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# A SENSITIVE AND RELIABLE ANALYTICAL METHOD FOR ESTIMATION OF OBETICHOLIC ACID IN TABLET DOSAGE FORM BY USING DIFFERENT MODES OF HPLC DETECTORS

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## ABSTRACT

A Specific, precise, and accurate RP-HPLC method has been developed and validated for the quantitative analysis of Obeticholic acid in tablet formulation by using refractive index detector and UV detector. An isocratic separation was achieved in RI detector using an Inertsil ODS 3V, 150 x 4.6mm; 5µ column with a flow rate of 1.2ml/min and Mobile phase pH 3.0 formic acid Buffer: Acetonitrile (30:70% v/v). An isocratic separation was achieved in UV detector using Cortecs C18$^+$, 150 x 4.6mm; 2.7µ column with 0.7ml/min flow rate at 192nm. Isocratic elution was performed with Mobile phase A and mobile phase B as 0.05% OPA Buffer and Acetonitrile respectively in the ratio of 45:55% v/v. These methods were validated for specificity, linearity, precision, accuracy and robustness. These methods were linear over the concentration range 125-1000ppm and 240-1500PPM ($r^2$ = 1.0) using RI and UV detectors respectively. The accuracy of the methods was between 98.1-101.7%. These methods were found to be Robust and suitable for the quantitative analysis of Obeticholic acid in a tablet formulation.

## KEYWORDS

Obeticholic acid, Non chromophoric molecule, Refractive Index detector, UV detector and RP-HPLC.

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## INTRODUCTON

Chenodeoxycholic acid is a natural bile acid which was identified as the most active physiological ligand for farnesoid X receptor (FXR). Obeticholic acid ((3α, 5β, 6α, 7α)-6-Ethyl-3, 7-dihydroxycholan-24-oic acid) is a novel semi synthetic bile acid analogue. OBT is the most potent FXR agonist. The presence of ethyl group in 6-alpha position allows to increase the activity of FXR. Obeticholic acid is a non chromophoric

molecule. It has low sensitivity towards uv region due to lack of strong chromophores in its molecule. It is detected at 192nm which results in decreased selectivity of detection. The carboxylic group increases the sensitivity of detection. Typical structure of obeticholic acidis shown in Figure No.1

During literature survey it was observed that the published methods for related substances of Obeticholic acid API by HPLC-UV[1] were not precise showing fluctuations in retention time as well as placebo interference for Obeticholic acid tablet formulation.

Hence the objective of the present work is to develop a new isocratic assay method for obeticholic acid tablet formulations by using the cortecs $C18^+$ columns are with high efficiency based on a solid-core particle that are designed to deliver excellent peak shape for at low pH at low wavelength UV detection by RP-HPLC. On same way estimation of Obeticholic acid by RI-HPLC detection was also developed as Obeticholic acid (as its non-chromophoric properties) for Obeticholic acid formulations.

## METHODS AND REQUIREMENTS

### Method A: Method Development Using Refractive Index Detector

#### Chemicals and reagents

HPLC grade Acetonitrile was purchased from Rankem (Hyderabad, India). Emparta grade formic acid was purchased from Merck Specialties PVT. Ltd, Mumbai, India. Buffers for the mobile phase were prepared with Milli Q (Millipore, Milford, MA, USA) grade water. Analytical standard obeticholic acid was purchased from Virupaksha organics limited, Hyderabad, India.

#### Chromatographic conditions

HPLC Waters alliance separation module 2695 series equipped with a quaternary pump, cooled auto sampler, column oven and Refractive index detector of 2410 series was used for method development of obeticholic acid. In addition to this electronic balance, microliter syringe, pipettes, ultrasonic apparatus and0.45µm nylon syringe filter filters were also used.

Chromatographic optimization was achieved by analytical column Inertsil ODS 3V, 150 x 4.6mm; 5µm using isocratic mobile phase as pH 3.0 formic acid Buffer: Acetonitrile (30:70% v/v) at the flow rate of 1.2mL/min. Column temperature and detector temperature was maintained at 40°C.

