A Validated Stability-Indicating UPLC Method for the Determination of Impurities in Maraviroc

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Maraviroc is an antiretroviral drug in the CCR5 receptor antagonist class, which is used in the treatment of HIV. Maraviroc has six impurities. A novel, stability-indicating reversed-phase ultra-performance liquid chromatography (RP-UPLC) method has been developed for the quantitative determination of maraviroc in active pharmaceutical ingredients, along with its six impurities. The method is applicable to the quantification of related compounds and the assay of maraviroc. Efficient chromatographic separation was achieved on a BEH Shield RP-18 column, 100 × 2.1 mm, 1.7 μm, in isocratic elution within 12 min. The mobile phase was 0.01 M ammonium acetate in water and acetonitrile in the ratio of 63:37 (v/v). The flow rate was 0.4 mL/min, column oven temperature was maintained at 40°C and detection was conducted at 210 nm. Stress degradation conditions were established for maraviroc by subjecting it to acid, base, oxidation, water, humidity, thermal and photolysis stress. The stress samples were assayed against a qualified reference standard and the mass balance was close to 98.0%. The developed UPLC method was validated according to the current International Conference on Harmonization guidelines for specificity, detection limit, quantitation limit, linearity, accuracy, precision, intermediate precision and robustness. The resolution between maraviroc and its six impurities was greater than 3.0. A regression analysis showed that the correlation coefficient value was greater than 0.999 for maraviroc and its six impurities.

Introduction

During the past decade, HIV infection, although an incurable disease, has become largely manageable. This is attributable to the advent of highly active antiretroviral therapy (HAART), in which patients are treated with a cocktail of drugs designed to reduce their viral loads to extremely low levels (1). Maraviroc, 4,4-difluoro-N-[(1S)-3-[(3-exo)-3-[3-methyl-5-(1-methyllethyl)-4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]octan-8-yl]-1-phenylpropyl]-cyclohexane carboxamide, is an antiretroviral drug in the CCR5 receptor antagonist class, which is used in the treatment of HIV infection (2, 3).

A new category of separation technique, ultra-performance liquid chromatography (UPLC), is one of the most promising developments in the area of fast chromatographic separations, with its unique characteristics of satisfactory chromatographic resolution (Table 1), high speed and sensitive analysis (4–7).

This study identified six process-related impurities: the current (1S)-3-[(3-exo)-3-[3-methyl-5-(1-methyllethyl)-4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyltert-butyl(1S)-3-[1,(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-exo-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropylcarbamate (Imp-2), 4,4-difluoro-N-[(1S)-3-[(3-end o)-3-[3-methyl-5-(1-methyllethyl)-4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl]-cyclohexane carboxamide (Imp-3), 1-((R)-3-[(3-Isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl)-3-((1S)-3-((3-isopropylpentyl)-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl) urca (Imp-4), 1-chloro-4,4-difluoro-N-[(1S)-3-[(3-exo)-3-[3-methyl-5-(1-methyllethyl)-4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl]-cyclohexane carboxamide (Imp-5) and N-((S)-3-((I R,3R,5S)-3-(3,5-diisopropyl-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl)-4,4-difluorocyclohexane carboxamide (Imp-6). The chemical structures of maraviroc and its impurities are shown in Figure 1.

A liquid chromatographic method has been described in the literature for the determination of maraviroc by reversed-phase high-performance liquid chromatography (RP-HPLC) (8). However, there are no methods in the literature for the quantification of maraviroc and its related compounds. Further, no official or draft monograph on maraviroc has been published in any of the pharmacopoeias for compendial applications.

The dearth of pharmacopoeial methods and established stress stability conditions necessitated the development of a stability-indicating UPLC method to separate all six impurities and degradants from maraviroc. The objective of the work was to develop a cheap, efficient RP-UPLC method to and demonstrate its stability-indicating capabilities by forced degradation. Forced degradation of maraviroc was conducted under acid hydrolysis, base hydrolysis, and oxidative, thermal, humid and photolytic stress conditions, because this is a part of developmental strategy under the recommendations of the International Conference on Harmonization (ICH). These studies provide valuable information on the stability, shelf life and storage of the drug and its degradation pathways. This paper also deals with the validation of the developed method, as per ICH guidelines.

