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Article

# Development of a Stability-Indicating RP-LC Method for the Separation of a Critical Pair of Impurities and their Degradants in Zafirlukast

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**A high-throughput reverse-phase liquid chromatographic (RP-LC) method is developed for the quantification of zafirlukast and its related impurities in drug substance. The separation of known impurities is accomplished using a short (50 mm) LC column with sub-2-μm particle size in a relatively short run-time. A linear gradient elution involves ammonium formate and acetonitrile as mobile phase. The critical impurity pair is the meta and para isomers of zafirlukast, which are known to be potential impurities of zafirlukast, whose resolution is sensitive to pH. The stability-indicating capability of the developed method is demonstrated using forced degradation samples from stress conditions such as hydrolysis, oxidation, thermal and photolytic degradation. The developed RP-LC method is validated in accordance with International Conference on Harmonization requirements. The results from the validation study indicate that this RP-LC method can be used for the determination of synthetic and degradation impurities in regular quality control analysis for the drug substance.**

## Introduction

Zafirlukast is a selective peptide leukotriene receptor antagonist with the chemical name 4- (5-cyclopentyloxy-carbonylamino-1-methyl-indol-3-ylmethyl)-3-methoxy-N-o-tolylsulfonylbenzamide, used for the prophylaxis and chronic treatment of asthma in adults. It has demonstrated an improvement in pulmonary function and shows reduction in the use of short-acting inhaled beta 2-adrenoceptor agonist therapy in patients with mild to moderate asthma treated. Zafirlukast is available as Accolate in 10 and 20 mg tablets. The oral administration of zafirlukast at the recommended dosage is 20 mg twice daily. Available data suggest that zafirlukast may significantly reduce the incidence of asthma exacerbations (1).

Few analytical methods have been reported for the quantification and characterization of zafirlukast and its impurities in the biological samples and in the formulations. The reported analytical methods employ quantification by high-performance liquid chromatography (HPLC) with fluorescent detection in normal phase (2), HPLC with ultraviolet (UV) detection in reverse phase (RP) (3, 4), electrochemical methods (5), UV-spectro photometry (6), differential pulse adsorptive spring voltammetry (7) and mass detection (8). An RP-HPLC assay method was also recently developed (9) for the drug substance. An article has been published on the identification and characterization of impurities; meta and para isomers of zafirlukast are two of the potential impurities in the active pharmaceutical ingredient (API) (10). The previously published methods were not targeted for the separation of regio isomers of zafirlukast along with the degradants. Another article described the separation of isomers in normal phase mode with chiral column as stationary phase (11); a specific test was performed for the content of regio isomer apart from the routinely used method. Until now, the quantitative separation of degradants from stress studies has been considered only with two methods. In one of the methods, there is no separation of regio isomers (12) and another involves complex ion pair reagents with a long run time (13), which cannot be used directly for LC–mass spectrometry (MS) analysis. It is important to consider using volatile buffers in HPLC methods for LC–MS compatibility, as defined in the reverse-phase HPLC buffers section of the Sigma–Aldrich catalog (http://ccc.chem.pitt.edu/wipf/Web/HPLC_RP_Buffers.pdf).

Recently, there has been a huge interest in high-throughput chromatographic separations for pharmaceutical applications using sub-2-micron 50 or 30 mm columns (14). Because no LC method has been reported with short analysis time and LC–MS compatibility for the separation and quantitation of zafirlukast and its potential impurities with degradants, we have developed a reverse-phase LC method with a 15-min analysis time, including regio isomers and its degradants. The goal of this research work is to optimize and implement a method that is LC–MS compatible for related substances, including the separation of critical pairs: meta and para isomers of zafirlukast. Additionally, this study describes the determination of the assay with a reasonably short run time and demonstrates the stability-indicating capability of the developed method. The forced degradation was performed as per International Conference on Harmonization (ICH) recommended conditions, i.e., acid and base hydrolysis, oxidative, photolytic and thermal stressed conditions. The method was validated as per ICH recommendations (15)

## Experimental

### *Chemicals and reagents*

Zafirlukast and its impurities (Figure 1) were synthesized and purified by the process research department of Active Pharmaceutical Ingredients, IPDO, Dr. Reddy's Laboratories (Hyderabad, India). For the mobile phase preparation and degradation studies, HPLC-grade acetonitrile and methanol and AR-grade sodium hydroxide, hydrochloric acid, hydrogen peroxide and formic acid were purchased from Rankem (Mumbai, India). HPLC-grade tetrahydrofuran was procured from