#### Preparation of solutions

#### Mobile phase preparation

pH 3.0 formic acid buffer: To 2000mL of Milli-Q water, formic acid was added drop wise to adjust the pH to 3.0±0.05. Filtered through 0.45 µ membrane filter.Mixed pH 3.0 formic acid buffer and Acetonitrile in the ratio 30:70 v/v respectively and sonicated.

#### Standard solution (0.5mg/ml)

Weigh and transfer accurately 25mg of obeticholic acid working standard in to a 50ml volumetric flask, add 30ml of Diluent(90% v/v acetonitrile) and sonicate if required, make up to the volume with diluent.

#### Sample solution: (0.5mg/ml)

10 tablets were transferred into 200mL flask for 10mg strength and 10 tablets were transferred to 100mL flask for 5mg strength. 10% of milli-Q water was added to remove the coating of tablets. Add about 60% of acetonitrile, sonicate for 45minutes with continuous shaking by maintaining the sonication temperature below 30°C. Make up the volume acetonitrile and mix. Filter through 0.45µm nylon syringe filter discarding initial 5mL of filtrate.

### Method B: Method Development Using UV Detector

#### Chemicals and reagents

HPLC grade Acetonitrile and analytical grade of Ortho phosphoric acid were purchased from Rankem (Hyderabad, India). Analytical standard obeticholic acid was produced from Virupaksha organics limited, Hyderabad, India.

#### Chromatographic conditions

HPLC Waters alliance separation module 2695 series equipped with an auto sampler, quaternary pump, column oven and UV detector of 2489 series was used for method development of obeticholic acid. In addition to this electronic balance,

microliter syringe, pipettes, ultrasonic apparatus and 0.45µm nylon syringe filter filters were also used.

Chromatographic optimization was achieved by isocratic elution technique using analytical column Cortecs C18$^+$, 150 x 4.6mm; 2.7µm using mobile phase A as 0.05% OPA Buffer and mobile phase B as Acetonitrile at the flow rate of 0.7mL/min. Column was maintained at 45°C. The UV detection was done at 192nm.

**Preparation of solutions**

**Standard solution (0.5mg/ml)**

Weigh and transfer accurately 50mg of obeticholic acid working standard in to a 100ml volumetric flask, add 70ml of diluent(80%v/v acetonitrile) and sonicate if required, make up to the volume with diluent.

**Sample solution: (0.5mg/ml)**

Due to lower strengths of Obeticholic acid tablets (5mg and 10mg) minimum 10 tablets were selected to prepare the sample preparation for both the strengths of Sample concentrations to equalize with standard concentration. 10 tablets were transferred into 200mL flask for 10mg strength and 10 tablets were transferred to 100mL flask for 5mg strength. 20% of milli-Q water was added to remove the coating of tablets. Add about 50% of acetonitrile, sonicate for 45 minutes with continuous shaking by maintaining the sonication temperature below 30°C. Make up the volume acetonitrile and mix. Filter through 0.45µm nylon syringe filter discarding initial 5mL of filtrate.

**Validation of proposed methods**

The methods were validated for parameters like specificity, linearity, precision, accuracy and robustness as per ICH guidelines.

## RESULTS AND DISCUSSION

**Method A: Method Development Using Refractive Index Detector**

**Method development**

The method development was initiated in isocratic mode of HPLC at different compositions of mobile phases consisting of pH 3.0 formic acid buffer and acetonitrile (in the ratio range of 45:55%v/v to 30:70%v/v) and at different flow rate (in the range of 1.0–1.4mL/min) were tested for complete chromatographic estimation of obeticholic acid (data not shown). Chromatographic optimization was achieved by pH 3.0 formic acid buffer: Acetonitrile (30:70%v/v) as mobile phase with a flow rate of 1.2mL/min on an Inertsil ODS 3V, 150 x 4.6mm; 5µm column and was found to be suitable for the determination of response for obeticholic acid. The blank and standard solution Chromatograms were represented below in Figure No.2 and 3. System suitability parameter of the optimized method is given in Table No.1.

**Method Validation**

**Specificity**

Blank (diluent), Placebo and impurities spiked sample were injected and compared with as such sample; there were no peak interference observed in the blank, placebo and degraded sample at retention time of obeticholic acid peak. Descriptive impurities spike chromatogram was represented in Figure No.4.

