An Elsevier Indexed Journal ISSN-2230-7346

*Journal of Global Trends in Pharmaceutical Sciences*

# TO DEVELOP A UPLC METHOD FOR EVOLUTION AND COMPUTATION OF QUINAPRIL AND TOLCAPONE

**C.P. Poornitha*, Meruva Sathish Kumar, S. Marakatham, Kanduri Valli Kumari**

Department of Pharmaceutical Analysis and Quality Assurance, Malla Reddy Institute of Pharmaceutical Sciences, JNTUH, Medchal-Malkajgiri, Telangana

*Corresponding author E-mail:**poornitharoy@gmail.com**

**ARTICLE INFO**

**Key Words**
**Quinapril, Tolcapone, UHPLC**

**ABSTRACT**

A simple and exact fast technique has been produced for the concurrent estimation of Quinapril and Tolcapone in pharmaceutical measurement shape by RP-UHPLC.The ideal wavelength for the assurance of Quinapril and Tolcapone was chose at 270 nm based on isobestic point.The Retention time of Quinapril and Tolcapone were observed to be 1.323 and 2.360 min separately. The adjustment bend was gotten by plotting top region versus the focus over the scope of 50-100 µg/mL For Tolcapone and25-75 µg/mL for Quinapril.The %RSD in the pinnacle region of medication was observed to be under 2%.The level of recuperation of Quinapril and Tolcapone were observed to be 99.9 and 99.8respectivelyshows that the proposed technique is exceptionally exact.

## INTRODUCTION

UPLC alludes to Ultra Performance Liquid Chromatography. UPLC gets emotional enhancements affectability, goals and speed of examination can be figured. It has instrumentation that works at high weight than that utilized in HPLC and in this framework utilizes fine particles(less than 2.5µm) and versatile stages at high straight speeds diminishes the length of segment, lessens dissolvable utilization and spares time.

### UPLC Column

Sections utilized for UPLC have been created and fabricated by the accompanying diverse organizations:

Waters: Acquity UPLC sections and Vanguard Pre-segments have been created. Agilgent innovation gives most astounding performing sections that give quick and reproducible outcomes. These incorporate Poroshell 120 sections, ZORBAX Rapid Resolution High definition segments, ZORBAX Eclipse in addition to segments and ZORBAX Rapid Reduction High Throughput segments.

**MATERIALS AND METHODS:** Quinparil and Tolcapone samples were obtained from Madras pharmaceuticals, Chennai, India. All solvents used were HPLC/AR grade. HPLC column of Phenomenex Cyano(50x2.1mm) 1.8µm was used.

**Instrumentation:** Analysis was carried out in UV-Visible Spectrophotometer with HPLC column Phenomenex

Cyano(50x2.1mm) 1.8µm.The ideal wavelength for the assurance of Quinapril and Tolcapone was chose at 270 nm based on isobestic point. A few preliminaries were performed with unique portable stages in divergent proportions, yet finally Ammonium acetic acid derivation Buffer pH 3.2: Acetonitrile (60:40) %v/v) was chosen as great pinnacle symmetry and goals between the pinnacles was observed.

**Preparation of Phosphate buffer pH 3.5:**

3.85 gm of Ammonium acetate was gauged and broke up in 1000 mL of water. Included 1mL of Triethylamine at that point Adjust the pH to3.2□0.02 utilizing weakened orthophosphoric corrosive. Cradle was separated through 0.45µm channels to expel every single fine molecule and gases.

**Preparation of Mobile Phase:** Prepared a mixture of buffer and Acetonitrile in the ratio of 60:40 (v/v). Mixed well. Sonicated for 10 min.

**Preparations for Methodology:**

**Preparation of standard stock solution:** About 10 mg of TOLCAPONE and 10mg of QUINAPRIL were weighed into a 50mL volumetric flask, to this 50 mL (200 µg/mL) of mobile phase was added,sonicated and the volume was made up to mark with the mobile phase.

***Dilutions:*** About 0.5 ml was transferred from standard stock solution (200 µg/mL) to get the concentration range of 10 µg/mL of TOLCAPONE and 10 µg/mL of QUINAPRIL. The wavelength of maximum absorption (λ max) of the solution of the drugs in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200– 400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 262 nm for TOLCAPONE (Fig.7.1), 249 nm for QUINAPRIL and at 270nm same absorbance for both the drugs (Fig.7.3), i.e., isobestic point. Thus 270nm was selected as detector wavelength for the HPLC chromatographic method.