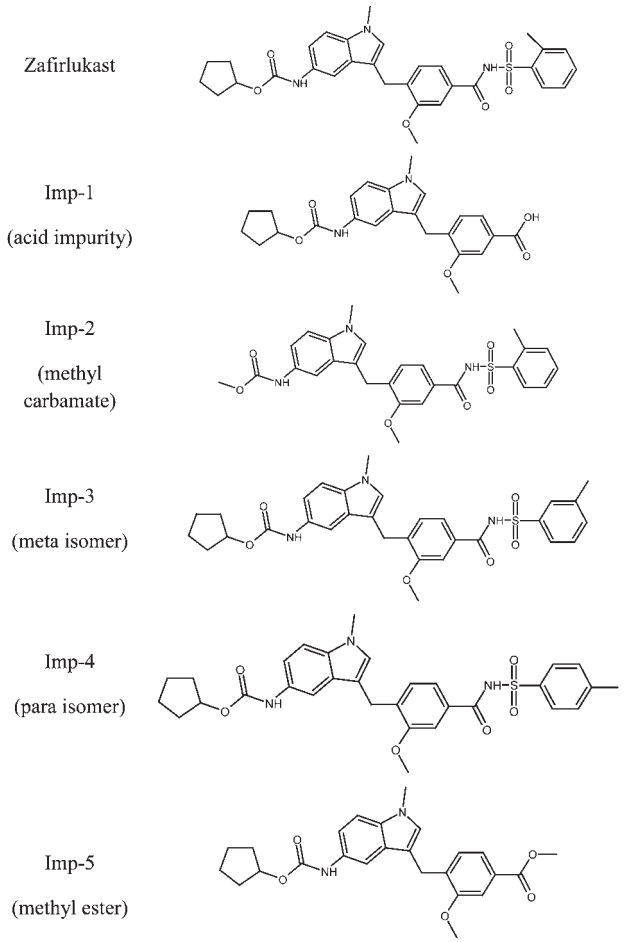


**Figure 1.** Chemical structure of zafirlukast and its impurities.

Qualigens (Germany). AR-grade of ammonium formate was used from Sigma–Aldrich (St. Louis, MO). Millipore purified water (Milli-Q Plus; Bangalore, India) was used to prepare mobile phase and washing solvents.

### *Instrumentation*

The attempts at method development, forced degradation studies and method validation were performed in Agilent 1100 series LC systems with a diode array and variable wave length detectors (Agilent Technologies; Waldbronn, Germany). The photolytic degradation was carried out using a Binder KBS240 photolytic chamber (New York, NY). The peak homogeneity was studied on an Agilent LCMS 6410 QqQ instrument (Palo Alto, CA). The data were collected and processed using Chemstation software.

### *Chromatographic conditions*

The chromatographic separation was optimized on a Zorbax SB C8 column with the dimensions of 50 × 2.1 mm and 1.8 μm as particle size. The gradient elution involved 20 mM ammonium formate with pH 4.4 as a mobile phase A and pure acetonitrile as mobile phase B. The HPLC gradient program was optimized as: time/percent mobile phase B: 0/30, 8/75, 10/75 and 11/30 with gradient delay of 4 min. The flow rate of the mobile phase and the column temperature were set as 0.3 mL/min and 40°C. The detection λ was set as 240 nm. The column loading was finalized as 1.25 μg of zafirlukast in 5 μL injection volume. A mixture of water and acetonitrile in the ratio of 3:1 was used as diluent.

### *Sample preparation*

Zafirlukast solution was prepared at target analyte concentration (TAC), which is 250 μg/mL in the diluent for related substances and assay determination. A stock solution with a blend of impurity-1 (acid impurity), impurity-2 (methyl carbamate impurity), impurity-3 (meta isomer), impurity-4 (para isomer) and impurity-5 (methyl ester) was also prepared in diluent for the preparation of system suitability solution with 0.15 % w/w (specification level) of each impurity at TAC of zafirlukast.

### *Method development and optimization*

The core objective of the current study is to develop a chromatographic method for the separation of all the potential impurities along with degraded impurities of zafirlukast during a short run time. All potential impurities were removed in the final re-crystallization process developed in Dr. Reddy's Laboratories for zafirlukast. The method was developed using a Zorbax SB C8, which provides good selectivity over impurities with better peak shapes, and with 20 mM ammonium formate buffer with the pH adjusted with formic acid. The desired resolution ($R < 1.5$) of all potential impurities and isomers was observed at pH 4.4 with acetonitrile as mobile phase B in the gradient elution. Zafirlukast showed good responses at 210 to 240 nm and 280 to 320 nm. The detection wavelength (240 nm) was chosen considering the peak signal of impurities and a non-drifting baseline. The relative response factor (RRF) of the potential impurities is close to the unit. The RRF of degradants was considered to be one, which was supported by the mass balance to a great extent.

Initial effort for the method development was made in ammonium acetate and acetonitrile as a mobile phase in an ODS column with 150 mm length. Buffer concentration with 20 mM ammonium acetate resulted in better peak shapes than 10 mM ammonium acetate; however, the run time was observed to be long. Less than 3-μm particle size columns Shimpak XR and Zorbax SB C18 and C8 were used for screening to reduce the run time. The Zorbax SB C8 with dimensions of 50 × 2.1 mm and 1.8 μm particle size was chosen for its better peak shape and for the acceptable resolution of critical pairs. The addition of methanol in acetonitrile led to a reduction in the resolution of isomers. Different pH was screened by adjusting the pH of mobile phase A using acetic acid. Optimum resolution was observed at pH 4.4.

Changing the mobile phase A to 20 mM ammonium formate and adjusting the pH to 4.4 with formic acid provided sharper peaks than ammonium acetate. Other modifications in mobile phase B, column temperature and stationary phases did not show any improvement on the method. It is evident that the separation of positional isomers of zafirlukast was more dependent on pH.