**Linearity**

Five linear concentration of Obeticholic acid (125ppm to 1000ppm) were prepared and analyzed as per the method. Correlation coefficient was observed to be 1.000. Linearity is plotted as figured in Figure No.5.

**Precision (Repeatability)**

Precision was evaluated by carrying out six different samples preparation and the results were found to be within the limits of acceptance criteria. The results are summarized in Table No.2.

**Robustness**

The robustness for a method is performed by varying parameters like organic percentage in mobile phase, pH, column oven temperature, flow rate etc., and determining the effect on the results of the method. The results are summarized in Table No.3.

**Accuracy**

Accuracy is to validate the closeness of test results obtained by the analytical procedure to the true value. The accuracy should be established across the specified range of the analyte concentration. Samples are prepared by spiking known amounts of analyte at low, medium and high sample concentrations of the target assay to placebo. The

accuracy is then calculated from the test results as the percentage of analyte recovered from the samples. Recovery was found to be 98.9%, 100% and 99.5% at low, medium and High sample concentrations respectively for obeticholic acid.

**Method B: Method development using UV detector**

**Method development**

The method development was initiated in isocratic mode of HPLC at different compositions of mobile phases A and mobile phase B consisting of 0.05% OPA buffer and acetonitrile respectively and at different flow rate.

Based upon the trials, 0.05% OPA buffer and Acetonitrile were maintained at the ratio of 45:55%v/v and Cortecs $C18^{+}$, 150 x 4.6mm; 2.7µ column was finalized for the evaluation of Obeticholic acid in tablets. The blank and standard solution Chromatograms were represented below in Figure No.6 and 7. System suitability parameters of optimized method are represented in Table No.1.

**Method Validation**

**Specificity**

Blank (diluent), Placebo and impurities spiked sample were injected and compared with as such sample; there were no peak interference observed in the blank, placebo and degraded sample at retention time of obeticholic acid peak. Descriptive Impurities spiked sample chromatogram was represented in Figure No.8.

**Linearity**

Five linear concentration of Obeticholic acid (240ppm to 1500ppm) were prepared and analysed as per the method. Correlation coefficient was observed to be 1.000. Linearity is plotted as figured in Figure No.9.

**Precision (Repeatability)**

Precision was evaluated by carrying out six different samples preparation and the results were found to be within the limits of acceptance criteria. The results are summarized in Table No.2.

**Robustness**

The robustness for a method is performed by varying parameters like organic percentage in mobile phase, pH, column oven temperature, flow rate etc., and determining the effect on the results of the method. The results are summarized in Table No.3.

**Accuracy**

Accuracy is to validate the closeness of test results obtained by the analytical procedure to the true value. The accuracy should be established across the specified range of the analyte concentration. Samples are prepared in duplicate (n=2) by spiking known amounts of analyte at 50%, 100%, 150%, 200% and 300% of target sample concentration levels. The accuracy is then calculated from the test results as the percentage of analyte recovered from the samples. % average Recovery was found to be 100.6%, 101.7%, 100.5%, 99.6% and98.1% at 50%, 100%, 150%, 200% and 300% of target sample concentrations respectively for obeticholic acid.

**Table No.1: System suitability parameters for Obeticholic acid**

| S.No | System Suitability | Evaluation Parameters | | | | |
|---|---|---|---|---|---|---|
| | | Area | Retention time | Tailing factor | Plate count | %RSD |
| 1 | OBT Standard by HPLC-RI | 2868297 | 5.882 | 1.1 | 4597 | 0.4 |
| 2 | OBT Standard by HPLC-UV | 863584 | 12.172 | 1.0 | 12546 | 0.1 |

**Table No.2: Precision data of Obeticholic acid**

| S.No | Sample | % Assay obtained by HPLC-RI | % Assay obtained by HPLC-UV |
|---|---|---|---|
| 1 | Sample -1 | 96.7 | 100.0 |
| 2 | Sample -2 | 95.0 | 100.0 |
| 3 | Sample -3 | 96.5 | 99.3 |
| 4 | Sample -4 | 95.6 | 99.5 |
| 5 | Sample -5 | 96.4 | 99.1 |
| 6 | Sample -6 | 94.2 | 99.3 |
| 7 | Average | 95.7 | 99.5 |
| 8 | % RSD | 1.0 | 0.4 |

