTA nasal spray has a number of compounds listed in Table-1.[6]

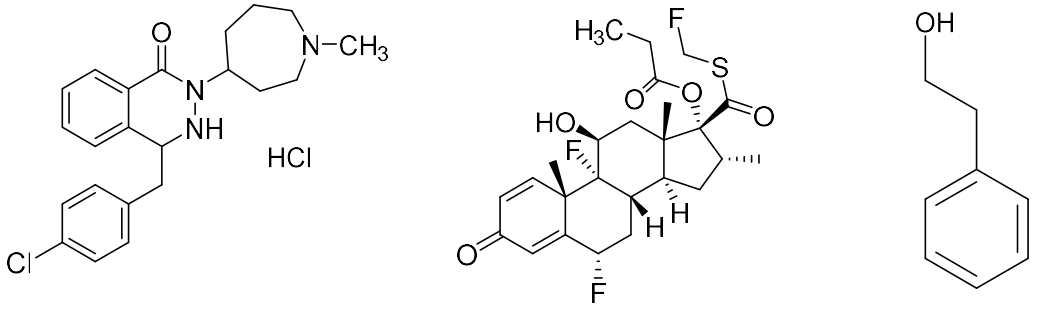
Table-1: List of Compounds in DYMISTA Nasal Spray

| Active ingredients | Strength |
|---|---|
| Azelastine Hydrochloride | 137µg per spray |
| Fluticasone propionate | 50 µg per spray |
| Phenylethyl Alcohol | 2.5 mg per gram |
| Benzalkonium Chloride | 0.1 mg per gram |
| Microcrystalline Cellulose | Quantity Sufficient |
| Carboxymethylcellulose Sodium | |
| Glycerin | |


http://doi.org/10.31788/RJC.2023.1616916

| | |
|---|---|
| Edetate Disodium | |
| Polysorbate 80 | |
| Purified Water | |

AZH (Fig.-1a) is efficient for bringing down cold, itchiness, sternutation, and dilation of blood vessels without relieving of blocked nose and also in the treatment of AR and Asthma. It is the second-generation H-1 receptor antagonist that inhibits the release of Histamine, Prostaglandins, and Leukotrienes.[7-10] It can be estimated by colorimetry, thin layer chromatography (TLC), and HPLC.[11-13] FP (Fig.-1b) is $\beta_2$ agonist and bronchodilator and is beneficial for itching of the nose, common cold, sternutation, congestion, and ocular symptoms of rhinoconjunctivitis.[14] It also shows good topical anti-inflammatory activity and is commonly used in the treatment of asthma and allergic rhinitis.[15] Phenylethyl alcohol (PEA) (Fig.-1c) is an antimicrobial preservative used in nasal spray.[16,17]




(a) (b) (c)

Fig.-1: Chemical Structure of AZH (a), FP (b), and PEA (c)

RP-HPLC method for simultaneous quantitative estimation of AZH, FP, and PEA in Nasal Spray formulation (DYMISTA) was developed in the present study.[18-20]

## EXPERIMENTAL

Simultaneous estimation of DYMISTA nasal spray was established by RP-HPLC method using isocratic elution. Various steps were comprised of chromatographic conditions, material, and equipment, mobile phase preparations, diluents, standard solutions, and sample preparations. Method validation was also implemented and obtained data are represented for each characteristic parameter.

### Chromatographic Conditions

Column: Inertsil ODS (25cm×46mm×5µm); Column temperature: 50ºC; Mobile phase: 40:60 ratio (Buffer: Solvent Mixture); Buffer: Potassium Dihydrogen Orthophosphate with Triethyl Amine (TEA) and pH 6.5 adjusted with diluted Ortho Phosphoric Acid (OPA); Solvent Mixture: 45:55 (Methanol: Acetonitrile); Injection volume: 20µL; Wavelength: 254nm; Runtime: 20 Minutes. The retention time (Rt) for the active ingredients is shown in Table-2.

