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## **Quantification of ioversol in injection dosage form using HPLC: Stability indicting method development and validation**

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## **Abstract**

### ***Background:***

Ioversol (IVL) is a radiographic contrast agent employed in the diagnostic radiography. In this investigation, our aim was to develop and validate a simple and rapid HPLC-DAD method for determination of IVL in bulk and injection dosage form.

### ***Methods:***

IVL separation and analysis was performed on Zodiac phenyl C 18 column (250 mm × 4.5 mm; 5 μ particle size) using water-methanol (90:10 by volume) as mobile phase system and with detection at 254 nm.

### ***Results:***

The retention value of IVL was 4.11 min. The method linearity range was found 254.5-763.5 μg/ml. The accuracy (~100%) and precision (< 2.0%) were within the acceptance criteria. Stability indicating ability of the method was proved by stress degradation studies. Adoptability of this method was assessed with application to marketed injection dosage form with good accuracy (recovery 100.49%) and precision (RSD 0.715%).

### ***Conclusion:***

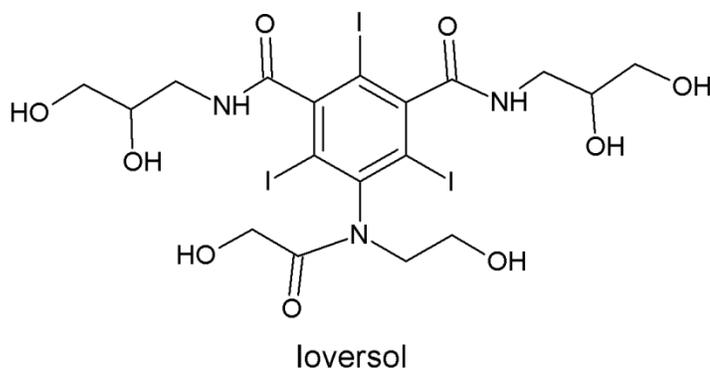
By adopting this method one can analyze IVL in injection dosage form in less than 10 min and hence this method is time saving and enables the estimation of large number of samples.

**Key words:** Ioversol, Optiray, Stability indicating, HPLC, Diode array, Analysis

## **Introduction**

Ioversol (IVL), an effective non-ionic and water soluble radiographic contrast agent, is employed mostly in the diagnostic radiographic procedures like ventriculography, angiocardiology, urography, angiography, venography, arthrography, myelography and arteriography<sup>1-3</sup>. IVL is a organoiodine substances with IUPAC name, 1-*N*,3-*N*-bis(2,3-dihydroxypropyl)-5-[2-hydroxy-*N*-(2-hydroxyethyl)acetamido]-2,4,6-triiodobenzene-1,3-dicarboxamide (Figure 1). Organoiodine substances block x-rays while passing through the body. This allows the delineation of body structures having iodine. On the contrary, the body structures without iodine are not delineated. The extent of opacity formed by organoiodine substances is directly related with the entire quantity of iodinated contrast agent in the x-rays

path. Following administration (intravascular) of IVL, the vessels are made opaque in the path of IVL flow. This makes a clear visualization of internal structures until considerable hemodilution happens<sup>4-6</sup>.



**Figure 1. Structure of the selected drug**

IVL is available in injection form with trade names, Optiray 240<sup>TM</sup> (IVL labeled claim 509 mg/ml), Optiray 300<sup>TM</sup> (IVL labeled claim 636 mg/ml), Optiray 320<sup>TM</sup> (IVL labeled claim 678 mg/ml), Optiray 350<sup>TM</sup> (IVL labeled claim 741 mg/ml)<sup>7</sup>. The over dosage of IVL have an effect mostly on the cardiovascular and pulmonary system, and are life threatening. IVL injections are to be used in prescribed dosages and those dosages should have exact content as mentioned in label claim. The over dosages will show adverse effects as mentioned above. Therefore, a reliable, simple, precise and accurate assay method is necessary to assay IVL content in injection.

Potentiometric titration method to estimate IVL is official in United States Pharmacopeia<sup>8</sup>. The method uses silver-silver chloride reference electrode and silver billet electrode and involves titration of IVL sample solution with 0.05 N silver nitrate in acidic media (2 N sulfuric acid). Liquid chromatography technique offers more specificity, sensitivity, precise and accuracy in comparison to potentiometry<sup>9</sup>. A stability indicating method quantifies accurately and precisely the active pharmaceutical ingredients with no interference from excipients, process impurities and degradation products. The major objective of a stability indicating assay method is to observe results throughout the stability studies so as to assure safety, efficacy and quality<sup>10,11</sup>.