**Table No.3: Robustness data of Obeticholic acid**

| S.No | Condition variations | Difference from initial condition | % RSD obtained by HPLC-RI | % RSD obtained by HPLC-UV |
|---|---|---|---|---|
| 1 | Column Temperature | +5°C | 0.7 | 0.2 |
| | | -5°C | 0.5 | 0.3 |
| 2 | Detector Temperature | +5°C | 0.7 | NA |
| | | -5°C | 0.5 | NA |
| 3 | Organic Phase | +10% | 0.4 | 0.3 |
| | | -10% | 0.3 | 0.5 |
| 4 | Flow rate | +0.2 | 0.6 | 0.2 |
| | | -0.2 | 0.4 | 0.4 |
| 5 | pH | +0.2 | 0.3 | NA |
| | | -0.2 | 0.2 | NA |

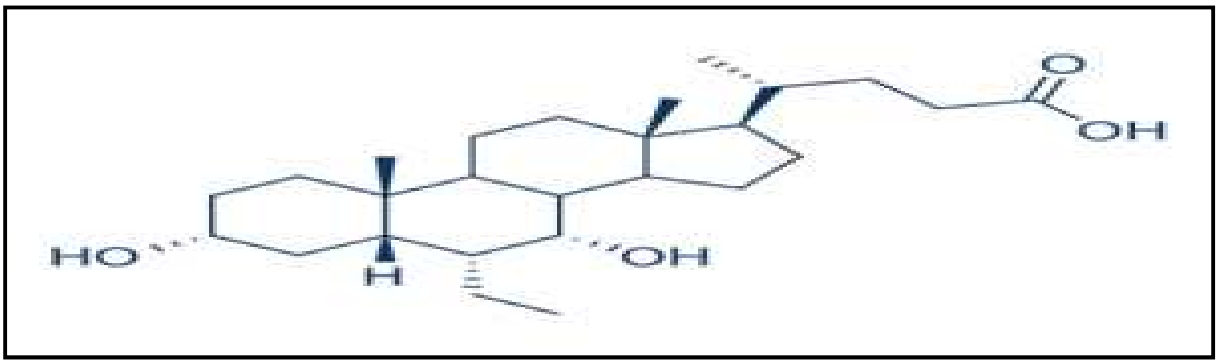