Under the optimized conditions, zafirlukast, impurity-1, impurity-2, impurity-3, impurity-4 and impurity-5 were well

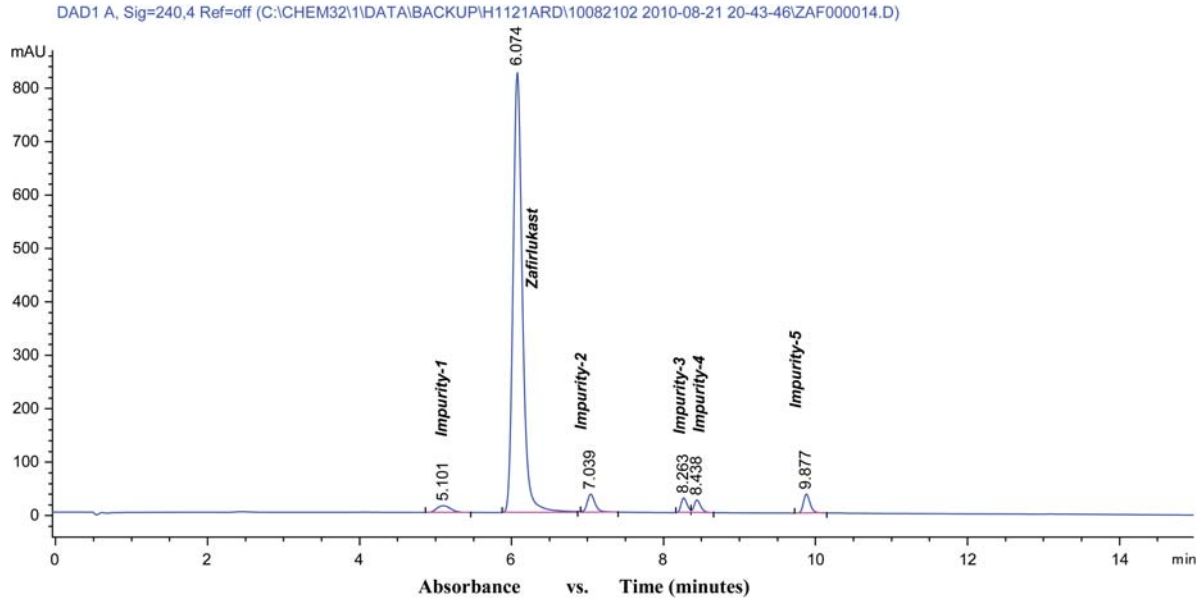


**Figure 2.** Typical system suitability test chromatogram.

**Table I**
System Suitability Test Results

| Compound ($n = 3$ ) | Capacity factor (k′) | Resolution (Rs) | USP tailing factor (T) | Number of theoretical plates (N-tangent method ) |
|---|---|---|---|---|
| Zafirulkast | 4.5 | — | 1.2 | 12,307 |
| Imp-1 | 3.6 | 3.5 | 1.1 | 61,297 |
| Imp-2 | 5.4 | 5.0 | 1.2 | 22,026 |
| Imp-3 | 6.5 | 7.5 | 1.2 | 77,647 |
| Imp-4 | 6.7 | 1.8 | 1.2 | 138,727 |
| Imp-5 | 8.0 | 9.3 | 1.1 | 171,945 |

separated with a resolution of greater than 1.8. The ratio of water and acetonitrile was optimized to 3:1 as diluent to achieve a better peak shape of impurity-1. Increasing the acetonitrile ratio raised the solubility of zafirlukast, however, the impurity-1 peak splits. The system suitability results were within the acceptance criteria and the developed LC method was found to be specific for zafirlukast, its potential impurities and its degradation products (Figure 2 and Table I).

### *Specificity*

Specificity is the ability of the method to measure the analyte in the presence of process-related products and degradation products. The specificity of the developed LC method for zafirlukast was demonstrated in the presence of its potential impurities, i.e., impurity-1, impurity-2, impurity-3, impurity-4 and impurity-5, in the process developed in Dr Reddy's Laboratories, and its degradation products. Thorough forced degradation studies were carried out on zafirlukast to ascertain the stability-indicating property of the developed method. The stress conditions performed for degradation studies as per the ICH preferred conditions consist of photolytic, thermal, oxidation and hydrolysis at acid and base. The photolytic stress studies were performed as per ICH Q1B (16). The thermal stress was studied at 60°C for seven days. The acid stress was performed at 0.5 N HCl at the concentrated sample solution at ambient temperature (25 ± 2°C) for seven days and further dilution was performed to analyte concentration during the analysis for the quantification of zafirlukast and its degradants. The alkali stress was performed at 0.5N NaOH for seven days at ambient temperature. The oxidation stress was studied using 3% hydrogen peroxide for six days at ambient temperature (17, 18). All stressed samples were quantified for zafirlukast and the impurities. Peak purity of stressed samples of zafirlukast and the spiked solutions of impurity-1, impurity-2, impurity-3, impurity-4 and impurity-5 were determined by an Agilent HPLC instrument with diode array detector (DAD). Additionally, the peak homogeneity was checked using LC–MS for all the stressed samples.