In this investigation, a simple, sensitive, precise and accurate stability indicating high performance liquid chromatographic method with diode array detector is developed and validated for the assay of IVL in bulk and injection forms. Validation parameters analyzed

include system suitability, linearity, selectivity, specificity, precision, accuracy, robustness, limits of detection and quantitation<sup>12,13</sup>.

## **Experimental**

### ***Materials***

Reference standard IVL sample was gifted by M/S Jodas Expoin Pvt. Ltd, Hyderabad, India. Commercial injections of Optiray 240<sup>TM</sup> injection dosage with labeled claim 509 mg/ml of IVL (manufactured by Mallinckrodt Inc., Hazelwood, USA) was obtained from the pharmacy market in Hyderabad, India. Methanol (HPLC Grade, Merck (India) Ltd., Mumbai, India), hydrochloric acid (analytical reagent grade, Sd. Fine Chemicals Ltd, Mumbai, India), sodium hydroxide (analytical reagent grade, Sd. Fine Chemicals Ltd, Mumbai, India) and hydrogen peroxide (analytical reagent grade, Sd. Fine Chemicals Ltd, Mumbai, India) were purchased. Water used in the investigation is produced by Milli-Q system (Millipore, MA, USA). Samples and solutions were filtered through a 0.45 µm membrane filter (Thermo Fisher Scientific India Pvt. Ltd. Mumbai, India).

For assay of IVL, HPLC system (model 1260 Infinity series, Agilent, USA) was equipped with a degasser quaternary pump, autosampler, 50 µl injector loop, column oven and diode array detector (DAD). HPLC system was controlled by Open Lab software. The separation and analysis of IVL was done in Zodiac phenyl C 18 column (250 mm × 4.5 mm; 5 µ particle size).

### ***Chromatographic conditions***

Mobile phase contains water and methanol mixture (90:10, v/v), with an isocratic elution at flow rate of 1.0 ml/min. The other conditions were: Detector wavelength 254 nm; Run time 10 min; Column temperature 35°C; Injection volume 50 µl.

### ***Stock and working standard solutions***

Stock standard of concentration (5090 µg/ml) was prepared by dissolving 509 mg IVL in mobile phase (100 ml). From stock standard solution, working standard solutions having concentrations 254.5 µg/ml, 381.75 µg/ml, 509 µg/ml, 610.8 µg/ml, 763.5 µg/ml were prepared through dilution with mobile phase.

### ***Calibration curve construction***

Calibration solution standards were prepared from stock solution (5090 µg/ml) to get IVL concentration range of 254.5 to 763.5 µg/ml (i.e., 50% to 150% of label claim). The peak area response of IVL at each concentration was measured following described chromatography conditions. IVL calibration curve was prepared by plotting IVL peak area versus IVL concentration. Also regression equation was calculated using calibration data.

### ***Injection Sample***

A volume of injection dosage form equivalent 509 mg of IVL was accurately measured and settled to a volumetric flask (100 ml) and ultrasonically extracted using 30 ml of mobile phase for 20 min. The resulting solution was diluted to 100 ml and filtered through 0.45 µm membrane filter. The prepared stock injection sample has a concentration of 5090 µg/ml IVL. This stock injection sample was diluted into working test concentration of 509 µg/ml IVL and used for the chromatographic analysis. The IVL content in injection was determined using calibration curve or regression equation.

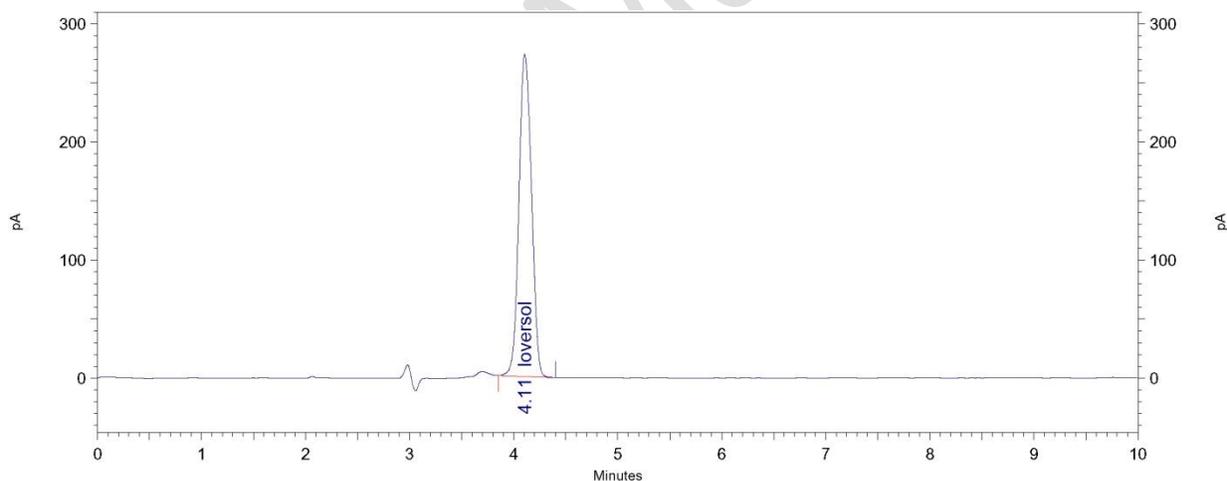
### ***Stress degradation study of IVL***

