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Reverse Phase Liquid Chromatography Method Development and Validation for Impurity Profiling of Donepezil Drug

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ABSTRACT
Reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for concurrent detection of five impurities in donepezil hydrochloride. Using the method employed an Hypersil ODS, 25 cm x 4.6 mm, 5.0µm), chosen for its superior retention, resolution, and reproducibility. The mobile phase was optimized to a gradient program consisting of solution A as 10 mM diammonium hydrogen orthophosphate in water (pH 6.0) and Solution B as acetonitrile and methanol (85:15v/v). This method demonstrated excellent peak symmetry, resolution, and reproducibility at wavelength of 230 nm and a column temperature at 35°C. Despite initial difficulties with peak shape, the final conditions produced reliable and high-quality chromatograms for impurity profiling. The RP-HPLC method effectively quantifies five impurities (Imp-A to Imp-E) and satisfies key validation criteria like accuracy, precision, linearity, specificity, and system suitability. It shows robust performance, though some impurities are challenging to detect at lower concentrations. System suitability tests confirm consistency in peak areas and retention times, validating the method as both reliable and cost-effective for impurity analysis in donepezil hydrochloride, ensuring the drug's quality and safety.

Keywords: HPLC Method, Validation, Impurities, System Suitability

INTRODUCTION
Controlling organic impurities in pharmaceutical products is essential for ensuring drug purity, as it directly affects patient safety and drug efficacy.[1] The precise, sensitive and robust stability-indicating analytical method is vital for monitoring drug stability and reducing production losses.[1] Key factors in developing such a method include selecting the appropriate detection wavelength for impurities, determining the limit of detection, and considering the drug's label claim in the finished product.[1]
Impurity profiling is crucial in pharmaceutical analysis for ensuring drug quality, efficacy, and safety.[2] We use contemporary analytical methodologies such as UPLC, LC-MS, HRMS, GC-MS, HPTLC, & NMR spectroscopy to establish impurity profiling tools.[2] These methods are necessary for identifying, characterizing, and quantifying impurities and degradation products in bulk drug synthesis and formulations.[3] High-performance liquid chromatography (HPLC) is widely used for separating and detecting active pharmaceutical ingredients (APIs) & related substances.[3] Method development is particularly important for new drug molecules, pro-drugs.[3] The goal is to showcase a more cost-effective, time-efficient, and highly reproducible approach. By optimizing chromatographic conditions and employing

advanced detection techniques, this new method ensures faster analysis with reduced solvent and reagent consumption, lowering operational costs. Additionally, its robustness offers consistent and reproducible results across various laboratories, providing a reliable tool for regulatory compliance and quality control in Donepezil manufacturing.

The process involves systematic development, optimization, and validation of analytical methods according to regulatory guidelines, for example ICH Q2(R2).[3] The forced degradation studies and stability-indicating assay methods are also crucial aspects of impurity profiling.[2] Alzheimer's disease affects around 50 million people worldwide, primarily in individuals over 65, and leads to a gradual decline in memory, thinking, behavior, and social skills.[4] Donepezil, an acetyl cholinesterase inhibitor, is commonly used in its treatment to increase both the level and duration of neurotransmitter action in the brain.[5] Given the prolonged nature of Alzheimer's treatment, drug quality is critical, with impurity profiling being a key factor.[6]

Most people use donepezil hydrochloride, a cholinesterase inhibitor, to treat Alzheimer's disease, and it is typically available in the hydrochloride salt form. The chemical structure is 2-[(1-benzyl-4-piperidyl)methyl].-5,6-Dimethoxy-2,3-dihydroinden-1-one hydrochloride. The molecular weight of donepezil HCl is 415.953, and it is a white-to-off-white-to-slightly yellow crystalline powder. It is easily soluble in chloroform, dichloromethane, and methanol; soluble in water; sparingly soluble in ethanol, n-butanol, and acetonitrile; and just faintly soluble in acetone.[4-6]

Several studies have aimed on developing and validating analytical methods for impurity profiling and quantification of donepezil hydrochloride. Mahalingam et al. (2017) developed the RP-UPLC method for analyzing donepezil including its impurities, which was compatible with LC-MS and suitable for stability testing.[7] Babu & Kavuri Naga Raju (2012) proposed a RP-HPLC method with high linearity and recovery rates for donepezil analysis in formulations.[8] Kafkala et al. (2008) developed a gradient HPLC method for simultaneous determination of donepezil assay and related substances in oral formulations, which was validated for linearity, precision, accuracy, and stability indication.[9] Liew et al. (2013) developed an optimized RP-HPLC method for quantifying donepezil in orally disintegrating tablets, demonstrating high precision, accuracy, and linearity.[10] These methods employed various columns, mobile phases, and detection wavelengths, with most utilizing C18 columns and UV detection. The developed methods offer improved efficiency, sensitivity, and applicability for quality control and stability assessment of donepezil formulations.[8-10]

RP-HPLC is widely employed to monitor impurities, offering a cost-effective method to purify, identify, and quantify drug compounds. A newly developed RP-HPLC technique for Donepezil allows monitoring of 5 impurities within a short duration without using expensive advanced techniques, ensuring both quality control and cost efficiency. This method was validated according to ICH Q2(R2) guidelines, evaluating system suitability, specificity, linearity, precision, accuracy, and detection limits, making it a sensitive, accurate, and economical tool for impurity profiling in Donepezil.