### *Method validation*

#### *Precision*

Precision is the closeness of agreement between a series of measurements obtained from multiple sampling of same sample under the prescribed conditions. Six individual preparations of zafirlukast solution were made with each of the known impurities at specification level to analyte concentration. Quantification of individual impurities and zafirlukast was performed for each of the preparations and the percent relative standard deviation (RSD) was determined for the content of the impurities and the assay. To evaluate the intermediate precision, the same experiment was repeated with a different lot of column and a different instrument in the same laboratory.

#### *Limit of detection and limit of quantification*

The limit of detection (LOD) and limit of quantification (LOQ) of an individual analytical procedure are the lowest amounts of analyte in a sample that can be detected and quantitatively

determined with suitable precision and accuracy, respectively. The LOD and LOQ for each of the components were established by attaining signal-to-noise ratio of approximately 3:1 and 10:1, respectively, from a series of dilute solutions with known concentrations (16). Six individual solutions of known impurities and zafirlukast were also prepared at LOQ level and the area of impurities and zafirlukast was recorded. The LOQ precision was established by calculating the %RSD of each of the areas. Accuracy of impurities at this level was established by spiking the known LOQ quantities of impurities in test sample and calculating the recovery.

*Linearity*

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to the amount of analyte in the sample. The linearity of method was demonstrated separately at impurity level and assay level. The solution of zafirlukast and its known impurities was prepared at five different concentrations from 0.05 to 0.30% (w/w) of analyte concentration for the linearity at impurity level. For the assay level, five different solid weighings of zafirlukast from 80 to 120% (w/w) with respect to analyte concentration were prepared and injected. Using least-squares analysis, the regression line was plotted with area versus concentration. The value of the slope and Y-intercept of the plot were calculated. The RRF of impurities was determined using the slopes of each component at impurity level.

*Accuracy*

The accuracy of an analytical procedure expresses the closeness of agreement between the values determined by the method and conventional true value or an accepted reference value. Accuracy of impurities at each level was established by standard addition of the known quantities of impurities in test sample and calculation of the recovery. The study was carried out in triplicate at 0.05, 0.15 and 0.225% (w/w) of the TAC. Recovery of impurities was calculated by calculating the amount of the impurities spiked and the amount of the impurity calculated from the spiked sample. The accuracy of the assay was evaluated in triplicate at three concentration levels from 80 to 120% (w/w) of analyte concentration by assaying the reference standard against 100% level of the same. The percentage recovery at each level was calculated against the zafirlukast standard, considered 99.1% (w/w) as the true value derived by the mass balance approach.

*Robustness*

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. Variations were made from original chromatographic conditions to record the resolution between zafirlukast and the impurities to determine the robustness of the developed method. Small variations made in column temperature, flow rate, gradient program and pH of the mobile phase A from optimized conditions.

*Solution stability and mobile phase stability*

Solution stability and mobile phase stability provide an indication of the method's reliability in normal usage during the storage of the solutions used in the method. The solution stability of zafirlukast was studied for 48 h at room temperature. The reference standard of zafirlukast and the sample spiked with impurities at specification level were injected every 6 h. The content of impurities and zafirlukast were quantified at each interval up to the study period. The mobile phase stability was also established by quantifying the freshly prepared sample solutions against freshly prepared reference standard solutions every 6 h. During the study period, the prepared mobile phase remained unchanged. The recovery of the assay of zafirlukast and the content of each impurity was calculated against the initial value for the study period.

## Results and Discussion

### *Forced degradation studies*

The degradation of the drug substance was only observed under oxidative conditions and acid hydrolysis. Degradation was not observed in other stressed conditions, which are photolytic, thermal, and treatment with alkali/base. Zafirlukast drug substance leads to the formation of a major unknown degradation peak at RRT 0.43 under oxidation stress conditions. The content of other degradants is less than 1.0% under this condition. A small unknown degradation peak was observed at the RRT of 0.67 under the acid stress condition (Figure 3). The peak purity factor is within the threshold limit, which demonstrates the specificity of the zafirlukast peak. Additionally, the peak homogeneity study by LC–MS confirmed that the zafirlukast peak is homogeneous and pure in all stressed samples (Figures 4 and 5). Almost no change in the assay value of zafirlukast was observed in the presence of impurity-1, impurity-2, impurity-3, impurity-4 and impurity-5. The RRF of the degradants were considered to be one, while impurities and the assay of the stressed samples were close to the area percentage of the degraded samples. Hence, the mass balance was between 98 and 102%, which confirms the specificity and stability-indicating ability of the developed method. The summary of the forced degradation is shown in Table II. During the peak homogeneity study, the mass pattern of all stressed samples matched the mass pattern of the sample before stressing and no additional signals were observed under the zafirlukast peak. It was also observed that the molecular weight of the impurities is less than the active component, except for the major impurity in oxidative stress condition, which shows that the impurities are the degradation products of zafirlukast. The molecular mass of the impurity from oxidative stress suggests that it could be an N-oxide impurity. Further studies can be performed on characterization of the degradation impurities to understand the metabolites of zafirlukast and other possible impurities during the stability.

