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A Validated Rapid Stability-Indicating Method for the Determination of Related Substances in Solifenacin Succinate by Ultra-Fast Liquid Chromatography

S. Radha Krishna[1], B.M. Rao[2,*], and **N. Someswara Rao[2]**

[1]Analytical Research, Custom Pharmaceutical Services, Dr. Reddy's Laboratories Ltd. and [2]Andhra University, Visakhapatnam

Abstract

A rapid, sensitive, and accurate ultra-fast liquid chromatographic method is developed for the determination of related substances and degradants of Solifenacin Succinate, an active pharmaceutical ingredient used in the treatment of overactive bladder. Chromatographic separation of Solifenacin Succinate, its related substances, and degradants was achieved using a Shimpack XR-ODS-II column and mobile phase system containing 10 mM potassium dihydrogen orthophosphate in water. The pH of the buffer was adjusted to 7.0 using triethyl amine (mobile phase A). LC-grade acetonitrile was used as mobile phase B, employing a binary-gradient program at a flow rate 0.5 mL/min. The resolution between the critical pair of peaks (Impurity A and analyte) was found to be greater than 3.5. The limits of detection and quantification (LOQ) of Impurity A, Impurity B, and the analyte were 0.2 and 0.6 µg/mL, respectively for a 5-µL injection volume. The percentage recovery of impurities in the presence of sample matrix ranged from 95 to 104 w/w. The test solution and mobile phase was observed to be stable up to 24 h after the preparation. The validated method yielded good results of precision, linearity, accuracy, robustness, and ruggedness. The proposed method is found to be rapid, accurate, and suitable for the quantitative determination of related substances and degradants during quality control of Solifenacin Succinate active pharmaceutical ingredient.

Introduction

Solifenacin Succinate, chemically known as 1-azabicyclo[2.2.2]oct-8-yl (1S)-1-phenyl-3,4-dihydro-1H-isoquinoline-2-carboxylate Succinate with an empirical formula of $C_{23}H_{26}N_2O_2.C_4H_6O_4$ and a molecular weight of 480.55, is a muscarinic receptor antagonist, it is used in the treatment of overactive bladder with or without urinary incontinence (1). Solifenacin is a competitive M3 receptor antagonist and has 90% bioavailability and a long half-life (45–68 h). It is available in two dosage strengths, a 5- or 10-mg once-daily tablet. (2)

Solifenacin (10 mg) was well tolerated in patients with renal disease. Solifenacin displays a higher exposure and a prolonged half-life in patients with renal impairment, especially when more severe. Therefore, while no special cautions are necessary for patients with mild or moderate renal impairment, patients with severe renal impairment should receive no more than 5 mg Solifenacin once daily (3).

Literature search revealed that few high-performance liquid chromatography (HPLC) and mass spectrometry (MS) methods were available for Solifenacin succinate. Hiren N. Mistri et al. reported a high sensitive and rapid LC–electrospray ionization-MS–MS method for the simultaneous quantification of uroselective alpha1-blocker, Alfuzosin, and an antimuscarinic agent, Solifenacin in human plasma (4). Determination of Solifenacin Succinate and its major metabolite in biological samples (rat plasma) by semi-micro HPLC was reported by Takamitsu Yanagihara et al. (5).

The available methods for Solifenacin succinate cited here can be used for the quantification of the drug in a biological matrix. However, the reported methods cannot be used for the determination of the related substances and degradation products present. The advantage of the method presented here is that it is simple, rapid, and stability-indicating. The presented ultra-fast liquid chromatography (UFLC) method can be conveniently used in the Quality Control Laboratories during the testing and cGMP release of drug substance. The same method also can be very well employed during the stability analysis of Solifenacin succinate. The key advantage of the developed method is that it is based on UFLC technology; hence a greater number of samples can be analyzed in a shorter time, with low consumption of organic solvents when compared with the existing HPLC methods.

Materials and Methods

Materials

Samples of Solifenacin Succinate (Figure 1) and its related substances Impurity A (Figure 2) and Impurity B (Figure 3) were received from a business unit of Dr. Reddy's Laboratories Ltd., (Hyderabad, India)

*Author to whom correspondence should be addressed: E-mail: drbmrao@hotmail.com

HPLC-grade acetonitrile was purchased from Rankem fine chemicals (India). HPLC-grade Potassium dihydrogen phosphate and AR grade Triethyl amine was purchased from Qualigens fine chemicals, and HPLC-grade water was produced internally by using Milli-Q, Millipore water purification system.

Instrumentation

The UFLC system used for method development and validation was Prominence-UFLC XR (Shimadzu Corporation, Kyoto, Japan). The output signal was monitored and processed using LC-Solution software on Pentium computer (Lenovo) (Laboratory A).