MATERIAL AND METHOD

Materials

For the quantitative determination of 3-(3,4-dimethoxyphenyl) propionic acid (Impurity-A), 5,6-dimethoxy indan-1-one (Impurity-B), benzaldehyde (Impurity-C), veratrole (Impurity-D), and 3-chloro-1-(3,4-dimethoxyphenyl) propan-1-one (Impurity-E) in Donepezil using reverse phase HPLC, acetonitrile (Qualigen, HPLC grade) and methanol (Qualigen, HPLC grade) were utilized as solvents. Additionally, the mobile phase was prepared using diammonium hydrogen orthophosphate (SDFCL) and orthophosphoric acid (Rankem, AR grade).

Chromatographic Condition

Chromatographic conditions for the analysis involve the use of Solution A, which consists of 10 mM diammonium hydrogen orthophosphate (anhydrous) in water, with the pH adjusted to 6.0±0.05 using dilute acetic acid, and Solution B, which is a mixture of acetonitrile and methanol in an 85:15v/v ratio. The separation is performed on a C18 column (Hypersil ODS, 25 cm x 4.6 mm, 5.0µm) with a detection wavelength of 230 nm. The column is maintained at 35°C, and the flow rate is set at 1.0 mL/min with an injection volume of 10µl. The diluent used is a 50:50 v/v mixture of acetonitrile and water.

Gradient Program

The gradient program for the analysis starts with 90% Solution A and 10% Solution B at 0.01 minutes. By 25.00 minute, the composition changes to 25% Solution A and 75% Solution B, which is maintained until 30.00 minutes. At 30.10 minutes, the gradient shifts back to the initial composition of 90% Solution A and 10% Solution B, which is held until

35.10 minutes.

Mobile Phase Selection

In the initial trials for mobile phase selection, using a 50:50v/v mixture of water and acetonitrile resulted in poor peak shapes. To improve peak symmetry, water was replaced with a buffer adjusted to an acidic pH using ortho-phosphoric acid, but this did not significantly enhance the peak shape. Further optimization focused on adjusting the mobile phase proportions to ensure proper analyte retention and achieve good resolution between Donepezil and its impurities. The organic phase composition was finalized as a mixture of 10 mM Diammonium Hydrogen Orthophosphate (anhydrous) in water, with the pH adjusted to 6.0±0.05 using dilute acetic acid Solution A, along with acetonitrile and methanol in an 85:15 v/v ratio – Solution B. A gradient method was then established.

Methods

Solution Preparation

Standard stock preparation

For the preparation of standard stock solutions, weigh accurately and transfer about 10.0 mg of Impurities A, B, C, D, and E into separate 10 mL volumetric flasks. Add about 5 mL of diluent to every flask, sonicate (for Imp-A and Imp-B) or shake well (for Imp-C, Imp-D, and Imp-E) until dissolved. Dilute every solution to the mark with diluent. From these stock solutions, take 5.0 mL of every & further dilute to 50 mL with diluent.[11]

Reference preparation

Transfer 1.0 mL of each standard stock preparation (A), (B), (C), (D), and (E) into a 50 mL volumetric flask with 10 mL of diluent, mix thoroughly, and dilute to the appropriate level with diluent.

Test preparation

Weigh and transfer about 50 mg of sample in 25 mL of volumetric flask, add diluent of 10 mL and sonicate to dissolve, shake well and dilute up to the mark with diluent.

Spiked Test preparation

Weigh accurately and transfer about 50 mg of sample in 25 mL of volumetric flask, add 10 mL of diluent and add 0.5 mL every of Standard stock preparation (A), (B), (C), (D) and (E), sonicate to dissolve, shake well and dilute up to the mark with diluent.

Identification Solution preparation

For the preparation of identification solutions, transfer 1.0 mL of the standard stock preparations (A, B, C, D, and E) into separate 10 mL volumetric flasks, every containing 5 mL of diluent. Dilute every solution up to the mark with diluent. For the procedure, prepare the standard stock solution, reference solution, and sample solution in duplicate. Inject 10.0 µL of every solution into the chromatographic system in the following sequence: Blank (diluent), Reference solution (1) in six replicates, Blank, identification solutions of Impurity A to E, Test solution, Spiked Test solution, Blank, and Reference solution (1). Record the chromatograms and check the system suitability parameters.[11]

Method Validation

The analytical method for determining the content of Impurity A to E by HPLC meets the acceptance criteria for key method validation parameters, including specificity, system suitability, limit of detection, limit of quantitation, linearity-range, precision, and accuracy.[12-15]