### *Precision*

All individual values of impurity content and the assay in the precision and intermediate precision studies fall well within the range of the average confidence interval, confirming the excellent precision of the method. The recommended precision values in terms of percentage RSD should be not more than 15 for the related substances and not more than 2.0 for the assay. However, the percentage RSD of the content of

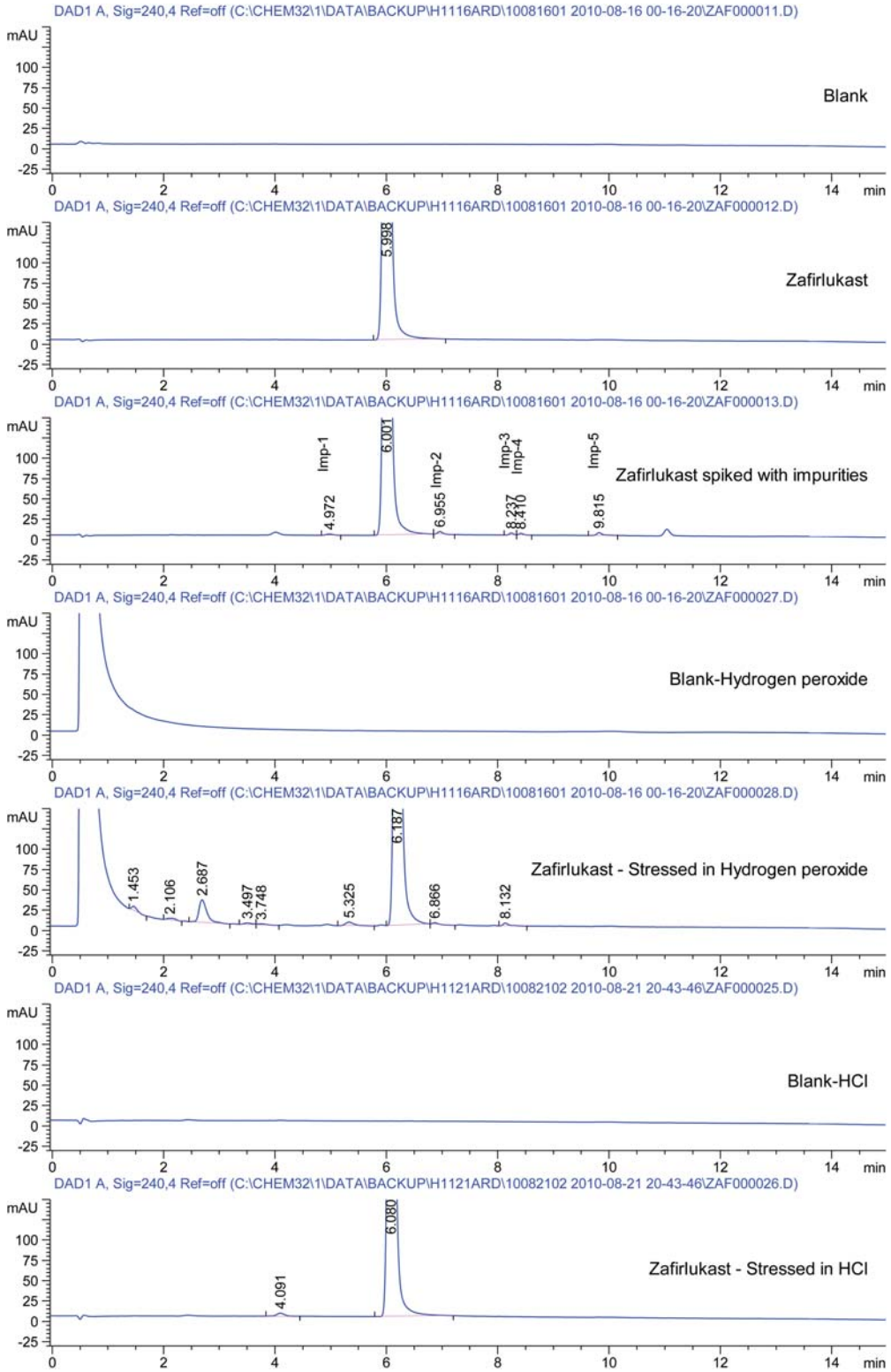


**Figure 3.** Chromatogram of zafirlukast, spiked with potential impurities and its degradation products.

impurities and the assay of zafirlukast in the precision study, including intermediate precision, were well within 3.5 and 0.42, respectively.