IVL stress degradation study was executed according to guidelines of ICH Q1A (R2)<sup>14</sup>. Degradation study was performed with injection stock sample solution (10 ml) with different solvents: 10 ml of water (neutral degradation), 10 ml of 0.1 N hydrochloric acid (acid degradation), 10 ml of 0.1 N sodium hydroxide (base degradation) and 10 ml of 3% H<sub>2</sub>O<sub>2</sub> (oxidative degradation). All samples, except neutral degradation, were subjected to chosen stress conditions for 24 hr at room temperature. For neutral degradation, the sample was treated for 3 hr at 60°C. For photolytic study, the sample solution (10 ml of injection stock solution) was exposed to UV light for 24 hr. The samples of acid and base degradation were neutralized with 0.1 N NaOH and 0.1 N HCl, respectively. After degradation, all the samples were diluted (100 ml) with mobile phase to a test sample concentration of 509 µg/ml IVL and analyzed employing proposed method. The percentage assay, degradation and peak purity of IVL in all the stress conditions were determined.

## Results and discussion

### *Method development*

Several analytical columns (Waters symmetry C8, 250 × 4.6 mm, 5 $\mu$ m; Develosil C18, 250 × 4.6 mm, 5 $\mu$ m; Zodiac phenyl C18, 250 × 4.6 mm, 5  $\mu$ m) and mobile phase systems (water and methanol in different ratios and flow rates) were examined for developing a novel high performance liquid chromatographic method for the determination of IVL in bulk and injection dosage form. The apt analytical column, mobile phase composition, mobile phase flow rate and associated chromatographic conditions were determined taking into account the values of system suitability (peak area response, number of theoretical plates and peak tailing). Considering those parameters for the separation and analysis of IVL, it was concluded that optimum HPLC conditions consisting of a mobile phase containing water and methanol (90:10, v/v) with a flow rate of 1.0 ml/min, column temperature of 35°C, injection volume of 50  $\mu$ l and detection wavelength of 254 nm were extremely suitable to obtain the optimal elution of IVL. The IVL was eluted from the column with retention time 4.11 min and total run time for every sample was 10 min.



**Figure 2. Representative chromatogram of IVL after HPLC conditions optimization**

### *Assay method validation*

The developed method was validated for system suitability, specificity, linearity, sensitivity, accuracy, robustness, precision, and selectivity as per the guidelines set by ICH Q2 (R1)<sup>12</sup> and FDA<sup>13</sup>.