System Suitability and Specificity

To assess the specificity and system compatibility of the approach, create standard stock solutions (A to E) by dissolving 10 mg of impurities (A to E) in 5 mL of diluent. Sonicate and dilute each sample to a final volume of 10 mL. Dilute 5 mL of each stock solution to a final volume of 50 mL using a diluent. Combine 1 mL of each stock solution in a 50 mL

flask, add 10 mL of diluent, then adjust the volume to make the reference solution. Weigh 50 mg of the sample for test preparation, add 10 mL of diluent, sonicate, and then dilute to a final volume of 25 mL. For the spiking test preparation, add 0.5 mL of each standard stock solution to the sample preparation. Prepare the identification solutions for Impurity A through Impurity E by diluting 1.0 mL of each corresponding stock solution to a final volume of 10 mL. Inject 10.0 µL of the blank reference solution (in six duplicates), identification solutions, test solution, and spiked test solution into the chromatographic apparatus. The acceptance requirements include that no interference should occur in the blank or test preparation during the retention durations of Impurity A to Impurity E, and any detected interference must be below 10%. The relative standard deviation (RSD) for the peak area responses of reference preparation duplicates must not surpass 5%, whilst the RSD for retention time should be within 1.0% for standard solutions and spiked test solutions.[13]

Limit of detection (LOD)

To determine the LOD for Impurity A to E, prepares standard stock solutions by accurately weighing 10.0 mg of every impurity into 10 mL volumetric flasks, adding 5 mL of diluent, sonicating to dissolve, and diluting to the mark. Dilute 5.0 mL of every stock solution to 50 mL with diluent. For the LOD level, transfer 0.12 mL of every standard stock solution (1, 2, 3, 4, and 5) into a 50 mL volumetric flask and dilute to the mark with diluent to achieve a concentration of 0.24 ppm (0.012% w.r.t. sample). Inject the LOD solution, ensuring the detection limits meet the acceptance criteria, including signal-to-noise ratio and %RSD for precision.[14] The signal-to-noise ratio (S/N) for the LOD should ≥3, ensuring adequate detection of the of impurities.

Limit of quantitation (LOQ)

To determine the limit of quantification (LOQ) for Impurities A to E, prepare a LOQ solution by adding 0.3 mL of each standard stock solution (1, 2, 3, 4, and 5) into a 50 mL volumetric flask. Next, dilute the solution to achieve a concentration of 0.6 ppm, which is 0.03% relative to the sample. Administer 10.0 µL of the blank solution and the LOQ solution in triplicate. The signal-to-noise ratio for the LOQ level should be at least 10 for the peak areas of Impurity A to E. Additionally, the %RSD for the peak area response should not exceed 5.0%, and for retention time, it should not exceed 1.0%.[14,15] The S/N ratio for the LOQ should be ≥10, confirming reliable quantification of impurities.

Linearity-Range

To assess the linearity of the method, prepare standard stock solutions of impurities A, B, C, D, and E (Impurity A-E) by accurately weighing 10.0 mg of each impurity into 10 mL volumetric flasks, adding 5 mL of diluent, sonicating to dissolve, and diluting to the mark with diluent. Dilute 5.0 mL of every solution to 50 mL with diluent. For every linearity level, prepare solutions by transferring 0.3 mL (LIN-I), 0.5 mL (LIN-II), 1.0 mL (LIN-III), and 1.5 mL (LIN-IV) of every standard stock solution to separate 50 mL volumetric flasks and dilute to the mark with diluent. Inject 10.0 µL of every linearity level solution: Level 1 (six replicates), Levels 2 and 3 (in duplicate), and Level 4 (six replicates). The correlation coefficient for the peak areas of Impurity A to E should be no less than 0.999, the % Y-intercept within ±3.0, and the %RSD for retention time and peak area response should not exceed 1.0% and 5.0%, respectively, for Linearity Levels 1 and 4.[13]

Precision

To estimate the precision of the method, prepare six replicate test solutions by accurately weighing 50 mg of the sample into 25 mL volumetric flasks, adding 10 mL of diluent, sonicating to dissolve, and diluting to the mark with diluent. Inject 10.0 µL of the blank, reference preparation (6 replicates), precision sample solutions, and reference preparation (1) into the chromatographic system, ensuring blanks are injected between every set. Record the chromatograms and check system suitability. The RSD for the peak area response of the respective peaks as of the replicate injections of the reference solution should not exceed 5.0%, while the RSD for retention time should not exceed 1.0%.[12]

Accuracy

The accuracy of the method is evaluated by preparing standard stock solutions of impurities A, B, C, D, and E at a concentration of 10.0 mg/10 mL in diluent, followed by dilution of 5.0 mL to 50 mL. A reference preparation is made by mixing 1.0 mL every of these standard stock solutions into a 50 mL volumetric flask with 10.0 mL of diluent, then diluting to the mark. Test samples of 50 mg are dissolved in 25 mL of diluent, and accuracy level solutions (LOQ, 50%,