### *LOD and LOQ*

The LOD of zafirlukast and all related compounds is approximately 0.01% (w/w) of TAC for a 5 μL injection volume. The

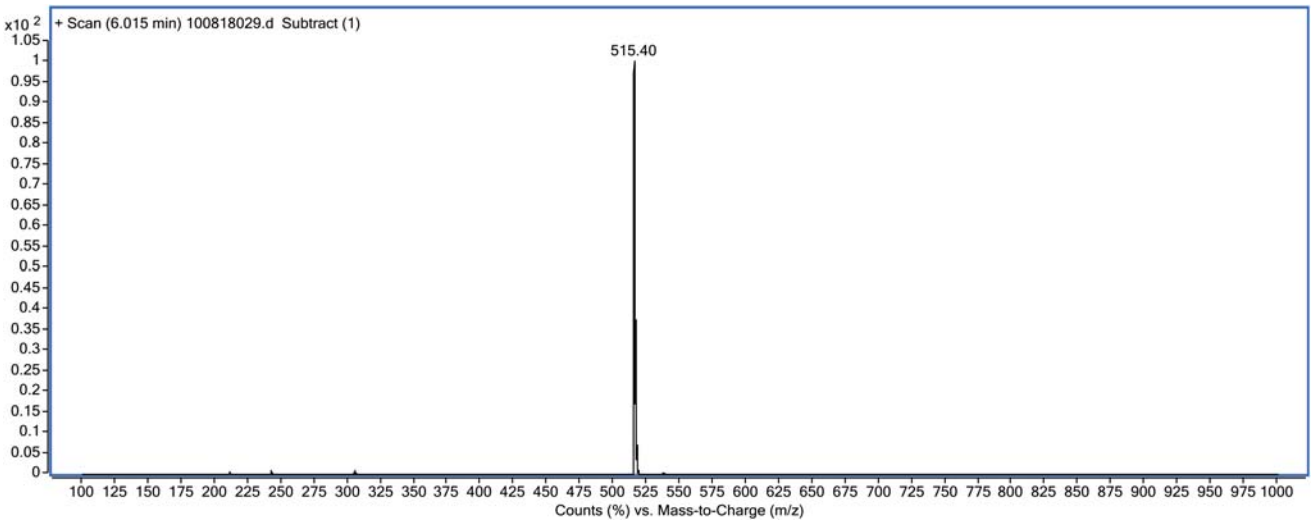


**Figure 4.** Peak homogeneity in acid degradation by LCMS.

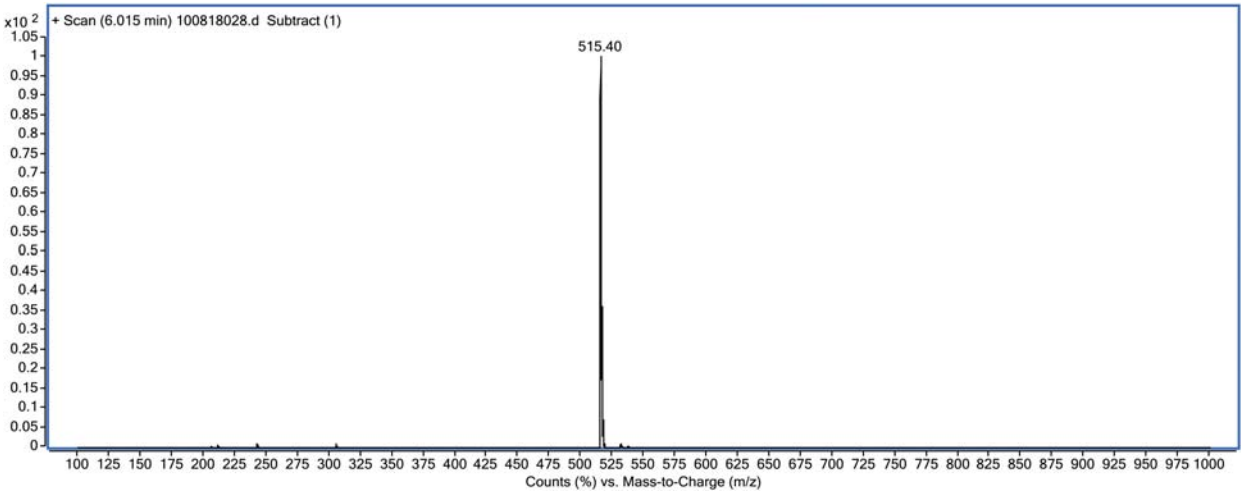


**Figure 5.** Peak homogeneity in oxidative degradation by LCMS.

**Table II**
Summary of Forced Degradation Results

| Stress condition | Duration | Assay of zafirulkast after forced degradation (% w/w) | Content of major degradants (% w/w) | Remarks |
|---|---|---|---|---|
| Acid hydrolysis | 7 days | 98.5 | 0.5 | Very mild degradation products formed |
| Base hydrolysis | 7 days | 99.2 | — | No degradation products formed |
| Oxidation | 6 days | 91.7 | 4.8 | Significant degradation product formed |
| Thermal (60°C) | 7 days | 99.1 | — | No degradation products formed |
| Photolytic, as per ICH | 11 days | 99.2 | — | No degradation products formed |

**Table III**
LOD and LOQ Results

| Compound | LOD | | LOQ | |
|---|---|---|---|---|
| | In ng/mL | % of analyte concentration | In ng/mL | % of analyte concentration |
| Imp-1 | 33.08 | 0.013 | 97 | 0.039 |
| Zafirulkast | 34.27 | 0.014 | 102 | 0.041 |
| Imp-2 | 36.46 | 0.015 | 112 | 0.045 |
| Imp-3 | 33.52 | 0.013 | 103 | 0.041 |
| Imp-4 | 33.68 | 0.013 | 100 | 0.040 |
| Imp-5 | 34.93 | 0.014 | 99 | 0.039 |

LOQ of all known impurities and zafirlukast is approximately 0.04% (w/w) (Table III). Because the RRF of the known impurities is close to one, no major difference is observed in the LOQ and LOD between the impurities and zafirlukast. The LOQ of all known impurities and zafirlukast is less than the reporting

level; hence, the method is capable of quantifying the unknown impurities. The concentration at LOQ meets the acceptance criteria for all other validation parameters.