Table-2: Retention Time of Active Ingredients

| Active | Rt (in minutes) |
|---|---|
| Azelastine Hydrochloride | 10.85 |
| Fluticasone Propionate | 17.01 |
| Phenylethyl Alcohol | 3.28 |

### Materials and Equipment

Drug (Active ingredients): AZH, FP, PEA; Placebo Sample and DYMISTA nasal spray (Sample) (Analytical Development Laboratory, Zydus Cadila, Ahmedabad, Gujrat, India); Buffer: Potassium Dihydrogen Orthophosphate ($KH_2PO_4$); Solvents: Methanol, Acetonitrile and TEA, all are of HPLC grade (Merck), and OPA of HPLC grade (Spectrochem); Deionized water: MiliQ water in house supply (metro ohm); HPLC: Shimadzu LC-2010 UV detector with LC solution software and Agilent 1100 DAD detector with Chromeleon software.

**Mobile Phase Preparation**

Buffer: 15Mm Potassium dihydrogen orthophosphate combined with 1000 mL of deionized water. Then 8 ml TEA was mixed in the solution and pH was adjusted to 6.5 with dilute OPA.
Solvent Mixture: 450 mL of Methanol and 550 mL of Acetonitrile were mixed thoroughly and sonicated.
Mobile Phase: 40:60 (Buffer: Solvent Mixture).
Diluent: 300 mL of Deionised Water and 700 mL of Acetonitrile were mixed thoroughly and sonicated.

**Standard Solution**

62.5 mg (AZH), 23 mg (FP), and 156 mg (PEA) were taken in 100 mL of a volumetric flask containing 50 mL of diluent. The flask was sonicated until the drug particles completely dissolved and make up the volume with diluent. After that 4 mL of stock standard solution was transferred into the 50 mL volumetric flask. Then volume makeup to the final mark and mix thoroughly. The final concentration of AZH (50 ppm), FP (18.4 ppm), and PEA (124.8 ppm) were achieved.

**Sample Preparation**

Six bottles of DYMISTA nasal spray were taken and mixed in another container and mixed thoroughly. Five grams of it was taken in a 100 mL volumetric flask containing 50 mL of diluent and then sonicated for 20 minutes. Then finally makeup to mark and mixed thoroughly. The suspension was centrifuged (4000 rpm) for 15 minutes and the resultant supernatant was used for the assay and development process.

## RESULTS AND DISCUSSION

**System Suitability**

System suitability was performed by preparing five replicate standards solutions for each active ingredient. % Relative standard deviation (%RSD) of peak area responses of AZH, FP, and PEA were less than 2%. The number of theoretical plates for AZH, FP, and PEA was more than 2000. The tailing factor for AZH, FP and PEA were less than 2. The outcome of system suitability was found under acceptance criteria indicating that the system was appropriate to analyze the sample for validation of the developed method. System suitability result is tabulated in Table-3.

Table-3: Results of System Suitability

| Parameters | Acceptance criteria | AZH | FP | PEA |
|---|---|---|---|---|
| Theoretical Plate (N) | >2000 | 7741 | 8839 | 5388 |
| Tailing Factor (T) | Not more than2.0 | 1.1 | 1.0 | 1.1 |
| %RSD (n=5) | Not more than 2.0 | 0.07 | 0.05 | 0.14 |

%RSD-% Relative standard deviation

**Results of Specificity**

Examining the blank: The sample blank was tested to prove that no peak was observed at particular Rt as AZH, FP, and PEA. No peaks were found in the blank chromatogram (Fig.-2).

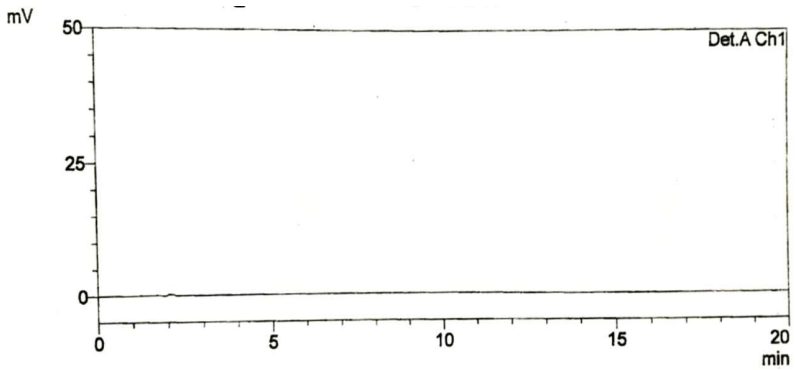



Fig.-2: Chromatogram for Blank

Examining the sample matrix (placebo): The sample matrix short of Active Pharmaceutical Ingredient (API) indicates that there are no peaks observed at particular Rt as AZH, FP, and PEA. There are no peaks were seen in the chromatogram beyond Rt of AZH, FP, and PEA (Fig.-3).