**Figure No.1: Structure of Obeticholic acid**

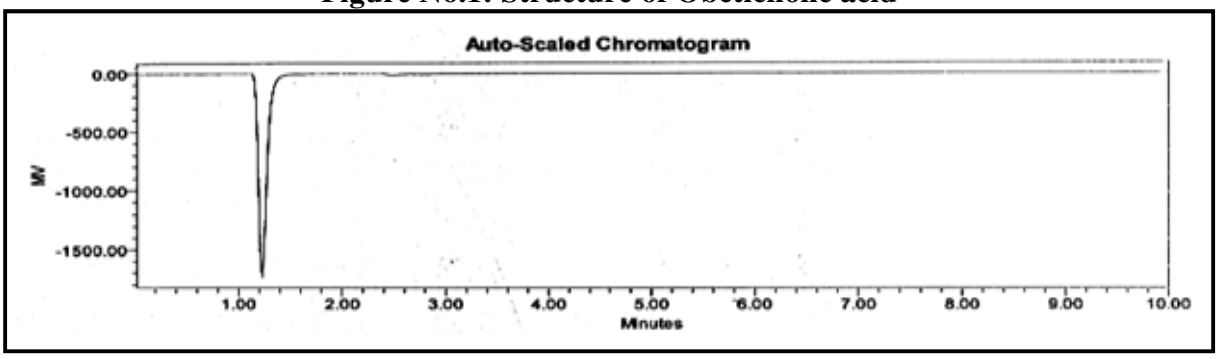


**Figure No.2: Typical chromatogram of Blank by HPLC-RI**

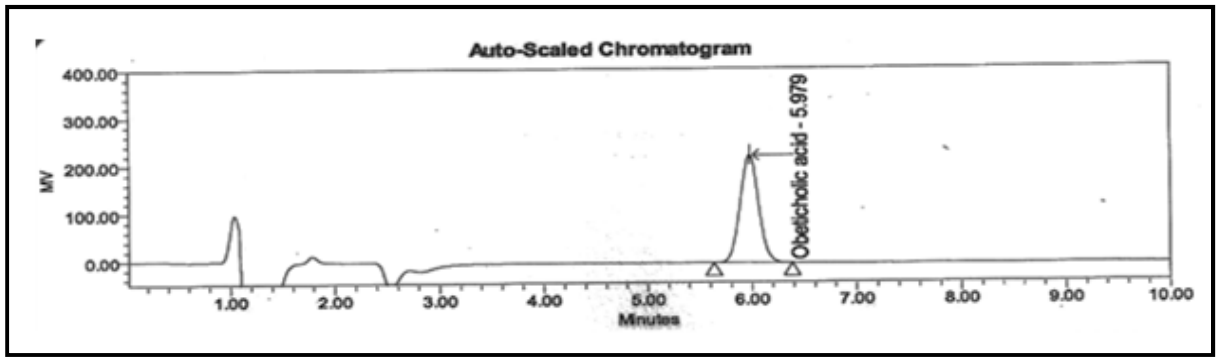


**Figure No.3: Typical chromatogram of Standard by HPLC-RI**

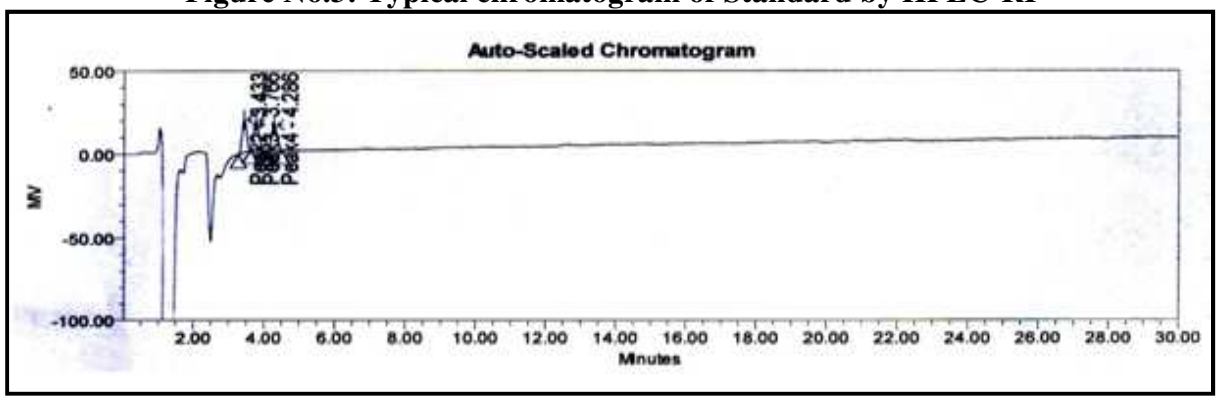


**Figure No.4: Typical chromatogram of impurity spiked sample by HPLC-RI**

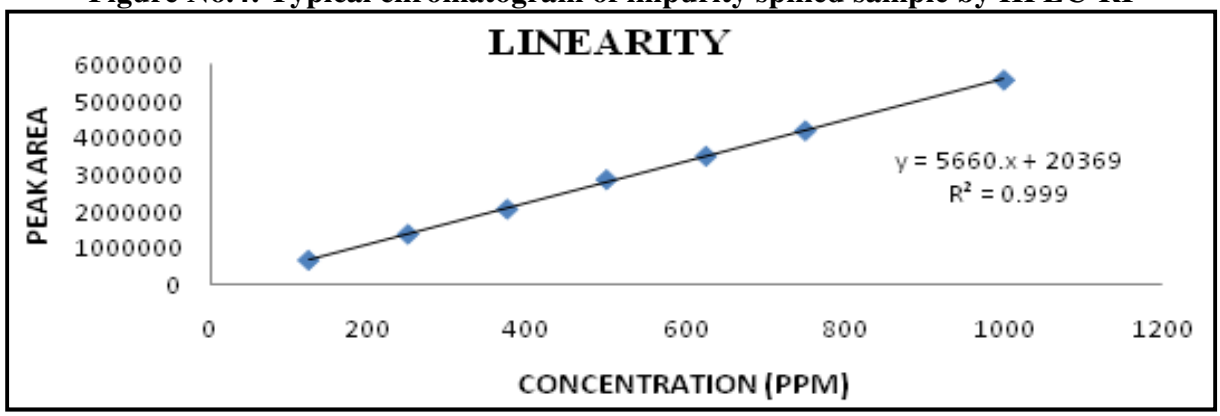


**Figure No.5: Linearity Graph of Obeticholic acid by HPLC-RI**

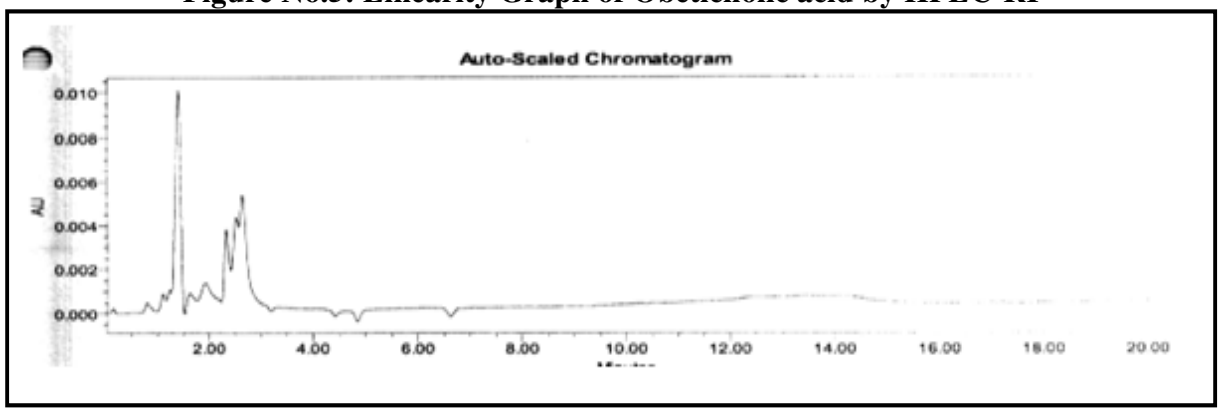


**Figure No.6: Typical chromatogram of Blank by HPLC-UV**

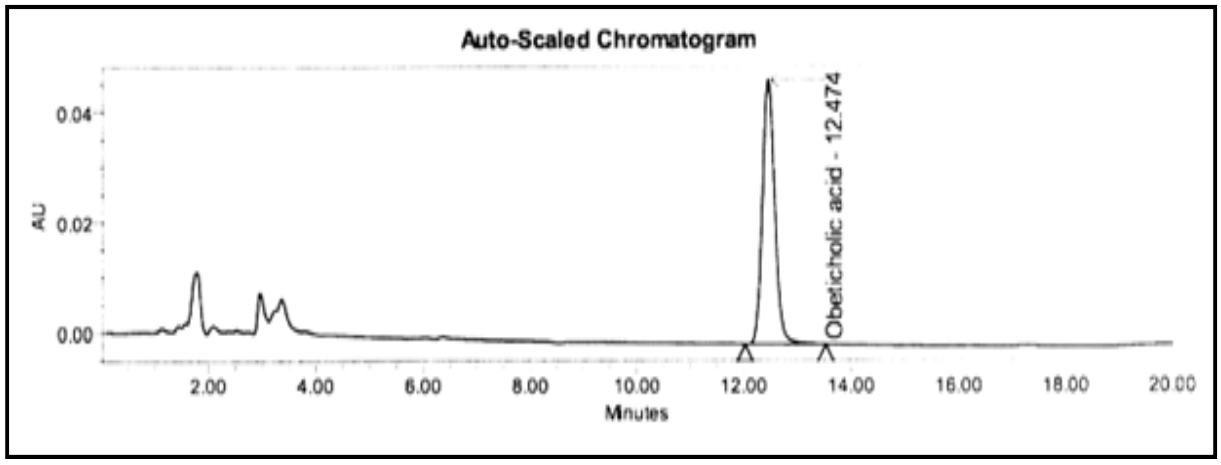