100%, 150%) are prepared by adding aliquots of the standard stocks (0.15 mL, 0.25 mL, 0.5 mL, 0.75 mL) to the sample, followed by sonication and dilution. Every solution is injected into the system along with blanks and references. Six replicates of the 100% accuracy level (Level-3) and duplicates of the other accuracy levels are run. The % recovery for every level must be between 80.0% to 120.0% with %RSD for the Level-3 replicates not exceeding 2.0%.[12] For low-level or higher variability ranges, for example content uniformity or potency testing, a wider acceptance criterion of 70.0% to 130.0% is acceptable, especially for Level I.[12]

RESULT AND DISCUSSION

Method Validation

The HPLC analytical method for quantifying Impurity A to E satisfies the acceptance criteria for essential method validation parameters, for example specificity, system suitability, detection limit, quantitation limit, linearity range, precision, and accuracy.

Specificity and System Suitability

The analysis of Standards A to E demonstrates consistent retention times and peak areas across multiple injections, with minimal standard deviation observed for both parameters. The %RSD values for the areas of Impurities A, B, C, D, and E are 0.3%, 0.1%, 3.6%, 0.2%, and 0.2%, respectively, while the %RSD for retention times are 0.07%, 0.02%, 0.02%, 0.01%, and 0.01%, all within acceptable limits. These low RSD values indicate excellent precision and stability in the chromatographic method. Furthermore, the analysis confirms that the blank solution and test preparation do not interfere with the retention times of Impurities A-E, as shown in Figure 1. The system suitability criteria for both peak areas and retention times are met, demonstrating the method's robustness and suitability for reliable impurity quantification, as detailed in Table 1.

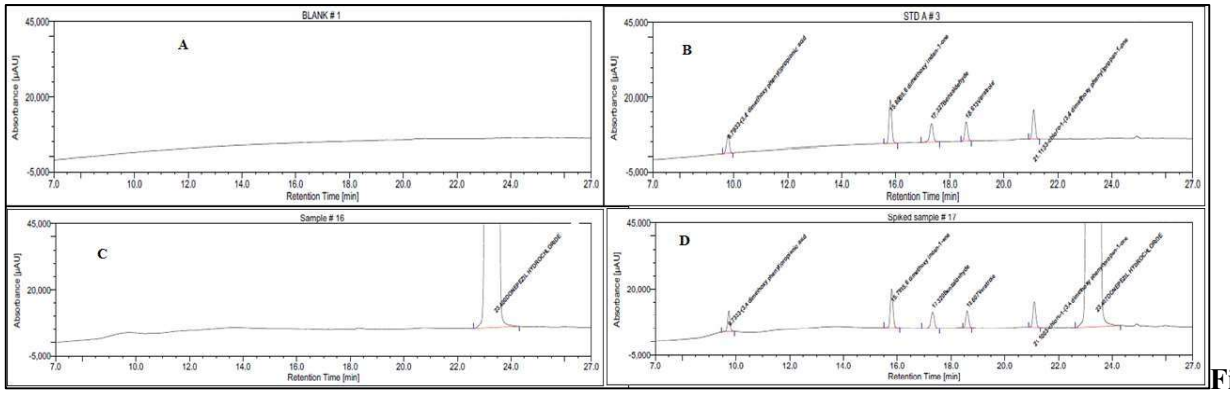

Figure 1: (A) Blank HPLC chromatogram; (B) Standard HPLC chromatogram of Donepezil HCl with Impurity I-V; (C) HPLC chromatogram Donepezil HCl sample; (D) Spiked HPLC chromatogram of Donepezil HCl with Impurity I-V

Table 1: System Suitability Parameters

Sr.No	Impurity Name	Area RSD in %	RT RSD in %
1	Impurity_A	0.3	0.07
2	Impurity_B	0.1	0.02
3	Impurity_C	3.6	0.02
4	Impurity_D	0.2	0.01
5	Impurity_E	0.2	0.01

Limit of detection (LOD)

The signal-to-noise (S/N) ratios for the limit of detection (LOD) solutions of Impurity-A, Impurity-B, Impurity-C, Impurity-D, and Impurity-E are 5, 48, 38, 23, and 40, respectively. These results indicate that all impurities meet the required S/N ratio range of 3 to 10 for LOD detection. While Impurity-B, Impurity-C, Impurity-D, and Impurity-E exhibit

higher-than-required S/N ratios, ensuring robust detection, Impurity-A also falls within the acceptable range, indicating that all impurities can be reliably detected at their respective LOD levels. Therefore, no concerns arise regarding the detection sensitivity of any impurity, as outlined in Table 2 and Figure 2.