### ***System suitability***

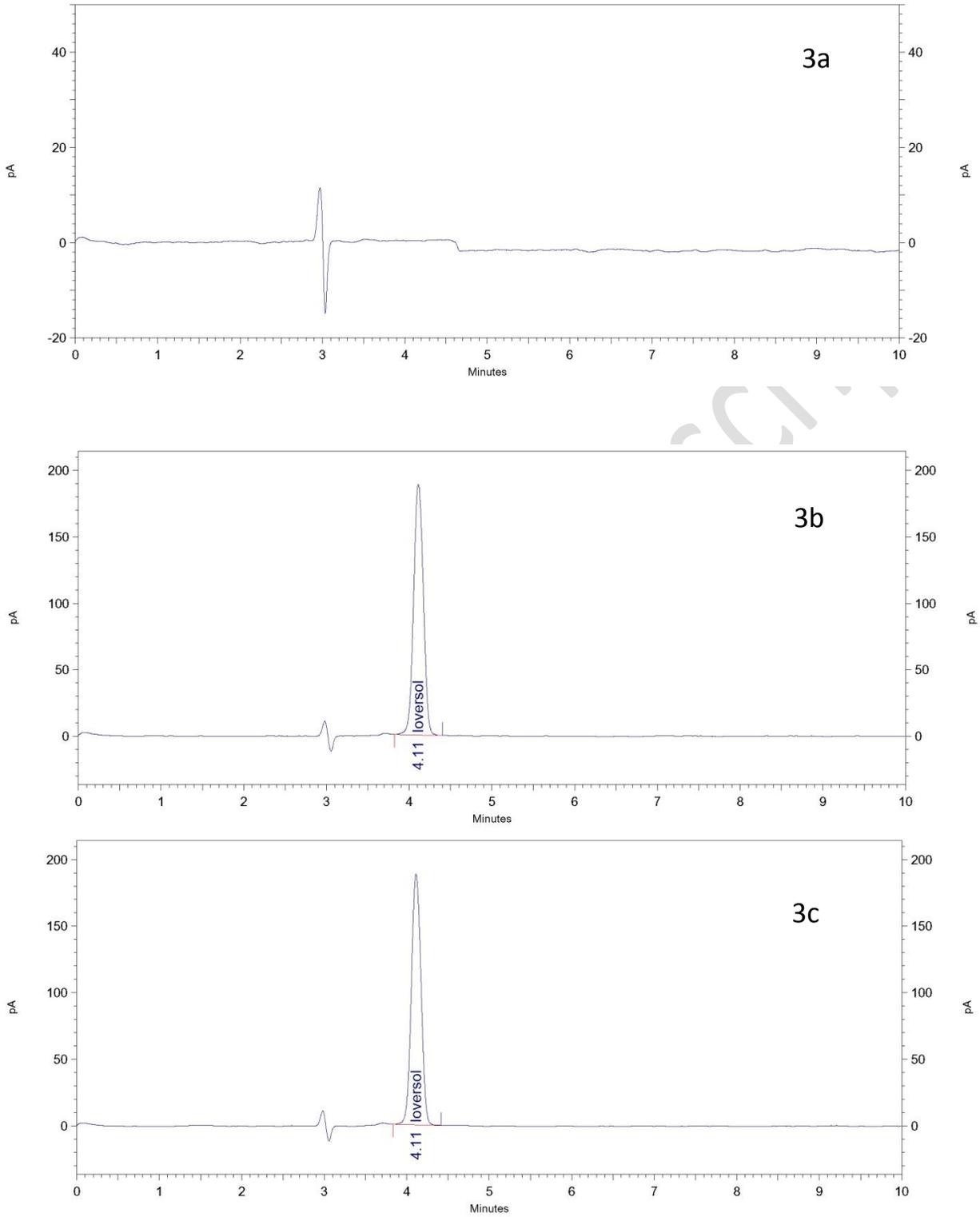
System suitability data was assessed based on the chromatograms of standard solution containing 509 µg/ml IVL. Results are acquired from 5 injections. Evaluation was by comparison with acceptance criteria. System suitability data are summarized in Table 1.

**Table 1. Data for System Suitability of IVL Standard Solution**

| <b>System suitability parameter</b> | <b>Value</b> | <b>Acceptance criteria<sup>12</sup></b>  |
|-------------------------------------|--------------|--|
| Tailing factor                      | 0.99         | Tailing factor of IVL peak should be not more than 2.0                                 |
| Theoretical plates                  | 7455         | Theoretical plates of IVL peak should be not less than 2000                            |
| %RSD of Peak area                   | 0.59         | Relative standard deviation should be not more than 2.0% obtained from five injections |

### ***Selectivity***

During the course of selectivity study, effect of excipients in injection dosage form and solvent composition of mobile phase on IVL peak response was analyzed<sup>15</sup>. Results are assessed based on the chromatograms of (Figure 3a) mobile phase blank (Figure 3b) IVL standard solution with concentration 509 µg/ml and (Figure 3c) IVL injection sample solution with concentration 509 µg/ml. The examined excipients and solvents did not give any detectable peak at the retention time of IVL ( $R_t = 4.11$  min). The retention time of IVL is same in both IVL standard ( $R_t = 4.11$  min) and injection sample ( $R_t = 4.11$  min) solutions. Hence the method is selective.



**Figure 3. Chromatogram of (a) Mobile phase blank (b) IVL standard solution,  $R_t = 4.11$  min (c) IVL injection sample solution,  $R_t = 4.11$  min**

### ***Calibration range***

Calibration range was determined by preparing IVL standard solutions at five concentrations from 254.5 to 763.5 µg/ml (50% to 150% of labeled claim), plotting a curve of IVL concentration against IVL peak area followed by determining the linearity by regression analysis. The developed method showed linear from 254.5 to 763.5 µg/ml for IVL ( $y = 11302892 x + 2253863$ ,  $y =$  IVL peak area and  $x =$  IVL concentration (µg/ml);  $R^2 = 0.9995$ ). Linear regression data was obtained at five levels of concentration (254.5, 381.75, 509, 610.8 and 763.5 µg/ml), from three parallel injections.

### ***Sensitivity***

The parameters to evaluate the method sensitivity are limit of detection (LOD) and limit of quantitation (LOQ)<sup>16</sup>. Signal to noise ratio method of 3:1 and 10:1 was employed to calculate LOD and LOQ, respectively. The determined LOQ is 2.3757 µg/ml and LOD is 0.7291 µg/ml for IVL. The values proved the sensitivity of method.