Experimental

Materials and reagents

Samples of maraviroc, maraviroc reference standard and its six impurities were received from Hetero Research Foundation (Hyderabad, India), along with their purity contents. All impurities and maraviroc reference standard were more than 95% pure; individual purity levels were as follows: maraviroc (99.7%), Imp-1 (95.8%), Imp-2 (99.3%), Imp-3 (97.4%), Imp-4 (96.2%), hydrochloride (Imp-1), tert-butyl(1S)-3-[3-(3-isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-exo-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropylcarbamate (Imp-2), 4,4-difluoro-N-[(1S)-3-[(3-end o)-3-[3-methyl-5-(1-methyllethyl)-4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl]-cyclohexane carboxamide (Imp-3), 1-((R)-3-[(3-Isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl)-3-((1S)-3-((3-isopropylpentyl)-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl) urca (Imp-4), 1-chloro-4,4-difluoro-N-[(1S)-3-[(3-exo)-3-[3-methyl-5-(1-methyllethyl)-4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl]-cyclohexane carboxamide (Imp-5) and N-((S)-3-((1R,3R,5S)-3-(3,5-diisopropyl-4H-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl)-4,4-difluorocyclohexane carboxamide (Imp-6). The chemical structures of maraviroc and its impurities are shown in Figure 1.

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Imp-5 (95.6%) and Imp-6 (95.4%). In addition, HPLC grade acetonitrile and acetic acid were purchased from Merck (Darmstadt, Germany). Ammonium acetate was purchased from Rankem (Mumbai, India). Highly pure water was prepared with a Millipore Milli-Q Plus water purification system (Millipore, Milford, MA).

**Chromatographic conditions**

The chromatographic separation was achieved on a BEH Shield RP-18 column, 100 x 2.1 mm, 1.7 μm (Waters). The mobile phase was composed of 0.01 M ammonium acetate buffer (0.077 g of ammonium acetate in 1,000 mL of water and adjusted to pH 6.5 ± 0.05 with acetic acid) and acetonitrile in the ratio of 63:37 (v/v). The mobile phase was filtered and degassed through a 0.22 μm membrane filter. The flow rate was kept at 0.4 mL/min.

**Equipment**

A UPLC (Waters Aquity H Class, Milford, MA) equipped with a photodiode array detector (PDA) with an autosampler was used. The output signal was monitored and processed by using Empower software on a Pentium computer (Digital Equipment Co.). Photostability studies were conducted in a photostability chamber (Atlas Suntest CPS+, Altenhasslau, Germany). Thermal stability studies were conducted in a dry hot air oven (Cintex precision hot air oven, Hyderabad, India).

![Chemical structures of maraviroc and its six impurities: maraviroc (A); Imp-1 (B); Imp-2 (C); Imp-3 (D); Imp-4 (E); Imp-5 (F); Imp-6 (G).](image-url)
The temperature of the column was maintained at 40°C and the detection wavelength was monitored at 210 nm. The injection volume was 1 μL and the mobile phase was used as diluent.

**Liquid chromatography–mass spectrometry conditions**

A liquid chromatography–mass spectrometry (LC–MS) system (Waters Micromass Quattro Micro-APPI-ESCI) was used to identify unknown compounds formed during forced degradation. A Symmetry Shield column, 100 × 4.6 mm, 3.5 μm, was used as the stationary phase, with a mobile phase containing a gradient of Solvent A, 0.01 M ammonium formate in water (0.065 g of ammonium formate in 1,000 mL of water and adjusted to pH 3.0 ± 0.05 with formic acid solution), and Solvent B, a mixture of water and acetonitrile in the ratio of 20:80 (v/v). The flow rate of the mobile phase was kept at 0.4 mL/min, with a gradient program of 0/45, 8/45, 30/60, 40/60, 45/45 and 55/45 [time (min) / B (%)]. The injection volume was 20 μL. The analysis was performed in positive and negative electrospray ionization (ESI) modes. The capillary and cone voltages were 3.50 KV and 25.00 V, respectively. The extractor and ratio-frequency (RF) lens voltages were 3.0 V and 0.3 V, respectively. The source and desolvation temperatures were 120 and 350°C, respectively, and the cone gas flow and desolvation gas flows were 100 and 650 L/h, respectively.