**Specificity Discussion**

This parameter is the ability to obtain no peak interference of the analytes in the presence of components such as placebo and placebo preparation (spiked API in placebo). Then it was found to be no peak interference of analytes at particular Rt of AZH, FP, and PEA, therefore we can say that this method is specific for the established method. No peaks were observed in the given chromatogram and this will be coming under acceptance criteria of specificity.

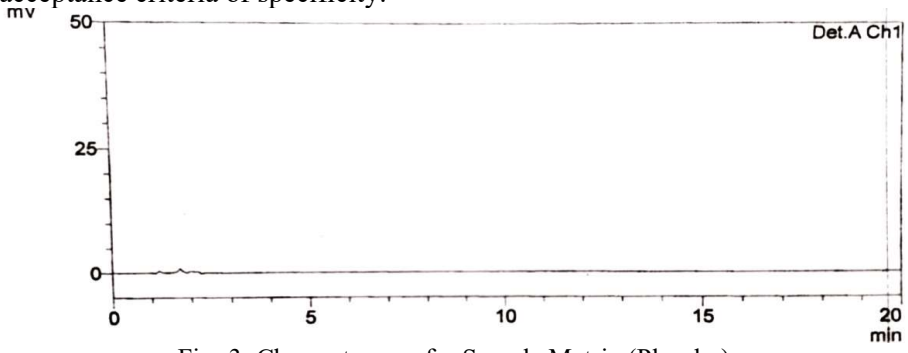



Fig.-3: Chromatogram for Sample Matrix (Placebo)

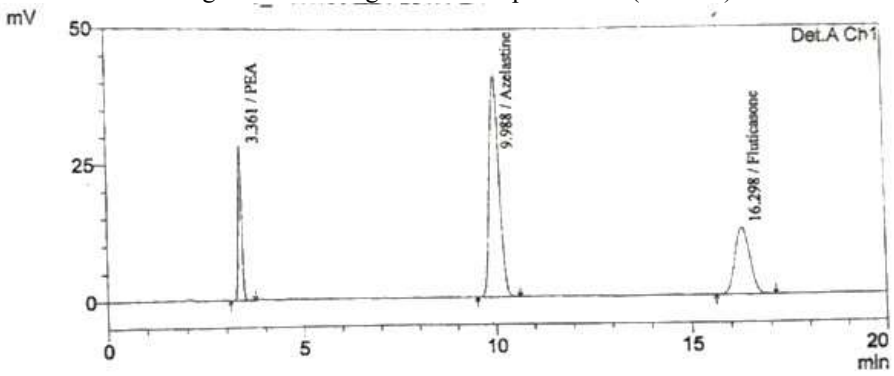



Fig.-4: Chromatogram for Standard Solution

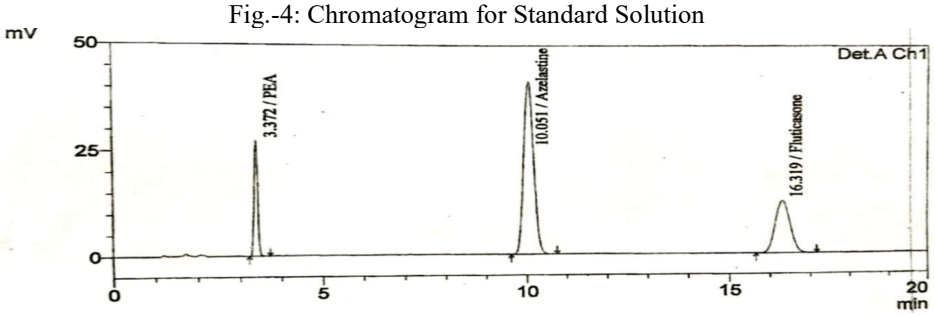



Fig.-5: Chromatogram for Sample Preparation

**Linearity Results**

Linearity gives the information that peak area is clearly related to the concentrations of analyte in the sample over a specific range. Linearity plots in the concentration range of 25 to 75 ppm, 9.2 to 27.6 ppm, and 62.4 to 124.8 ppm for AZH, FP, and PEA respectively were obtained with the correlation coefficient ($r^2$) of $\geq 0.9999$ (Table-5).