Table 2: Signal-to-noise ratio of limit of detection

Level	Concentration of LOD level solution (%w.r.t spl.)	Signal to Noise ratio
Impurity-A	0.012	5
Impurity-B	0.012	48
Impurity-C	0.012	38
Impurity-D	0.012	23
Impurity-E	0.012	40

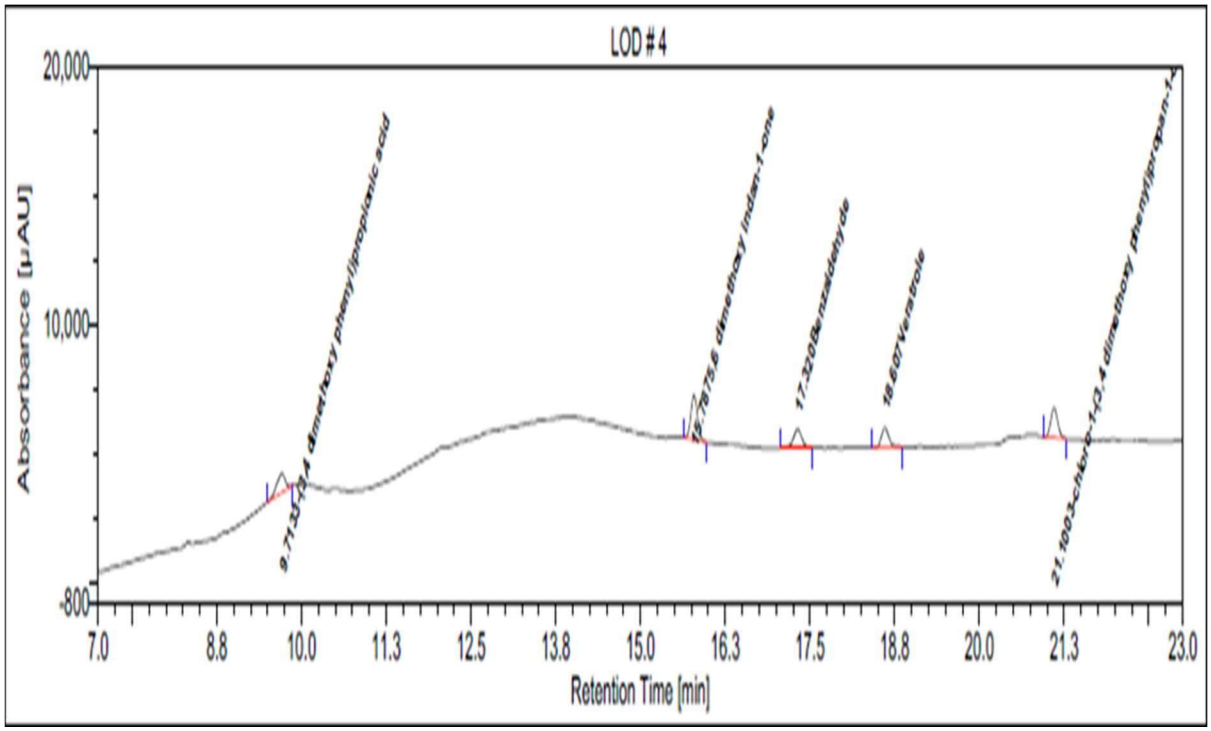

Figure 2: LOD Chromatogram

Limit of quantitation (LOQ)

The signal-to-noise (S/N) ratios for the LOQ solutions for Impurity-A-E are 12, 119, 93, 57, and 97, respectively. Since all impurities meet and exceed the required minimum S/N ratio of 10, reliable quantification is ensured at these concentrations. Impurity-B, with the highest S/N ratio, exhibits exceptional detection sensitivity. These findings confirm that every impurity can be accurately quantified without concern, as illustrated in Table 3 and Figure 3.

Table 3: Signal to noise ratio of limit of quantitation

Level	Concentration of LOQ level solution (%w.r.t spl)	Signal to Noise ratio
Impurity-A	0.03	12
Impurity-B	0.03	119
Impurity-C	0.03	93