### *Linearity*

Excellent correlation was achieved for the regression line of zafirlukast and its related impurities at LOQ to 200% of the specification level. The correlation coefficient obtained for all the plots was greater than 0.998. The RRF (calculated from linearity) of each impurity was very close to zafirlukast for all impurities at the optimized condition. The Y-intercept of each plots is below 3.3% of the response at 0.15% (w/w) level of the corresponding impurity. This indicates that the achieved RRF value is nearer to the true value because the plots almost go through the origin. Linear calibration plot for the assay was obtained over the calibration ranges tested, i.e., 200 to 300 µg/mL. An excellent correlation was obtained between the peak area and concentration of zafirlukast by achieving a correlation coefficient greater than 0.999. The Y-intercept for the assay concentration also supports that the plot goes almost through the origin.

### *Accuracy*

The recovery of each impurity falls in the range of 94 to 103% (Table IV). The individual assay value at each level in triplicate is close to the derived true value (Table V). All individual recovery values of the assay and impurities fell well within the confidence interval of mean values. Good recovery values reflect the accuracy of the method, as well as exact values of RRF of impurities.

**Table IV**
Results of Accuracy for Related Substances

| Compound | Level (%) | Spiked quantity $n = 3$ (ng/mL) | Recovered quantity $n = 3$ (ng/mL) | Recovery (%) | | |
|---|---|---|---|---|---|---|
| | | | | Individual | Mean | CI for mean |
| Imp-1 | 0.075 | 181.7 | 183.9 | 101.2 | 102.2 | ±2.7 |
| | 0.150 | 363.4 | 372.3 | 102.4 | | |
| | 0.225 | 545.1 | 561.5 | 103.0 | | |
| Imp-2 | 0.075 | 188.2 | 178.5 | 94.8 | 96.8 | ±3.7 |
| | 0.150 | 376.4 | 363.4 | 96.5 | | |
| | 0.225 | 564.6 | 559.4 | 99.1 | | |
| Imp-3 | 0.075 | 193.8 | 186.7 | 96.3 | 96.6 | ±0.73 |
| | 0.150 | 387.6 | 372.3 | 96.1 | | |
| | 0.225 | 581.4 | 565.5 | 97.3 | | |
| Imp-4 | 0.075 | 192.4 | 187.9 | 97.7 | 96.9 | ±3.2 |
| | 0.150 | 384.8 | 371.3 | 96.5 | | |
| | 0.225 | 577.2 | 557.5 | 96.6 | | |
| Imp-5 | 0.075 | 189.7 | 185.6 | 97.8 | 98.1 | ±1.8 |
| | 0.150 | 379.4 | 371.3 | 97.9 | | |
| | 0.225 | 569.1 | 560.4 | 98.5 | | |

**Table V**
Results of Accuracy for the Assay

| Compound | Level (%) | Assay by HPLC ($n = 3$) | True assay value % w/w | Recovery (%) |
|---|---|---|---|---|
| Zafirulkast | 80 | 99.31 | 99.1 | 100.2 |
| | 100 | 98.86 | 99.1 | 99.8 |
| | 120 | 98.52 | 99.1 | 99.4 |

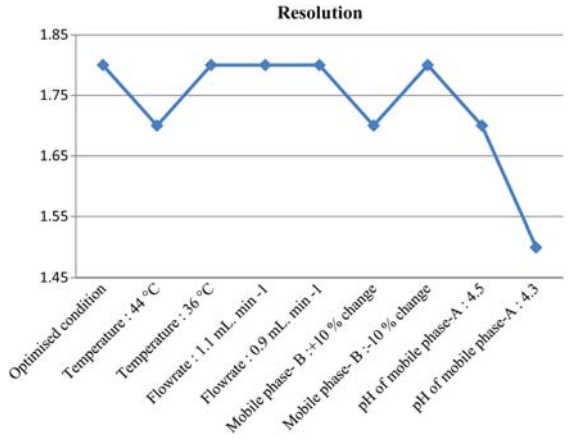


**Figure 6.** Resolution of critical pairs in robustness study – pH change.

### *Robustness*

The resolution between the critical pairs is the key parameter for robustness study. A change in pH to −0.2 units affects the resolution considerably (Figure 6). However, the rest of the deliberately varied chromatographic conditions did not have a significant effect on the resolution. Hence, the LC method is considered to be robust, except for the mobile phase pH, for which the recommendation is to stick with the optimized pH for mobile phase A.

### *Solution stability and mobile phase stability*

The RSD of the assay of zafirlukast during solution stability and mobile phase stability experiments was within 1.0%. No significant changes were experienced in the content of any of the impurities during solution stability and mobile phase stability experiments. The percentage recovery of the assay at each time point against the initial value is between 99.8 and 100.4. The percentage recovery of the content of each impurity against the initial value is between 96.7 and 103.0. The solution stability and mobile phase stability experiment data confirm that the mobile phase and sample solutions were stable up to 48 h.