**Method Validation:** About 100 mg of TOLCAPONE and 50mg of QUINAPRIL were weighed into a 100 mL volumetric flask (1000µg/mL TOLCAPONE and 500µg/mL of QUINAPRIL) , to this 70mL of mobile phase was added, sonicated and the volume was made up with the mobile phase. About 1 ml of standard was transferred into 100 mL volumetric flask and the volume was made up with the mobile phase (100µg/mL of TOLCAPONE) and About 1 ml of standard was transferred into 10 mL volumetric flask and the volume was made up with the mobile phase (50µg/mL of QUINAPRIL) **.**

**RESULT**

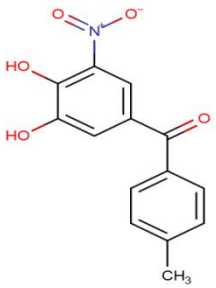
The plate check and following component results were observed to be attractive and are observed to be inside the limit. The % RSD was observed to be 0.58.

**Accuracy:** Accuracy of the method was determined by Recovery studies. To the formulation (pre-analysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug.

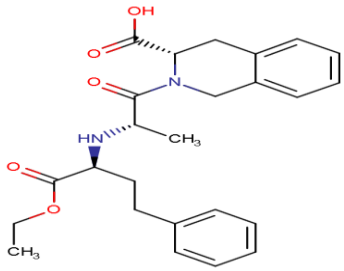
**Sample stock preparation:** Crush more than 20tablets then weigh a quantity of powder equivalent to 100mg of TOLCAPONE and 50mg of QUINAPRILin 100 mL volumetric flask and add70mL of mobile phase then sonicated it for 30min intermittent shacking after 30 min make up volume with mobile phase.

| Attribute | HPLC | UHPLC |
|---|---|---|
| Pressure in psi | 6000 | 100,000 |
| Particle Size (µm) | 5 | 1.7 |
| Flow Rate | ml/min | Ltr/min |
| Max Resolution | Relatively low | Relatively high |

- **DRUG NAME: TOLCAPONE**
- **Chemical structure:**



**Molecular Formula:** $C_{14}H_{11}NO_5$



**Chemical structure of quinapril**
**Molecular Formula: $C_{25}H_{30}N_2O_5$**

Table 1: Results for system suitability for drug TOLCAPONE

| Injection | Retention Time | Peak area | Theo. plates (TP) | Tail. factor (TF) |
|---|---|---|---|---|
| 1 | 1.323 | 14561651 | 3151 | 1.26 |
| 2 | 1.320 | 14256145 | 3145 | 1.24 |
| 3 | 1.320 | 14328763 | 3166 | 1.25 |
| 4 | 1.320 | 14370761 | 3130 | 1.30 |
| 5 | 1.320 | 14303452 | 3152 | 1.27 |
| 6 | 1.320 | 14303935 | 3146 | 1.27 |
| Mean | 1.321 | 14353924 | - | - |
| SD | 0.001 | 108334 | - | - |
| %RSD | 0.1 | 0.8 | - | - |

Table 2: Results for system suitability for drug TOLCAPONE

Table 10: Results for system suitability of drug QUINAPRIL.

| Injection | Retention time | Peak area | Theoretical plates | Tailing factor | Resolution |
|---|---|---|---|---|---|
| 1 | 2.360 | 18999753 | 6087 | 1.14 | 9.3 |
| 2 | 2.350 | 18792158 | 6045 | 1.16 | 9.6 |
| 3 | 2.343 | 18681457 | 6025 | 1.15 | 9.5 |
| 4 | 2.337 | 18743845 | 6037 | 1.16 | 9.3 |
| 5 | 2.330 | 18841274 | 6147 | 1.14 | 9.4 |
| 6 | 2.330 | 18841425 | 6255 | 1.18 | 9.3 |
| Mean | 2.342 | 18816674 | - | - | - |
| SD | 0.012 | 108557 | - | - | - |
| %RSD | 0.5 | 0.6 | - | - | - |