### ***Precision***

The method precision was done by preparing six replicate IVL standard solutions (509 µg/ml) and analyzed as per the described HPLC conditions<sup>17-19</sup>. The percent relative standard deviation of ILV peak area response was calculated. The values are found to be within the acceptance criteria (less than or equal to 2.0%). The relative standard deviation value is 0.54% for IVL. Hence the method is precise.

### ***Accuracy***

The method accuracy was done by preparing six replicate IVL standard solutions (509 µg/ml) and analyzed as per the described HPLC conditions<sup>20</sup>. The mean percent assay of ILV was determined. The values are found to be within the acceptance criteria (80% - 120%). The mean percent assay value is 100.70 % for IVL. Therefore, the method is accurate.

### ***Recovery test via standard addition technique***

For this test, pure IVL was spiked to preanalyzed injection sample at three levels of concentration (50%, 100% and 150% of labeled claim). The spiked samples were injected into

HPLC system (n=3) and analyzed following described HPLC conditions. The mean percent recovery was determined at each concentration level (Table 2). The values are within the acceptance criteria (80% - 120%). The percent recovery of IVL was 100.23% (at 50% spiked level), 101.30% (at 100% spiked level) and 99.93% (at 150% spiked level). The results indicated good recoveries with no obvious interference from excipients in injection dosage form. Hence the method is accurate and selective for the analysis of IVL.

**Table 2. Data for recovery of IVL**

| <b>IVL label claim (mg)</b> | <b>Spiked (mg)</b> | <b>Value assayed (mg)*</b> | <b>Recovery (%)**</b> |
|-----------------------------|--------------------|----------------------------|-----------------------|
| 509                         | 254.5              | 765.30                     | 100.24                |
| 509                         | 509                | 1031.22                    | 101.30                |
| 509                         | 763.5              | 1271.58                    | 99.93                 |

\* mean value of three assays; \*\* mean of three recoveries

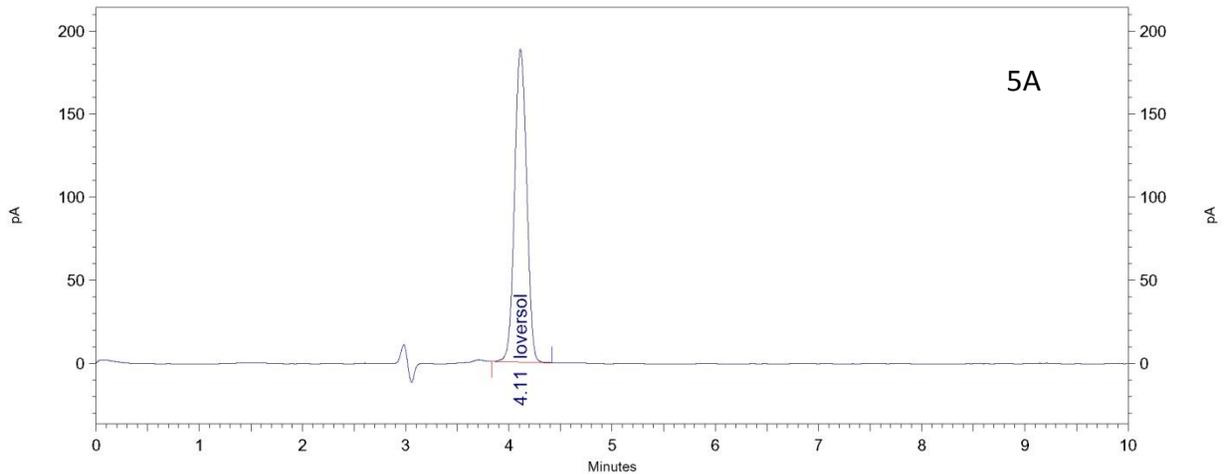
#### ***Stress degradation study of IVL***