## Conclusions

The developed stability-indicating analytical method for the related substance and assay determination of zafirlukast and its impurities is precise, accurate, linear and specific. The results of the validation carried out for the method satisfied the ICH requirements. The primary attributes of this method are its simplicity, adaptability to LC–MS environment and short analysis time. This method can be conveniently used for the routine analysis of production samples, and also to check the stability of the drug substance of zafirlukast during storage.

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## References


1. Adkins, J.C., Brogden, R.N.; Zafirlukast—A review of its pharmacology and therapeutic potential in the management of asthma; *Drugs*, (1998); 55: 121–44.
2. Bui, K.H., Kennedy, C.M., Azumaya, C.T., Birmingham, B.K.; Determination of zafirlukast, a selective leukotriene antagonist, human plasma by normal-phase high-performance liquid chromatography with fluorescence detection; *Journal of Chromatography B*, (1997); 696: 131–136.
3. Suslu, I., Altınoz, S.; A reversed-phase high-performance liquid chromatographic method for the determination of zafirlukast in pharmaceutical formulations and human plasma; *Journal of AOAC International*, (2006); 89: 1557–1572.
4. Ficarra, R., Ficarra, P., Tommasini, S., Melardi, S., Calabro, M.L., Furlanetto, S., *et al.*; Validation of a LC method for the analysis of zafirlukast in a pharmaceutical formulation; *Journal of Pharmaceutical and Biomedical Analysis*, (2000); 23: 169–174.
5. Suslu, I., Altınoz, S.; Electrochemical characteristics of zafirlukast and its determination in pharmaceutical formulations by voltammetric methods; *Journal of Pharmaceutical and Biomedical Analysis*, (2005); 39: 535–542.
6. Suslu, I., Altınoz, S.; UV spectrophotometric determination of zafirlukast in pharmaceutical formulations; *Hacettepe University Journal of the Faculty of Pharmacy*, (2007); 27: 33–46.
7. Suslu, I., Altınoz, S.; Differential pulse adsorptive stripping voltammetric determination of zafirlukast in pharmaceutical formulations; *Analytical Letters*, (2005); 38:1625–1639.
8. Vijaya Bharathi, D., Naidu, A., Jagadeesh, B., Maha Laxmi, K.N.K., Revathi Naga Laxmi, P., Reddy, P.R., *et al.*; Development and validation of a sensitive LC-MS/MS method with electrospray ionization for quantitation of zafirlukast, a selective leukotriene antagonist in human plasma: Application to a clinical pharmacokinetic study; *Biomedical Chromatography*, (2008); 22: 645–653.
9. Lakshmana Rao, A., Naga Jahnavi, V.; Development and validation of RP-HPLC method for the estimation of zafirlukast; *International Journal of Research in Pharmaceutical and Biomedical Sciences*, (2010); 1: 102–104.
10. Goverdhan, G., Reddy, A.R., Srinivas, K., Himabindu, V., Reddy, G.M.; Identification, characterization and synthesis of impurities of zafirlukast; *Journal of Pharmaceutical and Biomedical Analysis*, (2009); 49: 895–900.
11. Madhavi, A., Rao, D.V.S., Naidu, A., Raghuram, P.; LC Separation of para and meta isomers of zafirlukast in bulk drug samples and pharmaceutical dosage forms using a chiral stationary phase; *Chromatographia*, (2009); 70: 233–237.
12. Radhakrishna, T., Satyanarayana, J., Satyanarayana, A.; Determination of zafirlukast by stability-indicating LC and derivative spectrophotometry; *Journal of Pharmaceutical and Biomedical Analysis*, (2002); 30: 695–703.
13. Krishnaiah, Ch., Durga Prasad, B.J., Malathi, V., Goverdan, G., Reddy, A.R., Kumar, Ramesh, *et al.*; Development and validation of a regioselective, specific, stability-indicating LC and assay method for zafirlukast; *Indian Journal of Analytical Chemistry*, (2009); 8: 471–478.
14. Russo, R., Guillarme, D., Nguyen, D.T.T., Bicchi, C., Rudaz, S., Veuthey, J.L; Pharmaceutical applications on columns packed with sub-2 μm particles; *Journal of Chromatographic Science*, (2008); 46: 199–208.
15. International Conference on Harmonization, Q2 (R1) (2005) Validation of analytical procedures: Text and methodology. http ://www.ich.org/products/guidelines/quality/article/ quality-guidelines.html (accessed September 14, 2011)
16. International Conference on Harmonization, Q1B (1996) Photo stability testing of new drug substances and products. http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html (accessed September 14, 2011)
17. Bakshi, M., Singh, S.; Development of validated stability-indicating assay methods - critical review; *Journal of Pharmaceutical and Biomedical Analysis*, (2002); 28: 1011–1040.
18. Singh, B., Bakshi, M., Singh, A., Singh, S.; The ICH guidance in practice: Stress degradation studies on ornidazole and development of a validated stability-indicating assay; *Journal of Pharmaceutical and Biomedical Analysis*, (2001); 26: 891–897.