**Experiments**

Stock solutions for AZH, FP, and PEA were prepared at 50%, 75%, 100%, 125%, and 150% of the working standard. It was prepared over the concentration range from 0.025 to 0.075 mg/ml, 0.0092 to 0.0276 mg/ml, and 0.0624 to 0.1872 mg/ml for AZH, FP, and PEA respectively which is tabulated in Table-4.

**Linearity of the Actives**

All concentrations for AZH, FP, and PEA were analyzed and calculated the % recovery and Correlation coefficient ($r^2$).

**Linearity Analysis**

The $r^2$ from the response vs concentration linearity curve for AZH, FP, and PEA was 0.9999 which is more than 0.99. Calculated data was presented in Table-5. Linearity graphs were represented for AZH, FP, and PEA in Fig.-6, 7, and 8 respectively. Percentage recovery for AZH, FP, and PEA was also represented in Table-5. The obtained value is under acceptance criteria.

Table-4: Concentration of Compounds for Linearity and Accuracy

| Component | Standard weight (mg) | 50% Conc. (mg/ml) | 75% Conc. (mg/ml) | 100% Conc. (mg/ml) | 125% Conc. (mg/ml) | 125% Conc. (mg/ml) |
|---|---|---|---|---|---|---|
| AZH | 62.5 | 0.025 | 0.0375 | 0.05 | 0.0625 | 0.075 |
| FP | 23 | 0.0092 | 0.0138 | 0.0184 | 0.0230 | 0.0276 |
| PEA | 156 | 0.0624 | 0.0936 | 0.1248 | 0.156 | 0.1872 |

Conc.-Concentration

**Accuracy or Trueness Results**

It is proved by recovery of the analytes of known quantity which was added into the placebo preparations (spiking in placebo with a known quantity of AZH, FP, and PEA standard solution).

**Spiked Placebo Solutions**

The concentration prepared for Accuracy in the spiked placebo solution was the same as represented in Table-4.

Table-5: Results for Linearity

| Active | Conc.(%) | Speculativeconc. (mg/mL) | Actual conc. (mg/mL) | % Recovery | $r^2$ |
|---|---|---|---|---|---|
| AZH | 50 | 0.0250 | 0.0250 | 100.04 | 0.9999 |
| | 75 | 0.0375 | 0.0375 | 100.02 | |
| | 100 | 0.0500 | 0.0503 | 100.60 | |
| | 125 | 0.0625 | 0.0627 | 100.32 | |
| | 150 | 0.0750 | 0.0753 | 100.42 | |
| FP | 50 | 0.0092 | 0.0091 | 98.91 | 0.9999 |
| | 75 | 0.0138 | 0.0136 | 98.55 | |
| | 100 | 0.0184 | 0.0184 | 100.16 | |
| | 125 | 0.0230 | 0.0231 | 100.43 | |
| | 150 | 0.0276 | 0.0278 | 100.72 | |
| PEA | 50 | 0.0624 | 0.0622 | 99.67 | 0.9999 |
| | 75 | 0.0936 | 0.0931 | 99.46 | |
| | 100 | 0.1248 | 0.1243 | 99.59 | |
| | 125 | 0.1560 | 0.1570 | 100.60 | |
| | 150 | 0.1872 | 0.1877 | 100.20 | |

