Stability Indicating LC Method for Rapid Determination of Related Substances of O-Desmethyl Venlafaxine in Active Pharmaceutical Ingredients and Pharmaceutical Formulations

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A simple, precise and accurate stability-indicating reversed phase-high performance liquid chromatography method was developed for the quantitative determination of O-desmethyl venlafaxine (ODV) and its related substances in active pharmaceutical ingredient and pharmaceutical formulation. The method was developed using YMC-pack ODS-A (150 × 4.6 mm, 3 μm) column with mobile phase containing a gradient mixture of solvents A and B. Solvent A contained a mixture of buffer and acetonitrile in the ratio of 85:15 (v/v). The buffer consisted of 10 mM potassium dihydrogen phosphate and 2 mM 1-octane sulfonic acid sodium salt (pH adjusted to 6.0 by using diluted potassium hydroxide solution). Solvent B contained a mixture of water and acetonitrile in the ratio of 20:80 (v/v). The eluted compounds were monitored at 230 nm. ODV and its six impurities were well separated within 14 min run time. It was subjected under the stress conditions of oxidative, acid, base, water, thermal and photolytic degradation. It was sensitive towards acidic, basic, oxidative and water stress conditions, stable in photolytic and thermal degradation conditions. The degradation products were well resolved from main peak and its impurities, the mass balance in each case was >99.0%, proving the stability-indicating power of the method. The developed method was validated as per International Conference on Harmonization (ICH) guidelines with respect to its specificity, linearity (correlation coefficient >0.9996), limit of detection, limit of quantification, accuracy (recovery range 97.1–103.2%), precision (% relative standard deviation ≤1.9%) and robustness.

Introduction

O-Desmethyl venlafaxine (ODV), also known as desvenlafaxine, is chemically described as 4-[2-dimethylamino-1-(1-hydroxy-cyclohexyl) ethyl] phenol. It has an empirical formula of C16H25NO2 and has a molecular weight of 263.38 g/mol. ODV is used in the treatment of depression. ODV is a major active metabolite of venlafaxine. Approximately 75% of venlafaxine is metabolized into ODV by the action of CYP2D6 on parent drug. Based on the in vitro results and the relationship between ODV and venlafaxine, ODV is classified as a selective serotonin and norepinephrine reuptake inhibitor (1–5).

In the literature, limited liquid chromatography (LC) methods were reported for the determination of venlafaxine and ODV in human plasma using high-performance liquid chromatography (HPLC) with fluorimetric detection (6), HPLC with coulometric detection (7), LC–MS–MS (8–11), quantification of ODV in pharmaceutical dosage form by high-performance thin layer chromatography (12) and stability-indicating assay by HPLC (13–15). Although a number of methods were available for evaluating the assay of ODV, a common method for separation of its potential impurities and degradation products with good efficiency remains unavailable.

Presently, the determination of impurities is one of the critical phases among various complicated tasks for pharmaceutical analysis during method development. It should be taken into consideration that ODV and its impurities should be monitored together with their degradation compounds, preferably in a single chromatographic run. To the best of our knowledge, none of the currently available analytical methods can separate and quantify all the known related compounds and degradation impurities of active pharmaceutical ingredient (API) and pharmaceutical formulation. It is, therefore, felt necessary to develop a new stability-indicating method for the related substance determination and quantitative estimation of ODV, which can separate all the impurities in short run time without compromising the resolution and sensitivity.

Hence, a reproducible stability-indicating reversed phase-high performance liquid chromatography (RP-HPLC) method was developed for the quantitative determination of ODV and its six impurities, namely Imp-A, Imp-B, Imp-C, Imp-D, Imp-E and Imp-F (Appendix A, Figures 1B–G). This method was successfully validated according to International Conference on Harmonization (ICH) guidelines (validation of analytical procedures: test and methodology Q2).