**Figure No.7: Typical chromatogram of Standard by HPLC-UV**

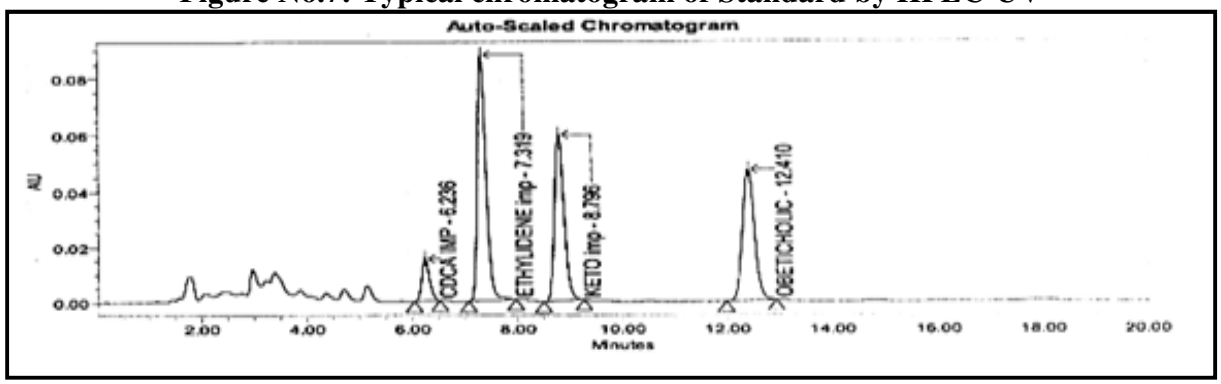


**Figure No.8: Typical chromatogram of impurity spiked sample by HPLC-UV**

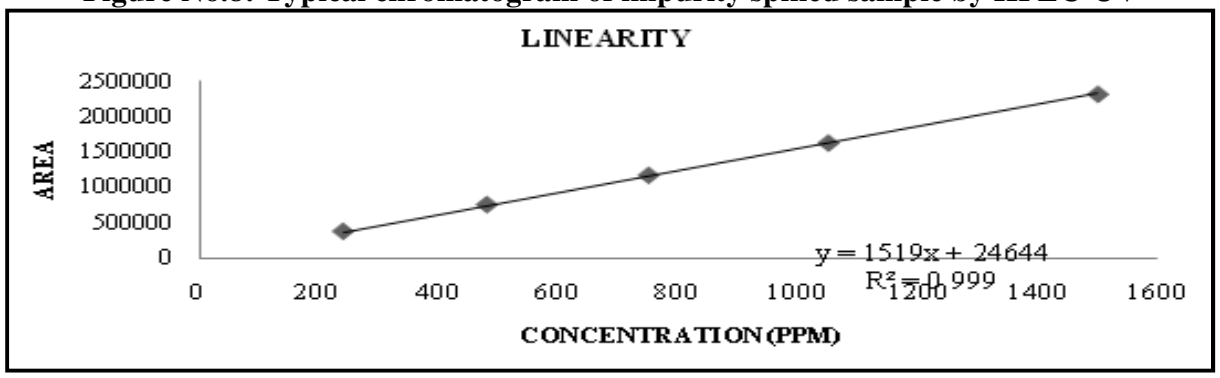


**Figure No.9: Linearity Graph of Obeticholic acid by HPLC-UV**

## CONCLUSION

A specific, accurate and reproducible Isocratic reverse phase HPLC method was developed for the estimation of Obeticholic acid in Tablet formulations by using RI and UV detectors. The developed methods were optimized prior to validation studies in terms of stationary phase, mobile phase composition, and flow rate and column oven temperature. The developed methods were validated as per ICH Q2A (R1) guidelines. These methods were found to be specific, accurate, precise, linear, rugged and robust. However UV detection is more precise and accurate than RI detection with respect to characterisation for the peak purity of sample. These methods can be used for routine analysis of Obeticholic acid in its tablet formulation.

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**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

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