$r^2$-Correlation coefficient, Conc.- Concentration

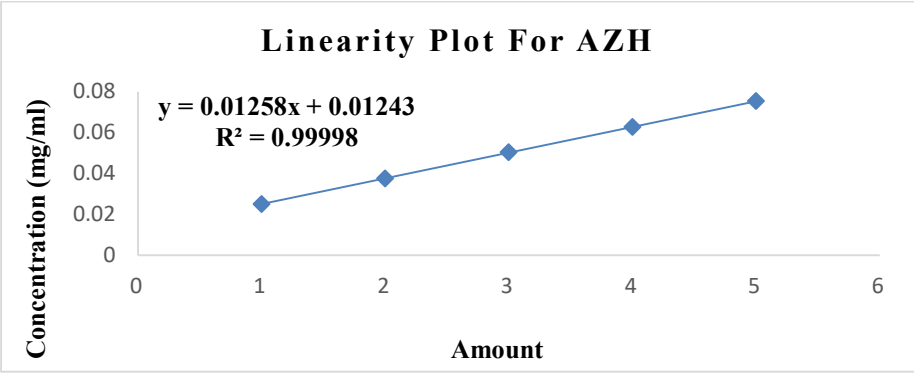



Fig.-6: Linearity Plot for AZH

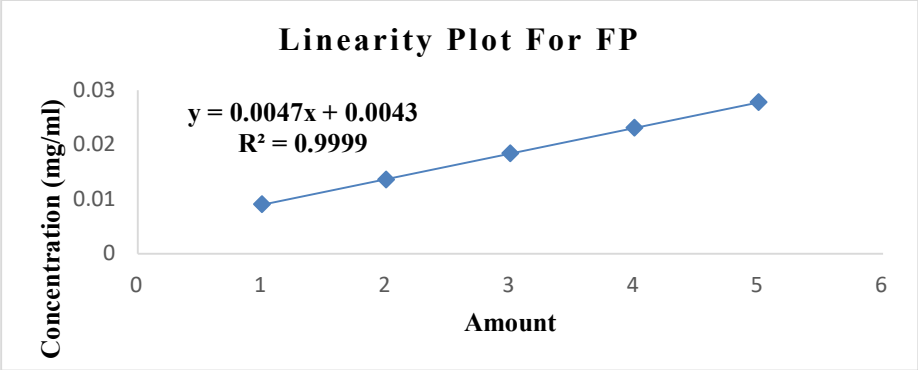



Fig.-7: Linearity Plot for FP

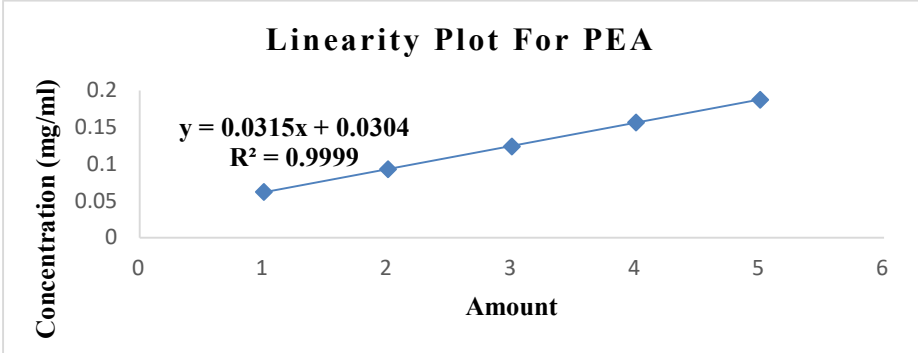



Fig.-8: Linearity Plot for PEA

**Accuracy of Actives**

Trueness or accuracy results are displayed in Table-6.

Table-6: Results for Trueness or Accuracy

| Active | Conc.(%) | Speculativeconc. (mg/ml) | Actual conc. (mg/ml) | % Recovery | % RSD |
|---|---|---|---|---|---|
| AZH | 50 | 0.0250 | 0.0252 | 100.96 | 0.02 |
| | 75 | 0.0375 | 0.0374 | 99.76 | |
| | 100 | 0.0500 | 0.0503 | 100.60 | |
| | 125 | 0.0625 | 0.0619 | 99.15 | |
| | 150 | 0.0750 | 0.0744 | 99.26 | |
| FP | 50 | 0.0092 | 0.0091 | 99.89 | 0.01 |
| | 75 | 0.0138 | 0.0138 | 100.14 | |
| | 100 | 0.0184 | 0.0183 | 99.89 | |
| | 125 | 0.0230 | 0.0229 | 99.56 | |
| | 150 | 0.0276 | 0.0275 | 99.96 | |
| PEA | 50 | 0.0624 | 0.0619 | 99.19 | 0.05 |
| | 75 | 0.0936 | 0.0931 | 99.55 | |
| | 100 | 0.1248 | 0.1248 | 100.07 | |
| | 125 | 0.1560 | 0.1570 | 100.64 | |
| | 150 | 0.1872 | 0.1869 | 99.83 | |

$r^2$-Corelation coefficient, Conc.- Concentration, RSD- Relative Standard Deviation

**Accuracy Discussion**

The percentage recovery of placebo preparations at the level of 50%, 75%, 100%, 125%, and 150% was prepared and analyzed. The concentration was found under acceptance criteria such as 98% to 102% with less than 2.0% RSD.