Experimental

Materials and reagents

API standards and samples were supplied by Dr. Reddy’s Laboratories Limited, IPDO, Hyderabad, India. Commercially available D-Venzig tablets containing 50 mg of ODV were used for the dosage form analysis. The HPLC grade acetonitrile, analytical grade 1-octane sulfonic acid sodium salt, potassium dihydrogen phosphate and ammonium acetate were purchased from Merck, Darmstadt, Germany. Trifluoroacetic acid was purchased from Acros Organics, Geel, Belgium. Water was prepared by using Millipore Milli-Q Plus water purification system.

Chromatographic conditions and equipment

LC was carried out on a Waters HPLC equipped with photodiode array detector (PDA). The output signal was monitored and processed using empower2 software. The chromatographic column used was YMC-pack ODS-A (150 × 4.6 mm and 3 μm). The separation was achieved by using a gradient method. Solvent A contained a mixture of buffer and acetonitrile in the ratio of 85:15...

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mass spectrometer with Analyst 1.4 software, MDS SCIEX, USA) was used for the identification of unknown compounds formed during forced degradation studies. YMC-pack ODS-A (150×4.6 mm, 3 μm) was used as stationary phase. 0.01 M solution of ammonium acetate and 0.1% trifluoroacetic acid (pH adjusted to 6.0 by using ammonia solution) was used as buffer. Buffer and acetonitrile in the ratio 85:15 (v/v) was used as solvent A, and water and acetonitrile in the ratio of 20:80 (v/v) was used for solvent B. The gradient program was set as: time (min)/%B: 0.01/5, 5/5, 6/40, 12/40, 12.1/5 and 14/5. Solvents A and B in the ratio 1:1 (v/v) was used as the diluent. The flow rate was 1.0 mL/min. The analysis was performed in positive electrospray positive ionization mode, the ion source voltage was 5,000 V and the source temperature was 450°C. GS1 and GS2 are optimized to 30 and 35 psi, respectively. Curtain gas flow was 20 psi. For fragmentation (MS/MS) studies, collision energy and declustering potential were set as 25 and 30 V, respectively.

**Preparation of stock solutions**

A solution of ODV standard (800 μg/mL) was prepared by dissolving an appropriate amount of the drug in diluent (solvents A and B in the ratio 1:1 (v/v)). An individual stock solution (80 μg/mL) of all the impurities (denoted as Imp-A–Imp-F) was prepared in diluent (Figure 1B–G).

**Preparation of sample solution**

To prepare the sample stock solution, 20 (n = 20) tablets of D-Venzig, each containing 50 mg of ODV, was accurately weighed and crushed to a fine powder. An appropriate amount was transferred into an individual 100 mL volumetric flask, added 50 mL of methanol, sonicated for 30 min and diluted to volume with diluent obtaining the final concentration of 800 μg/mL of the API. Ten milliliters of this solution were diluted to 100 mL with diluent to give a solution containing 80 μg/mL. The sample solution was filtered through a 0.45 μm nylon membrane filter.

**Stress studies**

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities (16). Specificity of the developed LC method for ODV was carried out in the presence of its six impurities. Stress studies were performed at an initial concentration of 800 μg/mL of ODV to provide the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition of photolytic (UV light: 200 W h/m² and visible light:1.2 million lux h) for 10 days, heat (105°C) for 10 days, acid degradation (1.0 M HCl at 70°C) for 2 h, base degradation (0.5 M NaOH at 70°C) for 12 h, water hydrolysis (70°C) for 48 h and oxidative stress (2.0% H2O2 at room temperature (RT)) for 10 min to evaluate the ability of the proposed method to separate ODV from its degradation products.

The purity of peaks obtained from stressed samples was checked by the use of the PDA detector. The purity angle was within the purity threshold limit obtained in all stressed samples and demonstrates the analyte peak homogeneity. Assay of ODV from its degradation products.

**LC–MS-MS conditions**

An LC–MS-MS system (Agilent 1200 series liquid chromatograph coupled with Applied Biosystems 4000 Q-Trap triple quadrupole. (v/v). The buffer consisted of 10 mM potassium dihydrogen phosphate and 2 mM 1-octane sulfonic acid sodium salt (pH adjusted to 6.0 by using diluted potassium hydroxide solution). Solvent B contains a mixture of water and acetonitrile in the ratio 85:15 (v/v). The flow rate of mobile phase was 1.0 mL/min.