**Preparation of standard solutions and sample solutions**

A standard solution was prepared by appropriate weighing and respective dilutions of the reference standard and impurities in the diluent to yield a final concentration of 0.1%, with respect to sample concentration, for maraviroc and each of its six impurities; this solution was used throughout the study. The drug substance (for degradation) powder equivalent to 100 mg of the sample was transferred to a 20 mL volumetric flask and 10 mL of diluent was added. The flask was attached to a rotary shaker and shaken for 2 min to completely disperse the powder. The mixture was sonicated for 2 min and diluted to the appropriate volume with diluent to make a solution containing 5.0 mg/mL. The solution was filtered through a 0.45 μm Nylon 66 membrane filter.

**Specificity Stress Studies**

Specificity is the ability of the method to measure the response of the analyte in the presence of its six impurities. The specificity of the developed UPLC method for maraviroc was determined in the presence of its six impurities and degradation products. Forced degradation studies were also performed on maraviroc to provide an indication of the stability-indicating property and specificity of the proposed method. The stress conditions employed for the degradation study included, light, 1.2 million lx/h (as per ICH Q1B Option 1); heat, 105°C; humidity, 90% relative humidity (RH); acid hydrolysis, 2 M of hydrogen chloride (HCl) heated at 80°C for 8 h; base hydrolysis, 2 M of sodium hydroxide (NaOH) heated at 80°C for 8 h; and oxidation, 5% hydrogen peroxide (H₂O₂) heated at 80°C for 15 min. For heat, humidity and light studies, the samples were exposed for five days and 1.2 million lx/h and 200 W hm², respectively (13). The peak purity of the principal peak in the chromatogram of the stressed samples of maraviroc was assessed by using a PDA.

**Analytical Method Validation**

The proposed method was validated as per ICH guidelines (9–12).

**Precision**

**Repeatability and reproducibility**

The repeatability of the related substances method was checked by a six-fold analysis of 5.0 mg/mL of maraviroc spiked with 0.10% of each of the six impurities. The relative standard deviation (RSD) of the peak area was calculated for each impurity.

**Intermediate precision**

Intra-day and intra-day variation and analyst variation were studied to determine the intermediate precision of the proposed method. Intra-day precision was determined by a six-fold analysis of 5.0 mg/mL of maraviroc spiked with 0.10% of each of the six impurities. Different analysts prepared different solutions on different days. The RSD of peak area was calculated for each impurity.

**Limits of detection and quantitation**

The limit of detection (LOD) and limit of quantitation (LOQ) for maraviroc and the six impurities were estimated at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. The LOQ values were confirmed by performing precision and accuracy verification.

**Linearity**

The linearity of the detector response to different concentrations was evaluated for maraviroc and its impurities by using six levels ranging from LOQ to 200% [LOQ, 50 (0.05%), 80 (0.08%), 100 (0.10%), 120 (0.12%) and 200 (0.20%)] with respect to sample concentration. The linear regression data were evaluated for all impurities.

**Accuracy**

The accuracy of the method, evaluated by the recovery values of all impurities, was determined by analyzing maraviroc sample solutions in triplicate, which were spiked with each impurity at four different concentration levels ranging from LOQ to 200% with respect to the specified limit.

**Robustness**

To determine the robustness of the developed method, the experimental conditions were purposely altered and the resolution between maraviroc and its six impurities was evaluated. The flow rate of the mobile phase was 0.4 mL/min. To study the effect of flow rate on the resolution parameter, it was changed instead of 40°C. In all of these varied conditions, the components of the mobile phase were held constant.