Impurity-D	0.03	57
Impurity-E	0.03	97

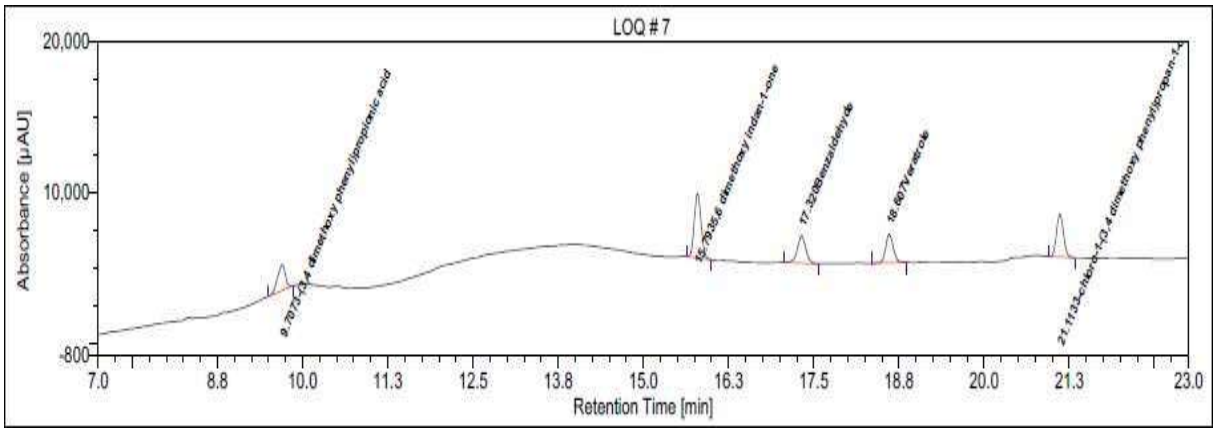

Figure 3: Limit of quantitation chromatogram

Linearity-Range

The linearity analysis for Impurities A through E shows consistent correlation coefficients of 1.0 for all impurities, demonstrating excellent linearity across the tested concentration ranges. The %Y intercept values for Impurities A, B, C, D, and E are 1.7%, -0.4%, -0.6%, 2.4%, and 0.0%, respectively, indicating minimal deviation from the origin. These results confirm that the method is reliable and precise for quantifying the impurities, ensuring accurate detection over the specified concentration range as shown in Figure 4.

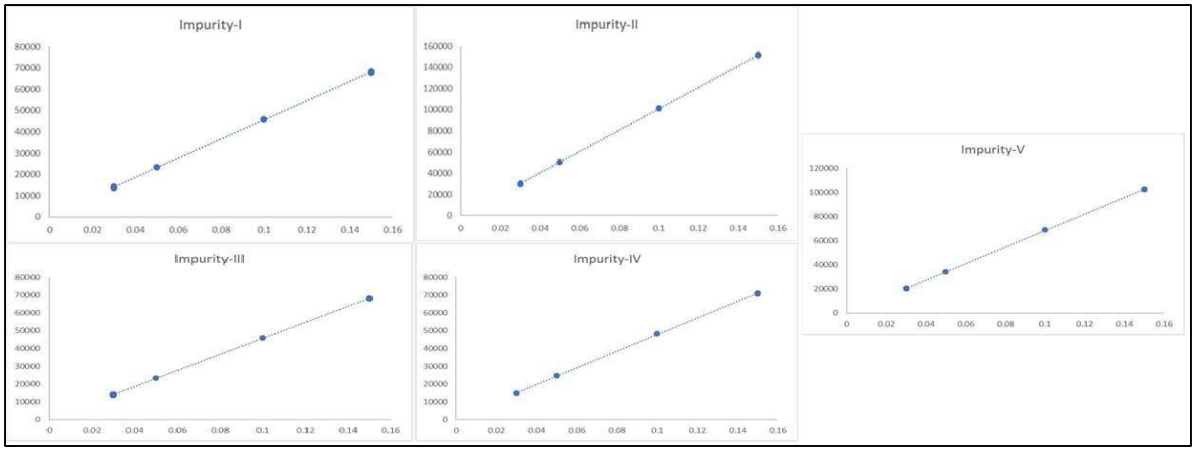

Figure 4: Linearity graph of Impurity A-E

Precision

Method Precision

The method precision was evaluated by calculating the RSD for areas and retention times of impurities (Impurity A to E) across six replicate injections. For Impurity-A, the %RSD for area was 0.93%, and for retention time, it was 0.03%, indicating good precision. Impurity-B demonstrated excellent precision with a %RSD of 0.11% for area and 0.00% for retention time. Impurity-C and Impurity-D both showed %RSD values for area of 0.46% and retention times of 0.02% and 0.00%, respectively, further confirming the method's consistency. Finally, Impurity-E had a %RSD of 0.19% for area and 0.02% for retention time, demonstrating the method's reliability across all impurities tested. All %RSD values were well below the generally accepted limit of 2.0%, confirming the method's high precision.

System Precision

The data indicates that all tested samples (Sample-I to Sample-VI) show no detectable levels of Impurity_A, Impurity_B,

Impurity_C, Impurity_D & Impurity_E, as all impurities are marked as "ND" (Not Detected). This suggests that the impurities are not present in any of the samples analyzed as shown in Table 4 and Figure 5.
Further spiking studies with known impurities at various levels performed under accuracy.

Table 4: Precision Data

Impurities	Sample-I	Sample-II	Sample-III	Sample-IV	Sample-V	Sample-VI
Impurity_A	ND	ND	ND	ND	ND	ND
Impurity_B	ND	ND	ND	ND	ND	ND
Impurity_C	ND	ND	ND	ND	ND	ND
Impurity_D	ND	ND	ND	ND	ND	ND
Impurity_E	ND	ND	ND	ND	ND	ND