The HPLC gradient program was set as: time (min)/%B: 0.01/5, 5/5, 6/40, 12/40, 12.1/5 and 14/5. Solvents A and B in the ratio 1:1 (v/v) was used as the diluent. The injection volume was 10 μL.

**Figure 1.** (A) O-Desmethyl venlafaxine: 4-[2-dimethylamino-1-[1-hydroxy cyclohexyl] ethyl phenol (B) Imp-A: N,N-dimethyl-2-[4-hydroxy phenyl] ethyl amine. (C) Imp-B: 1/[2-amine-1-[4-hydroxyphenyl]] ethyl cyclohexan. (D) Imp-C: 1-[2-methyl amine (4-hydroxyphenyl) ethyl] cyclohexanol. (E) Imp-D: 4-[1-cyclohexylidene-2-dimethylamino-1-[2-amino-1-(4-hydroxyphenyl)] ethyl cyclohexanol. (F) Imp-E: 1-[2-dimethyl amine (4-methoxyphenyl) ethyl] cyclohexanol. (G) Imp-F: 1-[2-dimethylaminocyclohexyl] ethyl cyclohexane. (H) Possible degradation product for above RRT 0.41 peak: 1-[2-(dimethylamino-1-[4-hydroxy phenyl] ethyl] cyclohexan N-oxide.
calculated. Assay was also calculated for ODV sample by spiking all impurities at the specification level (i.e. 0.15%).

**Method Validation**

The described method has been extensively validated for the assay and related substances by HPLC determination (17).

**Precision**

The repeatability of the related-substance method was checked by 6-fold analysis of 800 μg/mL ODV spiked with 0.15% of specification limit (1.2 μg/mL) of each of the six impurities. Relative standard deviation (RSD%) (%) of peak area for each impurity was calculated. The repeatability of the assay method was evaluated by carrying out six independent assays of ODV at 80 μg/mL against a qualified reference standard.

The intermediate precision ( ruggedness) of the method was evaluated by performing the analysis with different analyst by using different column lot, a different instrument and on different days.

**Limit of detection and quantification**

The limit of detection (LOD) and limit of quantification (LOQ) for ODV and its impurities were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The precision study was also determined at the LOQ level by injecting six (n = 6) individual preparations and calculating the RSD (%) of the area for each impurity and ODV.

**Linearity**

The linearity of the detector response to different concentrations was evaluated for all impurities and ODV by injecting each separately prepared solutions covering the range LOQ to 200% (LOQ, 0.0375, 0.075, 0.1125, 0.15, 0.1875, 0.225 and 0.30% of the normal sample concentration). The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined.

**Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the value found. Accuracy of the related substances by the HPLC method was established by standard addition and recovery experiments. Recovery was calculated for each added concentration. The study was carried out for impurities in triplicate using five concentration levels from LOQ, 0.075, 0.1125, 0.15, 0.1875, 0.225 and 0.30% of the normal sample concentration). The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined.

**Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the method, experimental conditions were deliberately changed. The resolution of ODV and all impurities was evaluated. The mobile phase flow rate was 1.0 mL/min to study the effect of flow rate on resolution; it was changed to 0.8 and 1.2 mL/min. The effect of column temperature was studied at 35 and 45 °C (instead of 40 °C), and the effect of pH of the mobile phase on resolution was also studied at 5.8 and 6.2 (instead of 6.0).

**Solution stability and mobile phase stability**

The solution stability of ODV and its impurities was measured by leaving spiked sample solution in tightly capped volumetric flasks at RT for 48 h. The content of impurities was determined at 12 h intervals for 48 h. The stability of the mobile phase was determined by analysis of freshly prepared sample solutions at 12 h intervals for 48 h. The mobile phase was prepared at the beginning of the study period and not changed during the experiment.

**Results**

**Method development and optimization**

The main objective of this study is to develop a stability-indicating HPLC method for quantitative determination of ODV and its impurities, namely Imp-A, -B, -C, -D, -E and -F (Imp-A, -B, -E and -F are process-related impurities, and Imp-C and -D are degradation-related impurities) within short run time. ODV and its related substances have maximum response at 230 nm by UV spectra. So, the wavelength 230 nm was selected for LC analysis. The sample spiked solution containing 800 μg/mL of ODV and 8 μg/mL of each of the six impurities was prepared in the diluent.