**Precision Results**

The system precision (reproducibility) and method precision (repeatability) were estimated by utilizingplacebo preparations (spiking placebo with a known quantity of AZH, FP, and PEA standard

solution). The system precision was evaluated by injecting the sample six times for every single preparation and also, and method precision was evaluated by injecting the multiple sample preparation.

**System Precision Results**

The system Precision result was shown in Table-7.

Table-7: Results for System Precision (Reproducibility)

| Analytes | Peak area %RSD, (n=6 injections) |
|---|---|
| AZH | 0.42 |
| FP | 0.33 |
| PEA | 1.19 |

**System Precision Discussion**

Percent RSD for AZH, FP, and PEA observed from peak area under acceptance criteria was not more than 2%.

**Method Precision Results**

The system Precision result was shown in Table-8.

Table-8: Results for Method Precision (Repeatability)

| Analytes | Peak area % RSD, (n=3 preparations) |
|---|---|
| AZH | 0.91 |
| FP | 0.68 |
| PEA | 1.07 |

**Method Precision Discussion**

Percent RSD for AZH, FP, and PEA were observed from peak area that is under acceptance criteria (not more than 2%).

**Robustness Results**

This parameter indicated that the analytical procedure is unaffected by small, but deliberate changes in method performance. The results of robustness were represented in Table-9.

Table-9: Results of Different Robustness

| Conditions | % RSD | Rt |
|---|---|---|
| For AZH | | |
| Normal condition | 0.15 | 9.763 |
| flow rate (+0.2 mL/min) | 0.03 | 8.58 |
| flow rate (-0.2 mL/min) | 0.07 | 11.373 |
| Column temperature (+5°C) | 0.11 | 10.007 |
| Column temperature (-5°C) | 0.03 | 9.523 |
| The organic ratio in the mobile phase (+2%) | 0.03 | 9.29 |
| The organic ratio in the mobile phase (-2%) | 0.07 | 10.89 |
| pH (+0.2 unit) | 0.6 | 11.873 |
| pH (-0.2 unit) | 0.08 | 8.2 |
| For FP | | |
| Normal condition | 0.14 | 15.933 |
| flow rate (+0.2 mL/min) | 0.23 | 13.88 |
| flow rate (-0.2 mL/min) | 0.11 | 18.71 |
| Column temperature (+5°C) | 0.14 | 14.503 |
| Column temperature (-5°C) | 0.07 | 17.587 |
| The organic ratio in the mobile phase (+2%) | 0.16 | 13.907 |
| The organic ratio in the mobile phase (-2%) | 0.08 | 21.1 |
| pH (+0.2 unit) | 0.18 | 16.42 |
| pH (-0.2 unit) | 0.07 | 15.23 |
| For PEA | | |
| Normal condition | 0.19 | 3.14 |
| flow rate (+0.2 mL/min) | 0.04 | 3.751 |
| flow rate (-0.2 mL/min) | 0.04 | 3.787 |

| | | |
|---|---|---|
| Column temperature (+5°C) | 0.13 | 3.177 |
| Column temperature (-5°C) | 0.03 | 3.31 |
| The organic ratio in the mobile phase (+2%) | 0.05 | 3.147 |
| The organic ratio in the mobile phase (-2%) | 0.2 | 3.45 |
| pH (+0.2 unit) | 0.12 | 3.26 |
| pH (-0.2 unit) | 0.05 | 3.22 |

**Robustness Discussion**

There was no significant change in the %RSD of AZH, FP, and PEA with slight variations in flow rate (±0.2 mL/min), Column temperature (±5°C), Organic ratio in the mobile phase (±2%) and pH (±0.2 unit). The % RSD observed under acceptance criteria was less than 2% (Table-4). Rt could be changed with some changes in chromatographic conditions but % RSD was under acceptance criteria.

## CONCLUSION

The RP-HPLC method was developed and confirmed for simultaneous estimation of AZH, FP, and PEA in DYMISTA nasal spray formulation. The developed method is simple, sensitive, precise, and accurate. The RP-HPLC method can be applied on a research scale and industrial scale for simultaneous estimation of AZH, FP, and PEA.

## ACKNOWLEDGMENTS

The authors wish to thank the Pulmonary Team, Analytical Development Lab, Zydus Cadila Ahmedabad, India, for providing me with a golden opportunity to carry out this research work. Also, I am thankful for my co-guide, and colleagues for making my successful work.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## AUTHORS CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The authors' ORCID ids are as follows.