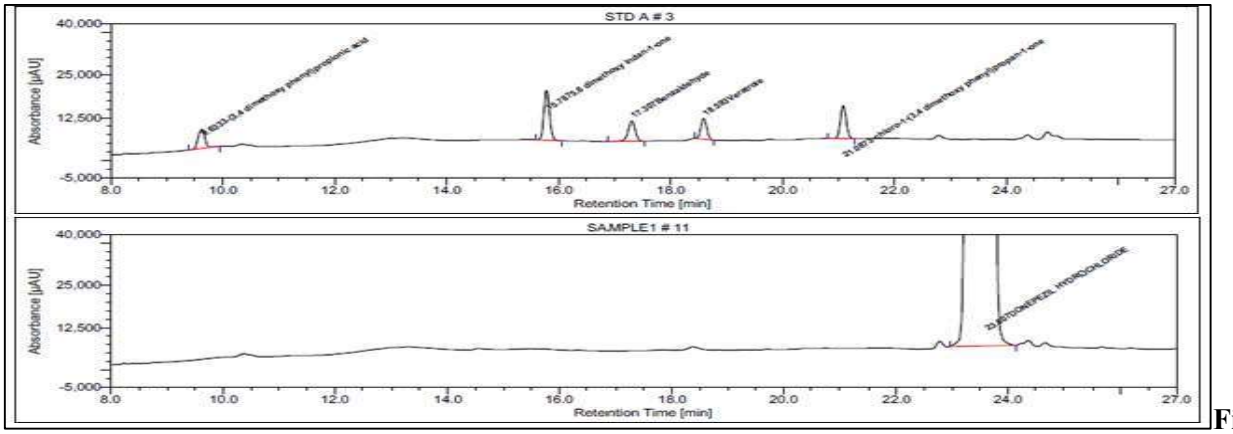

Figure 5: (A) Standard HPLC chromatograms for Impurity-A, Impurity-B, Impurity-C, Impurity-D, and Impurity-E. (B) HPLC chromatogram for the Precision Sample of Donepezil HCl.

Accuracy

The provided data assesses the percent recovery (ACC %) for impurities (Imp-I to Imp-V) across four levels (Level-I to Level-IV) in comparison to the standard mean area. The acceptance criteria require percent recovery to be within 80.0% to 120.0% for all levels, with a wider range of 70.0% to 130.0% allowed for Level-I due to higher variability at low level concentrations. Additionally, the RSD for Level-3 replicates should not exceed 2.0%. Impurity-I shows recovery values close to 90% in Level-I and 92-94% for Levels II-IV, with an RSD of 0.6%, indicating precision. Impurity-II exhibits recoveries between 98-100% across all levels, with an RSD of 0.6%, confirming high reproducibility. Impurity-III demonstrates recoveries of 95% in Level-I and 97-101% in Levels II-IV, with an excellent RSD of 0.01%. However, Impurity-IV has a recovery of 74% in Level-I, which is below the broader acceptance criterion, and slightly lowers recoveries (83-84%) in Level-II, but meets the criteria in Levels III-IV with recoveries of 96-99%, and an RSD of 1.2%. Impurity-V shows recoveries ranging from 88% in Level-I to 88-94% in Levels II and III, and 92% in Level-IV, with an RSD of 0.5%. Overall, the method demonstrates acceptable precision and accuracy, though improvements may be needed for Impurity-IV at lower levels to meet recovery expectations. The data presented in Table 5 and Figure 6.

Table 5: Accuracy Data

	Mean area of std.	Level-I		Level-II		Level-III		Level-IV	
		Area	ACC%	Area	ACC%	Area	ACC%	Area	ACC%
Imp-I	46088	12508	90	21293	92	43340	92	65104	94
		12590	90	21451	94	42967	94	64845	94
Imp-II	102238	30354	98	50746	98	102155	99	153629	100

		30519	99	50856	98	101302	98	153229	100
Imp-III	51290	14669	95	25192	97	51860	101	76948	100
		14814	95	25510	98	51854	100	76362	99
Imp-IV	44202	10047	74	19021	83	44259	98	64785	96
		10084	74	19150	84	45001	99	65228	97
Imp-V	68952	18334	88	31710	91	64913	94	95800	92
		18305	88	30746	88	64544	92	95387	92