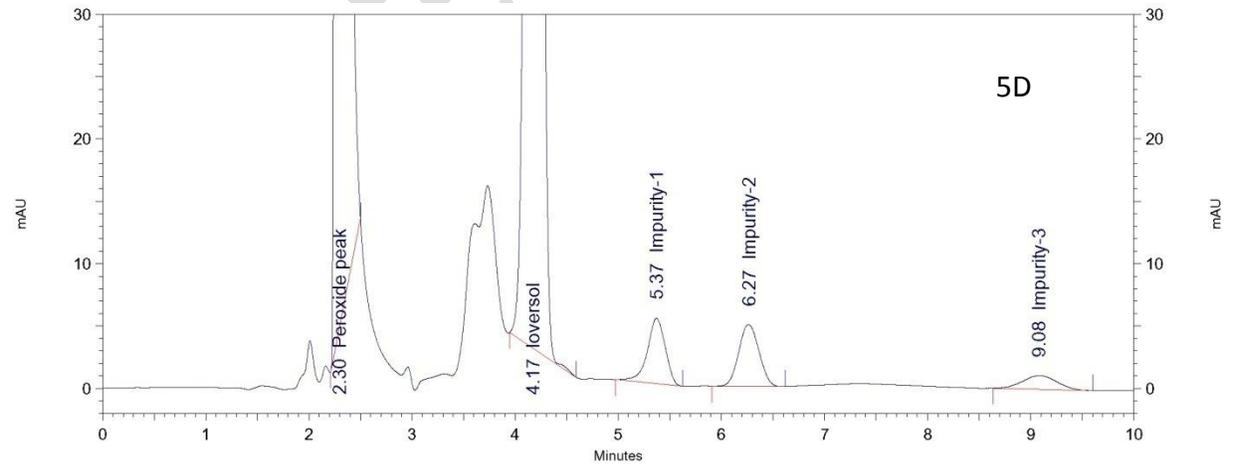
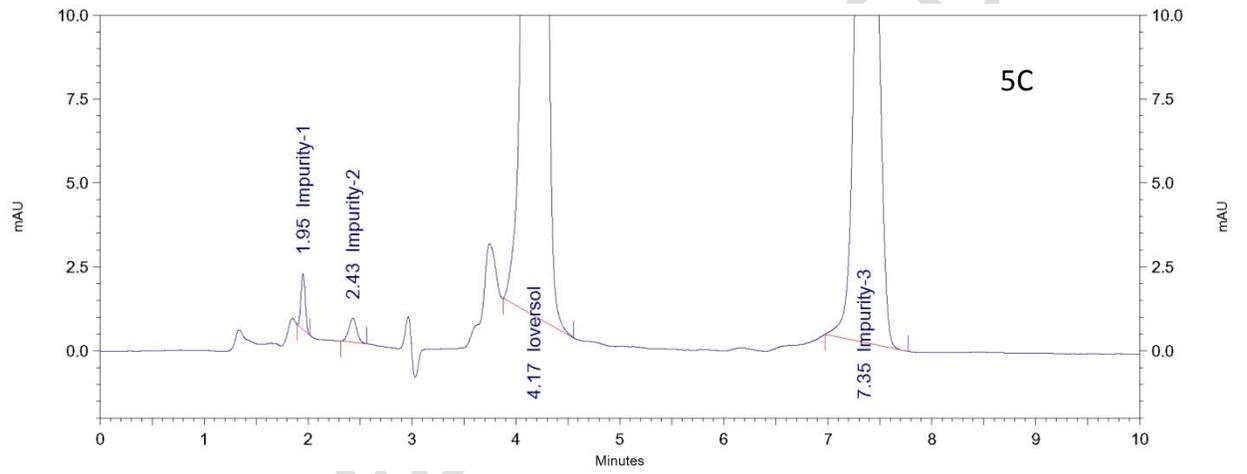
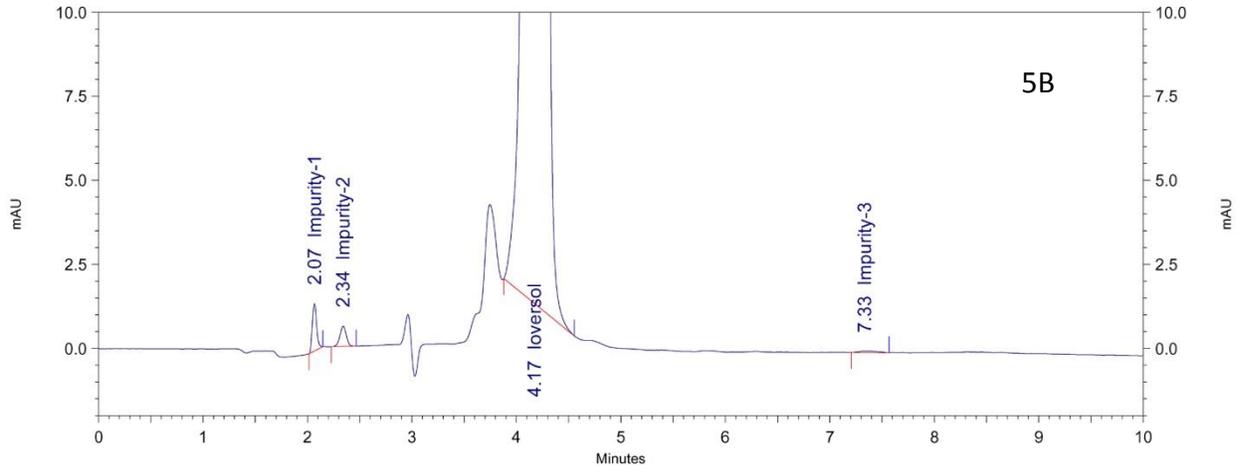
The study involves assessing the effect of acid (0.1N HCl, 24 hr at room temperature), base (0.1N NaOH, 24 hr at room temperature), water (3 hr at 60°C temperature), hydrogen peroxide (3%, 24 hr at room temperature) and UV light (7 hr) on IVL injection samples. The chromatograms obtained from various stress conditions are shown in Figure 5A–F. The percent assay, percent degradation and peak purity of IVL and retention time of degradants produced in all stress conditions are determined and summarized in Table 3. IVL was found to be more stable in applied water and acid stress conditions. IVL was sensitive to adopted stress conditions like photo, base and oxidation. The results proved that the developed assay method has good selectivity and specificity, and is suitable for assay of IVL in the presence of stress degradation products.