A. K. Gupta https://orcid.org/0000-0002-9286-0475
P. K. Singour https://orcid.org/0000-0002-4592-5934
S. K. Jain https://orcid.org/0000-0002-4798-7151



## REFERENCES

1. B. M. Prenner, N. J. Amar, F. C. Hampel, C. F. Caracta, and W. Wu, *Annals of Allergy, Asthma & Immunology*, **129(5)**, 618(2022), https://doi.org/10.1016/j.anai.2022.07.029
2. D. Price, S. Shah, S. Bhatia, C. Bachert, W. Berger, J. Bousquey, W. Carr, P. Hellings, U. Munzel, G. Scadding, and P. Lieberman, *Journal of Investigational Allergology and Clinical Immunology*, **23(7)**, 495(2013).
3. W. Alabsi, B. B. Eedara, D. Encinas-Basurto, R. Polt, and H. M. Mansour, *Pharmaceutics*, **14(9)**,1870(2022), https://doi.org/10.3390/pharmaceutics14091870
4. G. L. Plosker, and K. A. Lyseng-Williamson, *Drugs & Therapy Perspectives*, **31**, 425(2015), http://dx.doi.org/10.1007/s40267-015-0254-5
5. W. Berger, J. Bousquet, A. T. Fox, J. Just, A. Muraro, A. Nieto, E. Valovirta, M. Wickman, and U. Wahn, *Allergy*, **71**, 1219(2016), https://doi.org/10.1111/all.12903
6. DYMISTA-azelastine hydrochloride and fluticasone propionate spray, metered. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=4c557ec4-c4cf-11df-851a-0800200c9a66#section-3

7. M. E. M. Hassouna, M. M. Abdelrahman, and M. A. Mohamed, *World Journal of Applied Chemistry*, **2(2)**, 48(2017), http://dx.doi.org/10.11648/j.wjac.20170202.12
8. E. Trybus, T. Krol, and W. Trybus, *International Journal of Molecular Sciences,* **23(11)**, 5890(2022), http://dx.doi.org/10.3390/ijms23115890
9. A. V. Patil, and H. S. Mahajan, *Carbohydrate Polymer Technologies and Applications*, **2**, 100078(2021), https://doi.org/10.1016/j.carpta.2021.100078
10. A. Licari, G. Marseglia, and G. Ciprandi, *Current Treatment Options in Allergy*, **3**, 495(2016), https://doi.org/10.1007/s40521-016-0105-1
11. R. T. Sane, S. G. Joshi, M. Francis, and A. R. Khatri, *Indian drugs*, **36(5)**, 317(1999).
12. R. T. Sane, S. G. Joshi, M. Francis, A. R. Khatri, and P. Hijli, *Journalof Planar Chromatography*, **12(2)**, 158(1999).
13. Y. S. Park, S. H. Kim, Y. J. Kim, S. C. Yang, M. H. Lee, L. M. Shaw, and J. S. Kang, *International Journal of Biomedical Science*, **6(2)**, 120(2010).
14. K. Malik, D. Kumar, V. Tomar, S. Kaskhedikar, and L. Soni, *Der Pharmacia Sinica*, **2(6)**, 77(2011).
15. S. C. Martindale, The complete drug references, pharmaceutical press publication division of the Royal Pharmaceutical Society of Great Britain, USA, p. 1102(2005).
16. British Pharmacopoeia, her majesty's stationery office, London, p.191, 874(2005).
17. United states pharmacopoeia. United states pharmacopoeia Convention Washington DC, p. 2434, 3286(2009).
18. V. N. V. Kishore, G, V. Ramana, R. VenkataNadh and G. P. Gorumutchu, *RASĀYAN Journal of Chemistry*, **14**, 2236(2021), http://doi.org/10.31788/RJC.2021.1446412
19. S. Shiyan, T. Hertiani, R. Martien, and A. K. Nugroho, *Rasayan Journal of Chemistry*,**12(3)**, 1098(2019), http://dx.doi.org/10.31788/RJC.2019.1235276
20. J. S. Rani, and N. Devanna, *Rasayan Journal of Chemistry*, **11(2)**, 452(2018), http://dx.doi.org/10.31788/RJC.2018.1122079

[RJC-6916/2022]