**Table no. 2: Chromatographic conditions**

| **Mobile phase** | **Acetate Buffer pH 3.2: Acetonitrile CAN (60:40) %v/v** |
|---|---|
| **Column** | **PhenomenexCyano(50x2.1 ID) 1.8µm** |
| Flow rate ml/min | 0.5 |
| Column temperature | 30ºC |
| Sample temperature | 15ºC |
| Wave length | 270 nm |
| Injection volume | 20 µ L |
| 'Runtime' | five minutes |

**Table no. 4: Linearity**

| Concentration (µg/mL) | Area |
|---|---|
| 50 | 6505743 |
| 80 | 11298564 |
| 100 | 14229109 |
| 120 | 17544536 |
| 150 | 21936597 |

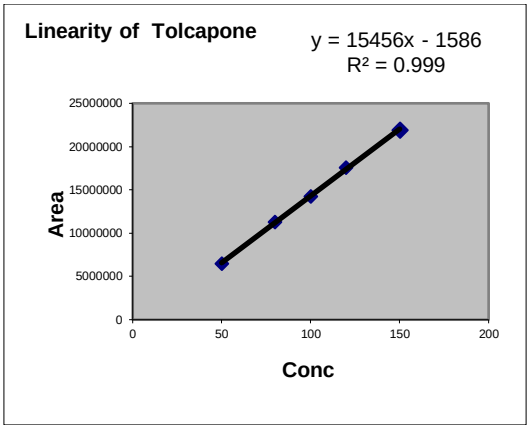



**Fig no.1 :Linearity graph of Tolcapone**

**Table no. 5: Linearity**

| Concentration (µg/mL) | Area |
|---|---|
| 25 | 8487692 |
| 40 | 14730739 |
| 50 | 18832059 |
| 60 | 22652163 |
| 75 | 28483383 |

**Fig no. 2: Linearity graph of Quinapril**

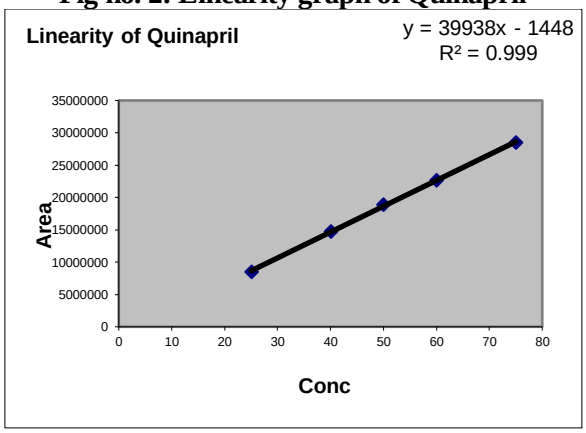



**Table 6: Results for linearity.**

| Parameter | TOLCAPONE | QUINAPRIL |
|---|---|---|
| **Correlation coefficient** | **0.9996** | **0.9996** |
| **Slope** | **154562** | **399384** |
| **Intercept** | **1153333** | **133** |

**Table 7: Accuracy**

**Table no.7 (a): Results for Recovery of TOLCAPONE**

| % Recovered | Area | Concentration Added | Concentration Recovered | %Recovery | Average |
|---|---|---|---|---|---|
| 50% _01 | 7132194 | 50 | 49.69 | 99.4 | |
| 50% _02 | 7152899 | 50 | 49.83 | 99.7 | |
| 50% _03 | 7171079 | 50 | 49.96 | 99.9 | |
| 100% _01 | 14269044 | 100 | 99.41 | 99.4 | |
| 100% _02 | 14286707 | 100 | 99.53 | 99.5 | **99.2** |
| 100% _03 | 14229355 | 100 | 99.13 | 99.1 | |
| 150% _01 | 21284249 | 150 | 148.28 | 98.9 | |
| 150% _02 | 21205193 | 150 | 147.73 | 98.5 | |
| 150% _03 | 21280624 | 150 | 148.26 | 98.8 | |

**Table no. 7 (b): Results for recovery of QUINAPRIL**

| % Recovered | Area | Concentration Added | Concentration Recovered | %Recovery | Average |
|---|---|---|---|---|---|
| 50% _01 | 25 | 9374038 | 25 | 24.91 | |
| 50% _02 | 25 | 9371143 | 25 | 24.90 | |
| 50% _03 | 25 | 9329351 | 25 | 24.79 | |
| 100% _01 | 50 | 18765131 | 50 | 49.86 | |
| 100% _02 | 50 | 18740887 | 50 | 49.80 | **99.5** |
| 100% _03 | 50 | 18687222 | 50 | 49.66 | |
| 150% _01 | 75 | 28045467 | 75 | 74.52 | |
| 150% _02 | 75 | 28083994 | 75 | 74.63 | |
| 150% _03 | 75 | 28097082 | 75 | 74.66 | |