The LC system used in the ruggedness study was Prominence-UFLC XR (Shimadzu Corporation, Japan). The output signal was monitored and processed using LC-Solution software on Pentium computer (HP) (Laboratory B).

Methods

Chromatographic separations were achieved on a Shimpack XR ODS-II 75 × 3.0 mm, 2.2 µm column. The mobile phase contained 10 mM Potassium dihydrogen orthophosphate in water; the pH was adjusted to 7.0 with triethyl amine and this was Mobile Phase A, and acetonitrile was Mobile Phase B. A binary linear gradient was employed as follows: time (min)/%B: 0 min, 50% B at 6 min raised to 90% B; a flow rate of 0.5 mL/min was used. The test sample concentration was 2.0 mg/mL in diluent, acetonitrile, and water in the ratio of 1:1. The column temperature was maintained at 40°C, and the detection was done using UV detector at a wavelength 220 nm. The injection volume was 5 µL. The analysis time for each run was ~ 6 min. Satisfactory resolution for all the impurities and degradants within short run time were observed. Typical retention times of Impurity A, Impurity B, and analyte peaks were 2.6, 4.5, and 3.4 min (Figure 4). The system suitability (6) test results were presented in Table I.

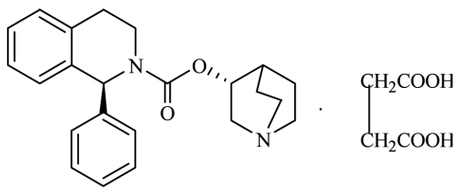

Figure 1. Chemical structures of Solifenacin Succinate.

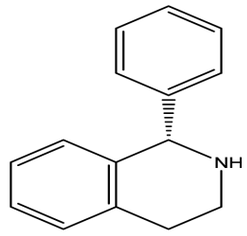

Figure 2. Chemical structures of Impurity-A: (S)-1-phenyl-1,2,3,4-tetrahydroisoquinoline.

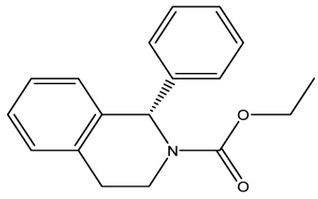

Figure 3. Chemical structures of Impurity-B: Ethyl-(1S)-1-phenyl-1,2,3,4-tetrahydro-2-isoquinoline carboxylate

Sample preparation

The stock solutions of Impurity A and B and Solifenacin Succinate were prepared separately by dissolving the appropriate amounts of the substances in diluent (water–acetonitrile, 1:1). The target analyte concentration was fixed as 2.0 mg/mL.

Results and Discussions

The objective of this work is to develop suitable stability-indicating HPLC method for quantification of related substances and degradation products that were present in Solifenacin Succinate drug substance. A test mixture consists of related compounds Impurity A, Impurity B, and Solifenacin Succinate was used in the method development. Various reverse phase stationary phases were employed during method development namely X-Terra MS (Waters Corporation, Ireland), Inertsil ODS (GL Science, Japan) and Shimpack XR ODS-II (Shimadzu Corporation, Japan). Initial trials were made by using HPLC, and the final method was optimized using UFLC to achieve shorter run time with high sensitivity by using a Shimpack XR UFLC column. The various trails attempted during the method development and optimization to achieve the separation between the Solifenacin succinate, related substances, and its degradants.

Method Validation

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradants, matrix, etc (7). Specificity was tested by injecting the spiked sample of Solifenacin Succinate with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Moreover, the identification of each impurity was confirmed with retention time as compared with those of pure standards.

Forced degradation studies were performed for bulk drug to

Table I. System Suitability Test Results

Name	Retention time (t_R) in min	Resolution (R_s) by Tangent method (USP)	USP Tailing factor (T)
Impurity A	2.6	–	1.5
Solifenacin	3.4	3.9	2.5
Impurity B	4.5	5.6	1.3

provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of photolytic degradation as per ICH Q1B, Thermal degradation (at 60°C), acid hydrolysis (using 0.5 N HCl), base hydrolysis (using 0.5 N NaOH), and oxidative degradation (using 3.0% H_2O_2) to evaluate the ability of the proposed method to separate Solifenacin from its degradation products generated during the described stress degradation studies. For heat and light studies, the study period was 10 days, where as for acid, base, and oxidative degradation it was 48 h. Peak homogeneity of Solifenacin in the stressed sample solutions was confirmed by using a photo diode array detector.