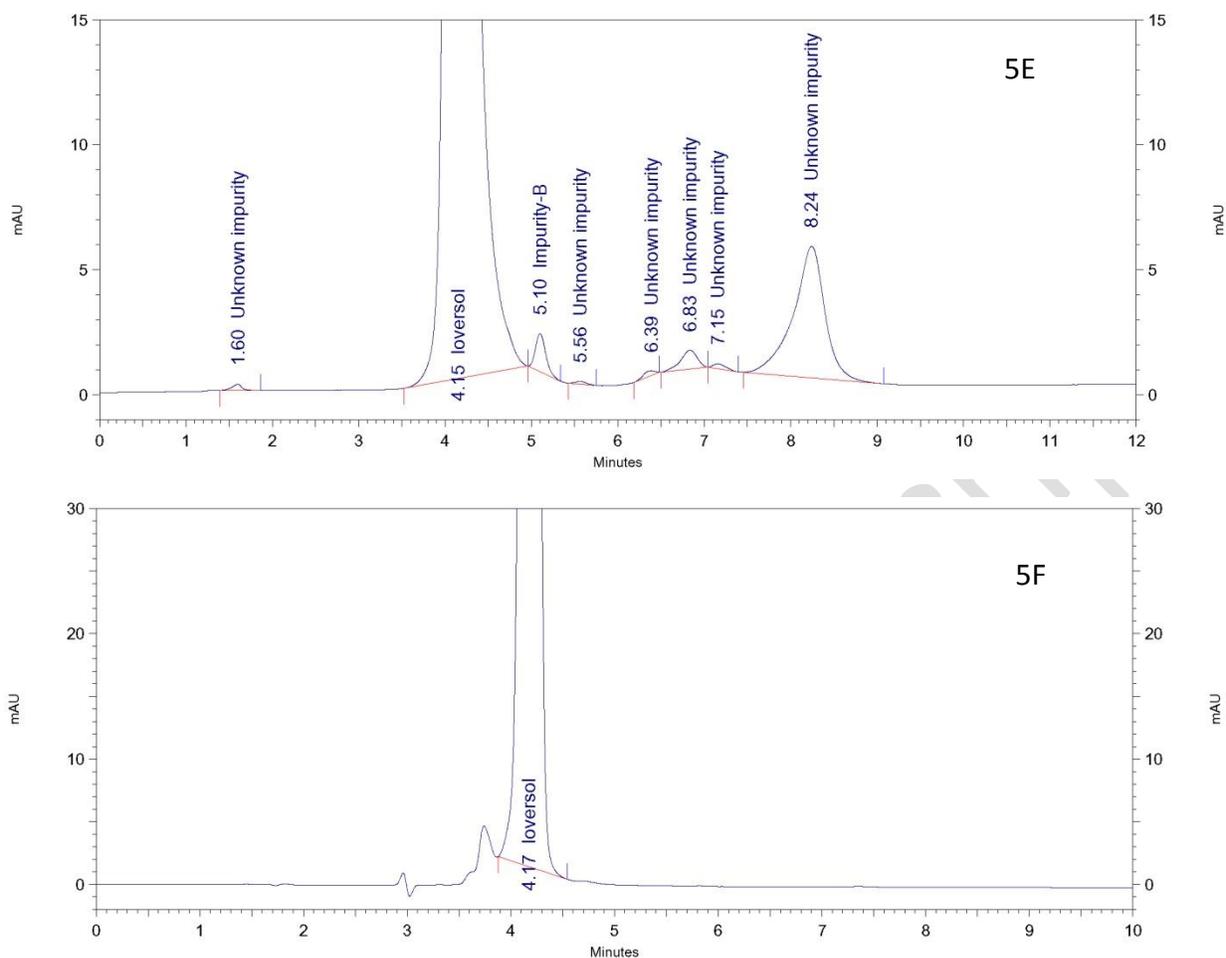
**Table 3 Summary of degradation of IVL under various stress conditions**