**Solution stability and mobile phase stability**

The solution stability of maraviroc in the related compounds method was determined by leaving both the sample and
reference standard solutions in tightly closed volumetric flasks at room temperature for 48 h. The same sample solutions were assayed in 6-h intervals over the study period. The stability of the mobile phase was also examined by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions in 24-h intervals up to five days. The prepared mobile phase remained constant during the study period. The solution stability of maraviroc and its impurities in the method is determined by leaving a spiked sample solution in a tightly closed volumetric flask at room temperature for 48 h. The contents of the six impurities were determined at each 6-h interval throughout the study period. The stability of the mobile phase was also investigated for five days by injecting the sample solutions for every 24-h interval. The contents of the six impurities were determined in the test solutions.

The prepared mobile phase remained constant during the study period.

Results

Method validation

Repeatability and reproducibility

The RSD of maraviroc during the precision study was within 0.81% and the RSD values for the areas of six impurities were within 2.46% in the related substances test. The results are tabulated in Table II.

Intermediate precision

The RSD of the results obtained in the intermediate precision study was within 1.53% for maraviroc and the RSD of the areas of six impurities were within 2.02%, which reveals that the method is highly precise. The results are tabulated in Table II.

Table II

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Imp-1</th>
<th>Imp-4</th>
<th>Imp-5</th>
<th>Imp-6</th>
<th>Imp-2</th>
<th>Imp-3</th>
<th>Imp-7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linearity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>0.9999</td>
<td>0.9997</td>
<td>0.998</td>
<td>0.997</td>
<td>0.997</td>
<td>0.995</td>
<td></td>
</tr>
<tr>
<td>Slope (m)</td>
<td>155.016</td>
<td>204.470</td>
<td>214.032</td>
<td>194.366</td>
<td>152.261</td>
<td>239.120</td>
<td></td>
</tr>
<tr>
<td>Y-intercept (C)</td>
<td>–298</td>
<td>–901</td>
<td>–118</td>
<td>–968</td>
<td>–308</td>
<td>–591</td>
<td></td>
</tr>
<tr>
<td>Residual sum of squares</td>
<td>163282</td>
<td>129960</td>
<td>459783</td>
<td>1315185</td>
<td>1125465</td>
<td>1180403</td>
<td></td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.20</td>
<td>0.20</td>
<td>0.10</td>
<td>0.20</td>
<td>0.35</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Accuracy/recovery (%)</td>
<td>100.1</td>
<td>97.6</td>
<td>—</td>
<td>88.7</td>
<td>90.8</td>
<td>95.8</td>
<td></td>
</tr>
<tr>
<td>50% level ($n$ = 3)</td>
<td>99.8</td>
<td>98.3</td>
<td>—</td>
<td>100.5</td>
<td>103.4</td>
<td>98.0</td>
<td></td>
</tr>
<tr>
<td>100% level ($n$ = 3)</td>
<td>99.6</td>
<td>95.9</td>
<td>—</td>
<td>118.4</td>
<td>100.3</td>
<td>105.6</td>
<td></td>
</tr>
<tr>
<td>200% level ($n$ = 3)</td>
<td>99.9</td>
<td>100.5</td>
<td>—</td>
<td>104.6</td>
<td>102.2</td>
<td>98.5</td>
<td></td>
</tr>
<tr>
<td>Precision/RSD (%)</td>
<td>3.45</td>
<td>2.95</td>
<td>4.11</td>
<td>1.98</td>
<td>2.89</td>
<td>3.45</td>
<td></td>
</tr>
<tr>
<td>LOD level ($n$ = 6)</td>
<td>0.43</td>
<td>1.83</td>
<td>0.81</td>
<td>2.29</td>
<td>1.86</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>100% level ($n$ = 6)</td>
<td>0.38</td>
<td>0.95</td>
<td>0.56</td>
<td>0.85</td>
<td>0.95</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>200% level ($n$ = 6)</td>
<td>1.58</td>
<td>2.02</td>
<td>1.53</td>
<td>0.85</td>
<td>0.94</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Intermediate precision</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% level ($n$ = 6)</td>
<td>5.758</td>
<td>9.256</td>
<td>10.052</td>
<td>3.878</td>
<td>8.637</td>
<td>14.378</td>
<td></td>
</tr>
<tr>
<td>Robustness (resolution)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual conditions: 0.4 mL/min</td>
<td>5.958</td>
<td>10.056</td>
<td>10.923</td>
<td>4.012</td>
<td>8.987</td>
<td>15.154</td>
<td></td>
</tr>
<tr>
<td>Different flow: 0.3 mL/min</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Different flow: 0.5 mL/min</td>
<td>4.123</td>
<td>7.546</td>
<td>8.136</td>
<td>3.052</td>
<td>7.232</td>
<td>13.678</td>
<td></td>
</tr>
<tr>
<td>Column temperature: 35°C</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Column temperature: 45°C</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