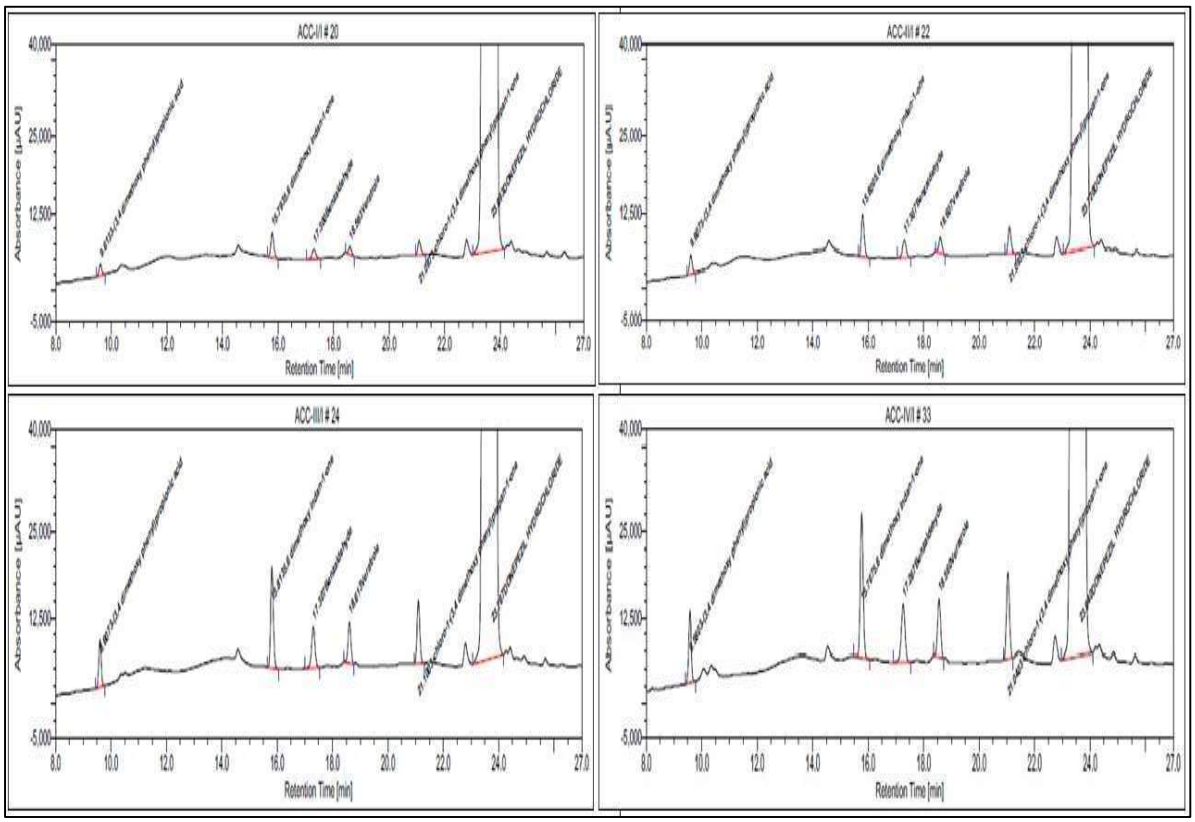

Figure 6: HPLC chromatogram of Accuracy Level I-IV

The HPLC analytical method effectively quantifies Impurity A-E, meeting all essential validation criteria including specificity, system suitability, detection limit, quantitation limit, linearity, precision, and accuracy. Accuracy results show that most impurities are detected with high precision and accuracy. The percent recovery (ACC%) for Impurities I-V across four levels shows that all impurities, except Impurity-IV, meet the acceptance criteria of 80.0%-120.0%, with an expanded range of 70.0%-130.0% for Level-I. Impurity-I recovers 90% at Level-I and 92-94% at higher levels, with an RSD of 0.6%, indicating good precision. Impurity-II achieves 98-100% recovery across all levels with an RSD of 0.6%, while Impurity-III shows 95% at Level-I and 97-101% at higher levels, with an exceptional RSD of 0.01%. Impurity-IV falls short at 74% in Level-I and 83-84% in Level-II but meets criteria in higher levels with recoveries of 96-99% and an RSD of 1.2%. Impurity-V recovers 88-94% across levels with an RSD of 0.5%. The method overall demonstrates acceptable precision and accuracy, though improvement is needed for Impurity-IV at lower levels.
Repeatability and accuracy analysis confirms the method precision & system precision. Linearity data demonstrates a perfect correlation between concentration and response, while specificity analysis ensures no interference at relevant retention times. LOD and LOQ results confirm reliable detection and quantification for all impurities, Impurities B-E show strong detection capabilities with higher-than-required S/N ratios, while Impurity-A also falls within the acceptable range for LOD detection. System suitability parameters reflect excellent precision and consistency across all impurities. Overall, the method is validated as robust and reliable for impurity analysis in Donepezil HCL.

CONCLUSION

The developed RP-HPLC method for analyzing Donepezil hydrochloride and its impurities offers notable advantages over other available methods, particularly in terms of robustness, precision, and reproducibility. It complies with all ICH guidelines for essential validation parameters, including specificity, system suitability, LOD, LOQ, linearity, precision, and accuracy. Optimized conditions, for example specific mobile phase composition and gradient elution, ensure excellent peak symmetry and resolution, while %RSD values for peak areas and retention times confirm consistent performance. A key strength of this method is its ability to detect and quantify impurities at low concentrations, with all impurities meeting signal-to-noise ratio requirements for LOD and LOQ. Impurity-IV, although showing lower recovery at Level-I, meets ICH's broader acceptance limits for lower concentration levels, highlighting the method's reliability and precision. Compared to other methods, this RP-HPLC technique offers superior accuracy, with recovery rates of 80-120% across levels, even accommodating higher variability at Level-I. The method provides a cost-effective, reliable solution for impurity profiling of Donepezil hydrochloride, demonstrating strong performance in detecting impurities and ensuring compliance with international regulatory standards.

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