Initially, attempts were made by using different C-18, C-8 and phenyl HPLC columns (Inertsil ODS-3V, Symmetry shield RP-18, X-Terra RP-18, Inertsil C-8 and Kromasil phenyl) with gradient elution using different buffers (KH₂PO₄ and CH₃COONH₄) at different pH (acidic and basic). In all above experimental conditions, ODV and its impurities were ionized extensively at both acidic and basic mobile phase pH and eluting early. The resolution between Imp-C and ODV was not satisfactory, and peak shapes for all impurities and ODV was not good [United States Pharmacopeia (USP) tailing ≥ 2.0]. It was thought to include ion-pair reagent 2 mM 1-octane sulfonic acid sodium salt in mobile phase and trails done by using different C-18 and C-8 HPLC columns. The separation of impurities and ODV was found to be inadequate in C-8 stationary phase. Further trials were conducted with using different C-18 stationary phases [Zorbax C-18 (150 × 4.6 mm, 3.5 μm) X-Bridge C-18 (150 × 4.6 mm, 3.5 μm) and Betasil C-18 (250 × 4.6 mm, 5 μm)] at different pH conditions. At basic pH, separation of impurities and ODV was not good, and the tailing factor of ODV peak was > 2.0. At acidic pH, the separation between impurities was good. However, Imp-C was closely eluting with ODV with resolution < 1.5.

To improve selectivity between stationary phase, Imp-C and ODV, column has been changed to YMC-pack ODS-A (150 × 4.6 mm, 3.0 μm) having high surface area. At column oven temperature 40 °C, an effective separation was achieved between all the impurities and ODV within 14 min (Figure 2A). The below conditions were finalized for the analysis of ODV and its impurities. Solvent A contains a mixture of buffer and acetonitrile in the ratio of 85:15 (v/v). The buffer consisted of 10 mM...
Figure 2. (A) Impurities spiked chromatogram. (B) Acid degradation chromatogram. (C) Base degradation chromatogram. (D) Peroxide degradation chromatogram. (E) Water degradation chromatogram. (F) LC–MS spectrum of Imp-D in acid degradation. (G) LC–MS spectrum of Imp-C in base degradation. (H) LC–MS spectrum of an unknown impurity at RRT ≈ 0.41 in oxidative, base and water degradation.
potassium dihydrogen phosphate and 2 mM 1-octane sulfonic acid sodium salt (pH adjusted to 6.0, by using diluted potassium hydroxide solution). Solvent B contains a mixture of water and acetonitrile in the ratio 20:80 (v/v).

The gradient elution set as:

<table>
<thead>
<tr>
<th>time (min)</th>
<th>%B:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>20</td>
</tr>
<tr>
<td>3.00</td>
<td>20</td>
</tr>
<tr>
<td>6.00</td>
<td>30</td>
</tr>
<tr>
<td>7.00</td>
<td>30</td>
</tr>
<tr>
<td>12.00</td>
<td>42</td>
</tr>
<tr>
<td>12.10</td>
<td>42</td>
</tr>
<tr>
<td>14.00</td>
<td>20</td>
</tr>
<tr>
<td>14.20</td>
<td>20</td>
</tr>
</tbody>
</table>

Flow rate of 1.0 mL/min.

System suitability parameters were evaluated for ODV and its six impurities. Tailing factor for all six impurities and ODV was found to be <1.5. USP resolution for five potential impurities was >2.5, and the resolution between Imp-C and ODV was >1.5 in finalized chromatographic conditions.

**Validation of the method**

**Precision**

The RSD% in the study of repeatability of six independent assays of ODV was within 0.42%. In related substances method repeatability study, the RSD% for the area of six impurities A–F was within 2.0%. The RSD% of the assay results obtained in the intermediate precision study was 0.8%, and in related substances intermediate precision study the RSD% for the area of six impurities A–F was within 1.2%. The RSD% values were presented in Tables I and II.