LOD and LOQ

The LOD and LOQ values for maraviroc and its six impurities were 0.35 and 1.0 µg/mL, respectively, with respect to test concentration. RSD values were in the range of 1.98–5.02% for the LOQ. The results are reported in Table II.

Linearity

The linearity calibration plots for the maraviroc and its six impurities were obtained over the tested calibration ranges, i.e., LOQ to 200% of the specification level of each component (i.e., LOQ, 0.05, 0.08, 0.10, 0.12 and 0.20%). The correlation coefficient ($r$) was greater than 0.999 (Table II). These results show that an excellent correlation existed between the peak areas and concentrations of the six impurities and maraviroc.

Accuracy

The percentage recovery of Impurities 1, 2, 3, 4, 5 and 6 ranged from 88.7 to 118.4%. The percentage of recovery values for maraviroc impurities are presented in Table II. The UPLC chromatogram of the spiked sample at 0.10% level of all six impurities in the maraviroc sample is shown in Figure 2.

Robustness

In all of the deliberately varied chromatographic conditions (flow rate and column temperature), all analytes were adequately resolved and elution orders remained unchanged. The resolution between the critical pairs, i.e., Imp-2 and Imp-6, was greater than 3.05 and resolution between all other components was greater than 3.78 for all flow rates (0.3 to 0.5 mL/min) and column temperatures (35 and 45°C). The resolutions between the impurities under various conditions are listed in Table II.

Solution and mobile phase stability

No significant changes were observed in the contents of the six impurities during solution stability and mobile phase stability.
experiments, when performed using the related substances method. The results of the solution and mobile phase stability experiments confirmed that the sample solutions used during related substances determinations were stable up to 48 h; the mobile phase was stable for up to five days.

Results of forced degradation studies
Degradation was not observed in maraviroc stressed samples subjected to light, humidity, heat, water and base stress conditions. Significant degradation observed in the drug substance under acid and oxidation hydrolysis, leading to the formation of Imp-1 (4.50%) under acid degradation conditions and one major unknown degradant at relative retention time (RRT) of 0.32 (4.12%) in oxidation hydrolysis. Peak purity test results derived from the PDA detector confirmed that the maraviroc and degraded peaks were homogeneous and pure in all of the analyzed stress samples. The mass balance results (percentage assay of maraviroc + percentage of total impurities) were calculated for all degradation samples and found to be more than 98.0%. The assay of maraviroc was unaffected by the presence of related compounds and degradation products, which confirms that the method is stability indicating. The forced degradation and mass balance results are shown in Table III. Typical UPLC chromatograms of the stress degradation of maraviroc are shown in Figures 3 and 4.

The purity angle was within the purity threshold limit in all of the stressed samples, demonstrating the homogeneity of the peak of the analyte.

Discussion
Method development and optimization
The primary objective of the chromatographic method was to separate six impurities, maraviroc and its generated degradation products from the peak of the analyte during stress studies. The ultraviolet (UV) profiling determined that the suitable wavelength for maraviroc and its impurities was 210 nm. Hence, the method was developed at 210 nm. The impurities and maraviroc were co-eluted by using different stationary phases, such as C18 with various mobile phases, and organic modifiers in the mobile phase, including acetonitrile and methanol. The pH change in the mobile phase from 7.5 to 8.0 showed significant variation in
the retention time (RT) of the maraviroc peak; i.e., from 3.2 min to 4.5 min. To increase the separation between the impurities and maraviroc, considering the nature of the product and the sensitivity of the mobile phase pH, the pH of the mobile phase was reduced to be slightly acidic, i.e., approximately 6.5, and a mobile phase ratio of 50:50 (v/v) was used for acetate buffer and acetonitrile; a flow rate of 0.6 mL/min was used with the stationary phase of a Shim-pack ODS-II column, 75 × 3.0 mm, 2.2 μm (Shimadzu), and an impurity spiked solution was injected. This analysis revealed that the two impurity peaks (Imp-1 and Imp-4) merged and the remaining impurities almost co-eluted with the peak of the analyte (Figure 2). To improve the resolution between the impurities and the analyte, the ratio of the mobile phase was adjusted to 60:40 (v/v) of acetate buffer and acetonitrile and the impurity spiked solution was injected. The resolution between the impurities and the analyte was very poor (0.902 between maraviroc and Imp-2) and no separation occurred between Imp-2 and the analyte peak. To further improve