| Degradation condition         | Assay (%) | Degradation (%) | Peak purity    |                     | Rt of degradants (min)                    |
|-------------------------------|-----------|-----------------|----------------|---------------------|---|
|                               |           |                 | Obtained value | Acceptance criteria |   |
| Non degraded sample           | 100.70    | NA              | 1.00           | $\geq 0.99$         | NA  |
| Base degradation              | 86.18     | 13.82           | 1.00           | $\geq 0.99$         | 1.95, 2.43 & 7.35                         |
| Acid degradation              | 99.70     | 0.30            | 1.00           | $\geq 0.99$         | 2.07, 2.34 & 7.33                         |
| Water degradation             | 99.92     | 0.08            | 1.00           | $\geq 0.99$         | -   |
| Hydrogen Peroxide degradation | 87.20     | 12.80           | 1.00           | $\geq 0.99$         | 5.37, 6.27 & 9.08                         |
| Photo degradation             | 85.17     | 14.83           | 1.00           | $\geq 0.99$         | 1.60, 5.10, 5.56, 6.39, 6.83, 7.15 & 8.24 |

NA = not available; Rt = retention time







**Figure 5. Chromatograms taken after stress degradation of IVL injection sample: IVL standard solution (A); IVL under acid condition (B); IVL under base condition (C); IVL under oxidative condition (D); IVL under photolytic condition (E); IVL under water degradation (F)**

***Robustness test:***

Robustness test was done to demonstrate the influence of deliberate varying experimental conditions such as mobile phase components ratio ( $\pm 5\%$ ), flow rate ( $\pm 0.1$  mL/min), column oven temperature ( $\pm 2^\circ\text{C}$ ) and detection wavelength ( $\pm 2$  nm) on the performance of proposed. The test was carried out with IVL standard solution ( $509 \mu\text{g/mL}$ ). In each varying condition, peak area and their relative standard deviation values were calculated. The determined relative standard values are less than 2%. The calculated values were within the acceptance limits (Table 4). Therefore the method is proved as robust.

**Table 4. Data for robustness of IVL**

| <b>Parameter</b>                        | <b>Value tested</b> | <b>Peak area</b> | <b>RSD (%)</b> |
|---|---------------------|------------------|----------------|
| Mobile phase ratio (water and methanol) | 85:15               | 1118902794       | 0.383          |
|   | 90:10               | 1111521891       |                |
|   | 95:05               | 1118960734       |                |
| Flow rate (mL/min)                      | 0.9                 | 1141916388       | 1.540          |
|   | 1.0                 | 1111521891       |                |
|   | 1.1                 | 1112455179       |                |
| Column temperature (oC)                 | 33                  | 1126390982       | 0.910          |
|   | 35                  | 1111521891       |                |
|   | 37                  | 1106970632       |                |
| Detection wavelength (nm)               | 252                 | 1123135125       | 0.547          |
|   | 254                 | 1111521891       |                |
|   | 256                 | 1114020384       |                |

***Method application for the assay of IVL in injection***

The developed method was applied for the determination of IVL in injection formulations obtainable in the local market. The test injection sample was prepared at a concentration of 509 µg/ml and analyzed by the proposed method three times. The percent recovery and relative standard deviation of IVL was calculated by determining their contents from the IVL chromatograms. The mean percent recovery and relative standard deviation of IVL was found to be 100.49% and 0.715% (Table 5). The obtained values proved the accuracy and precision of proposed method for the assay of IVL in injection formulation.

**Table 5. Assay of IVL in injection formulation**

| <b>IVL Label claim</b> | <b>Assay value</b> | <b>Recovery (%)</b> | <b>Mean recovery (%)</b> | <b>RSD (%)</b> |
|------------------------|--------------------|---------------------|--------------------------|----------------|
| 509                    | 510.20             | 100.24              |                          |                |
| 509                    | 515.61             | 101.30              | 100.49                   | 0.715          |
| 509                    | 508.63             | 99.93               |                          |                |

**Conclusion**

A simple and rapid HPLC-DAD method was developed and validated for the assay of IVL in pure and injection dosage form with good linearity, selectivity, sensitivity, accuracy, and precision. The content of IVL in the pharmaceutical product tested (Optiray 240<sup>TM</sup> injection, 509 mg IVL) was assayed three times and found to be 100.49% ± 0.715. In addition, the stability of

IVL was assessed under acid, base, water, oxidative and photo degradation. The order of stability of IVL in the applied conditions was water > acid > oxidation > base > photo. The adaptability of the developed method to injection dosage form was proved by its good performance in terms of selectivity, specificity and recovery of IVL in the injection sample.

### Conflicts of interest

None

### Acknowledgements

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