Precision

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions (7). The system and method precision for 2 mg/mL Solifenacin Succinate spiked with 0.15% of Impurity A and Impurity B with respect to analyte concentration the percentage relative standard deviation (%RSD) of method repeatability and system repeatability for impurities was found to be between 0.5% to 2% confirms good precision of the method.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample (7). The linearity of the method was checked at six concentration levels (i.e. from LOQ to 6 µg/mL of Impurity A, Impurity B, and Solifenacin Succinate). The coefficient of regression of the calibration curve was found to be greater than 0.99, thus confirming the excellent correlation existed between the peak area and concentration of the impurities.

Limits of Detection and Quantification

The limit of detection (LOD) represents the concentration of analyte that would yield a signal-to-noise ratio of 3 (7). The limit of detection for Impurity A, Impurity B, and Solifenacin was found to be 0.2 µg/mL for 5 µL of injection volume. The limit of quantification (LOQ) represents the concentration of analyte that would yield a signal to noise ratio of 10 (7). The limit of quantification for Impurity A, Impurity B, and Solifenacin was found to be 0.6 µg/mL for 5 µL of injection volume. The precision for Impurity A, Impurity B, and Solifenacin at LOQ level was good, and the RSD was found to be below 3.5%.

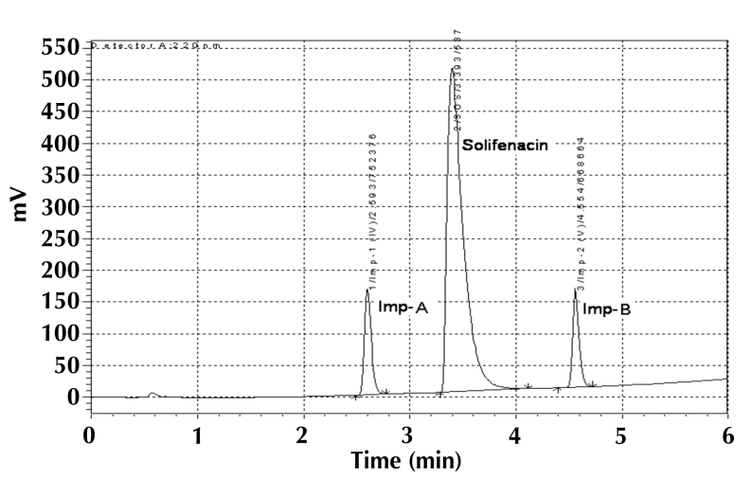

Figure 4. The HPLC chromatogram representing the final optimized conditions.

Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification Impurity A and Impurity B. The study was carried out at LOQ, 0.15% and 0.3% of target analyte concentration (2 mg/mL) of Impurity A and Impurity B. The percentage recoveries of impurities were ranged from 95 to 104 in samples of Solifenacin Succinate.

Ruggedness and robustness

The ruggedness of the method was defined as the degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions, such as different laboratories, different analysts, different instruments, different days, and different lots of reagents. Precision studies were carried out for Impurity A and Impurity B in Solifenacin Succinate bulk samples at the same concentration levels tested in Laboratory A were again carried out at Laboratory B using different instrument and a different analyst. The data obtained from Laboratory B was well in agreement with the results obtained in Laboratory A; thus proving that the method was rugged.

The robustness of an analytical procedure is the measure of its capability to remain unaffected by small, but deliberate, variations in method parameters, which provides an indication of its reliability during normal usage. The varied chromatographic conditions were flow rate and mobile phase composition. The resolution between the peaks of Solifenacin, Impurity A and Impurity B was found to be > 3.0, illustrating the robustness of the method.

Solution stability and mobile phase stability

Solution stability was studied by keeping the test solution spiked with impurities in tightly capped volumetric flask at room temperature (25 ± 2°C) on a laboratory bench for 24 h. The content of impurities was checked at 6-h intervals and compared with a freshly prepared solution. No variation was observed in the content of impurities for the study period, indicating that Solifenacin Succinate sample solutions prepared in diluent were stable up to 24 h at room temperature (25 ± 2°C). Mobile phase stability was carried out by evaluating the content of impurities in sample solution spiked with impurities, which were prepared freshly at 6 h intervals for 24 h. The same mobile phase was used during the study period. No variation was observed in the content of impurities for the study period, and it indicates prepared mobile phase was found to be stable up to 24 h.

Conclusion

In the present research, a simple, rapid, sensitive, and accurate stability-indicating UFLC method for the determination of related substances and degradation products in Solifenacin Succinate was described. The developed rapid stability-indicating LC method was very much useful during the quality monitoring of bulk samples as well the stability samples of Solifenacin succinate.

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