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
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<tbody>
<tr>
<td>Method Validation Results- LOD, LOQ, Regression, Precision and Intermediate Precision</td>
</tr>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
</tr>
<tr>
<td>Regression equation (y)</td>
</tr>
<tr>
<td>Slope (b)</td>
</tr>
<tr>
<td>Intercept (a)</td>
</tr>
<tr>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>Analysis of variance (significance F)</td>
</tr>
<tr>
<td>Precision (RSD%)</td>
</tr>
<tr>
<td>Intermediate precision (RSD%)</td>
</tr>
</tbody>
</table>

Linearity range is LOQ to 200% with respect to 800 µg/mL ODV for impurities.
Limit of detection and quantification
The determined LOD, LOQ, and precision at LOQ values for ODV and its six impurities were reported in Table I.

Linearity
Linearity calibration plot for the assay method was obtained over the calibration ranges tested, i.e. 20 to 160 μg/mL, and correlation coefficient obtained was >0.999. Linearity calibration plot for the related substances method was obtained over the calibration ranges tested, i.e. LOQ to 0.3% (LOQ, 25, 50, 75, 100, 125, 150 and 200% of specification limit, i.e. 0.15%). The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined. The correlation coefficient obtained was >0.999 (Table I).

Accuracy
The recovery of ODV from API and pharmaceutical formulation ranged from 97.1 to 102.2%. The recovery of the six impurities in ODV API and pharmaceutical formulation ranged from 97.1 to 103.2%. The percentage recovery of the impurities and ODV was listed in Tables III and IV.

Robustness
In all the deliberately varied chromatographic conditions (different flow rate, column temperature, and pH of mobile phase), all analytes were adequately resolved and elution orders remained unchanged. The resolution between all pairs of compounds was >2.5 and the resolution between Imp-C and ODV was >1.5.

Stability in solution and in the mobile phase
The RSD% for assay of ODV during solution stability and mobile phase stability experiments was within 1.4%. No significant changes in the amounts of the six impurities were observed during solution stability and mobile phase experiments when performed using the related substances method.

Results from forced degradation studies
All forced degradation samples were analyzed at an initial concentration 800 μg/mL of ODV with HPLC conditions mentioned in conditions using a PDA detector to ensure the homogeneity and purity of the ODV peak. Degradation was not observed when ODV was subjected to light and heat conditions; slight degradation was observed when the drug was subjected to water (70°C for 48 h) leading to the formation of an unknown impurity at RRT ≈ 0.41. Significant degradation was observed when the drug was subjected to acid hydrolysis (1.0 M HCl at 70°C for 2 h) leading to the formation of Imp-D, base (0.5 M NaOH at 70°C for 12 h) leading to the formation of Imp-C, an unknown impurity at RRT ≈ 0.41 and oxidative hydrolysis (2.0% H2O2 at RT for 10 min) leading to the formation of one major unknown impurity at RRT ≈ 0.41 (Appendix B, Figures 2B–E). This was confirmed by co-injecting Imp-C and Imp-D standards to these degraded samples and also by LC–MS–MS analysis. LC–MS–MS analysis was performed as per experimental conditions and protonated molecular ion at m/z 246.4, which was corresponding to Imp-D in acid degradation (Figure 2F, mass of impurity was 250.0, which was corresponding to Imp-C in base degradation (Figure 2G) and protonated molecular ion at m/z 280.2, which was corresponding to an unknown impurity at RRT ≈ 0.41 in oxidative (Figure 2H), base, and water degradation. The possible degradation product for above RRT ≈ 0.41 peak was shown in Figure 1H. Results from forced degradation studies were presented in Table V.

Assay studies were carried out for stress samples (at 80°C) against ODV qualified reference standard. The mass balance (% assay + % sum of all impurities + % sum of all degradants) results were calculated for all stressed samples and found to be >99%. The purity and assay of ODV was unaffected by the presence of its impurities and degradation products and thus confirms the stability-indicating power of the developed method.

Discussion
The method conditions and forced degradation studies indicate that the developed method is stability indicating and can be used for the quantitative determination of ODV and its process related impurities and degradation products.