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>TI (%)</th>
<th>Imp-1 (%)</th>
<th>Imp-2 (%)</th>
<th>Imp-3 (%)</th>
<th>Imp-4 (%)</th>
<th>Imp-5 (%)</th>
<th>Imp-6 (%)</th>
<th>Maraviroc (%)</th>
<th>Mass balance†</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-is sample</td>
<td>0.36</td>
<td>ND</td>
<td>ND</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
<td>99.5</td>
<td>99.9</td>
</tr>
<tr>
<td>Acid hydrolysis</td>
<td>5.10</td>
<td>0.02</td>
<td>ND</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
<td>98.5</td>
<td>99.0</td>
</tr>
<tr>
<td>Base hydrolysis</td>
<td>0.50</td>
<td>0.2</td>
<td>ND</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
<td>96.7</td>
<td>98.0</td>
</tr>
<tr>
<td>Oxidative degradation</td>
<td>6.30</td>
<td>0.02</td>
<td>ND</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
<td>0.08</td>
<td>91.7</td>
<td>98.0</td>
</tr>
<tr>
<td>Water degradation</td>
<td>0.36</td>
<td>ND</td>
<td>ND</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
<td>99.6</td>
<td>99.9</td>
</tr>
<tr>
<td>Thermal degradation</td>
<td>0.45</td>
<td>ND</td>
<td>ND</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
<td>99.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Photolytic degradation</td>
<td>0.40</td>
<td>ND</td>
<td>ND</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
<td>99.5</td>
<td>99.9</td>
</tr>
<tr>
<td>Humidity degradation</td>
<td>0.38</td>
<td>ND</td>
<td>ND</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.09</td>
<td>99.5</td>
<td>99.9</td>
</tr>
</tbody>
</table>

*Total impurities (TI).
†Calculated by: assay of maraviroc (%) + TI (%).

Figure 3. Typical chromatograms of maraviroc stress degradations: acid (A); base (B); oxidation (C).
the resolution between the impurities, the mobile phase ratio was adjusted to 63:37 (v/v) of acetate buffer (pH adjusted to 6.5 with acetic acid) and acetonitrile, the flow rate was changed to 0.4 mL/min and a stationary phase BEH Shield RP-18 column, 100 x 2.1 mm, 1.7 μm, was used. Satisfactory results were obtained: all of the impurities were well resolved from each other and from maraviroc (with a resolution between each other of at least 3).

Identification of major degradation product formed in oxidation hydrolysis

An LC–MS study was conducted to determine the m/z value of the major degradation product formed under oxidation hydrolysis using a Waters Micromass Quattro Micro-API-ESCI LC–MS. The conditions of the method were described previously. The m/z value obtained for the degradation product, resolved in ESI positive mode, was 530.21 (M + 1). Based on the m/z value the degradation product was identified as maraviroc N-oxide, with a molecular weight of 530.21. A typical LC–MS chromatogram and mass spectrum of the major oxidation degradant are given in Figure 5.

Conclusions

The degradation conditions of maraviroc were established as per ICH recommendations. The developed UPLC method, which was used for stress studies, also fit for the quantitative estimation of related substances and the determination of maraviroc. The behavior of maraviroc under various stress conditions was studied and all degradation products and process-related impurities were well separated from the drug substance, which demonstrates that the method is stability-indicating. The method was validated as per ICH recommendations. The developed UPLC method is stability-indicating and can be used to routinely analyze maraviroc in production and stability samples.

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References