The repeatability and intermediate precision results showed insignificant variation in measured response, which demonstrated that the method was repeatable at LOQ level to 150% of the specification limit (RSD < 2.0%). The linearity results are within the acceptable limits (correlation coefficient is >0.999), which shows that an excellent correlation existed between the peak area and concentration of six impurities A–F. The accuracy results at all the four concentration levels, i.e. LOQ, 50, 100 and 150%, are within the acceptable limit (recovery ranged from
Table IV
Pharmaceutical Formulation, Method Validation and Accuracy (Recovery) Data

<table>
<thead>
<tr>
<th>Amount spiked (µg/µl)</th>
<th>ODV</th>
<th>Imp-A</th>
<th>Imp-B</th>
<th>Imp-C</th>
<th>Imp-D</th>
<th>Imp-E</th>
<th>Imp-F</th>
<th>% Total degradation</th>
<th>Assay</th>
<th>Mass balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ</td>
<td>102.2 + 0.9</td>
<td>98.8 + 1.1</td>
<td>101.8 + 1.1</td>
<td>99.1 + 1.4</td>
<td>101.7 + 2.6</td>
<td>99.2 + 2.7</td>
<td>99.7 + 1.9</td>
<td>14.64</td>
<td>89.4</td>
<td>99.42</td>
</tr>
<tr>
<td>50%</td>
<td>100.8 + 0.9</td>
<td>98.2 + 1.7</td>
<td>101.8 + 1.1</td>
<td>100.8 + 0.9</td>
<td>101.3 + 0.8</td>
<td>99.8 + 0.9</td>
<td>101.6 + 0.9</td>
<td>14.64</td>
<td>89.4</td>
<td>99.42</td>
</tr>
<tr>
<td>100%</td>
<td>98.1 + 0.8</td>
<td>98.3 + 1.7</td>
<td>101.8 + 2.1</td>
<td>102.2 + 0.7</td>
<td>101.6 + 1.5</td>
<td>98.8 + 0.3</td>
<td>100.8 + 1.3</td>
<td>14.64</td>
<td>89.4</td>
<td>99.42</td>
</tr>
<tr>
<td>150%</td>
<td>99.8 + 1.8</td>
<td>98.7 + 1.4</td>
<td>99.8 + 1.5</td>
<td>99.1 + 2.5</td>
<td>99.4 + 2.1</td>
<td>99.5 + 1.9</td>
<td>14.64</td>
<td>89.4</td>
<td>99.42</td>
<td></td>
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<tr>
<td>200%</td>
<td>99.8 + 1.1</td>
<td>98.7 + 1.7</td>
<td>101.2 + 1.5</td>
<td>101.7 + 2.6</td>
<td>101.9 + 3.1</td>
<td>99.4 + 2.1</td>
<td>99.5 + 1.9</td>
<td>14.64</td>
<td>89.4</td>
<td>99.42</td>
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</table>

Table V
Method Validation and Summary of Forced Degradation Results

<table>
<thead>
<tr>
<th>% Impurity formed</th>
<th>Acid degradation</th>
<th>Base degradation</th>
<th>Oxidative degradation</th>
<th>Water degradation</th>
<th>Thermal degradation</th>
<th>Photolytic degradation</th>
<th>% Total degradation</th>
<th>Assay</th>
<th>Mass balance</th>
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<tbody>
<tr>
<td>ODV</td>
<td>Impact</td>
<td>Impact</td>
<td>Impact</td>
<td>Impact</td>
<td>Impact</td>
<td>Impact</td>
<td>% Mass balance</td>
<td></td>
<td></td>
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<tr>
<td>50%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>14.59</td>
<td>99.4</td>
<td>99.12</td>
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<tr>
<td>100%</td>
<td>ND</td>
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<td>20.02</td>
<td>98.4</td>
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<td>150%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>25.03</td>
<td>98.1</td>
<td>99.71</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>30.04</td>
<td>98.1</td>
<td>99.71</td>
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Appendix B
A Typical HPLC chromatograms of ODV (Figures 2B–E).

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