**Specificity**

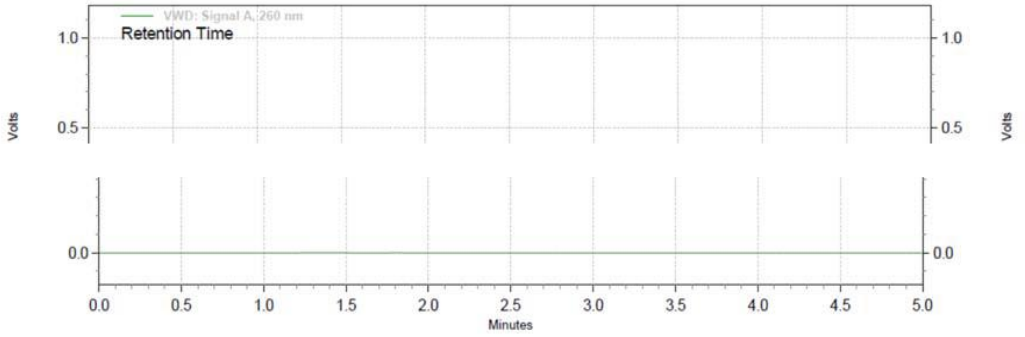



**Fig no. 3: Chromatogram of Quinapril and Tolcapone Blank**

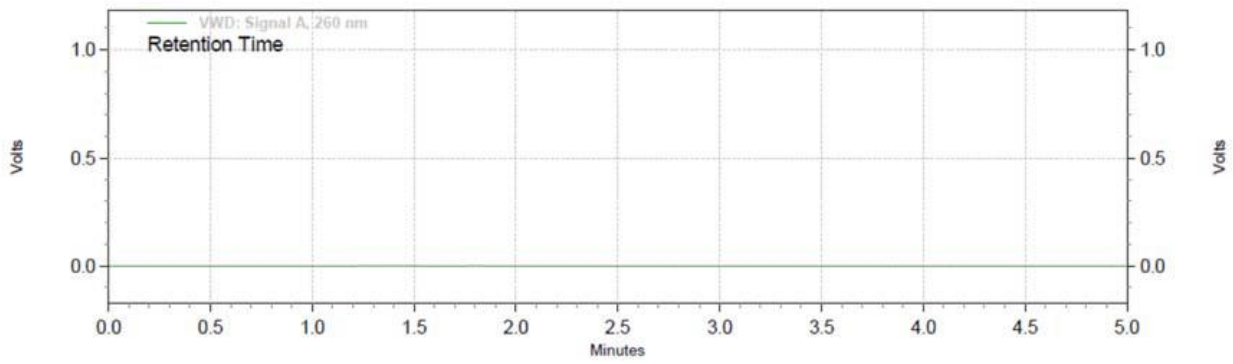


**Fig no. 4: Chromatogram of Placebo**

**Table no. 8: Precision**

| Injection | TOLCAPONE | | QUINAPRIL | |
|---|---|---|---|---|
| | Area | %Assay | Area | %Assay |
| 1 | 14107654 | 98.9 | 18518473 | 98.2 |
| 2 | 14255777 | 98.9 | 18575390 | 98.5 |
| 3 | 14223044 | 98.7 | 18674178 | 99.0 |
| 4 | 14398647 | 99.9 | 18783295 | 99.6 |
| 5 | 14176918 | 98.4 | 18539666 | 98.3 |
| 6 | 14272862 | 99.0 | 18896466 | 100.2 |
| Average | - | 98.8 | - | 99.0 |
| SD | - | 0.7 | - | 0.8 |
| %RSD | - | 0.7 | - | 0.8 |

Pipetted 5 mL of the clear solution in to 50 mL (5 µg/mL) volumetric flask and make up volume with mobile phase. Filter the solution through 0.45µm filter paper. The resulting solution is used to record the chromatogram.

**Method Precision:** The Precision of the method was determined by sample preparation. Calculated % of assay using formula

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{\text{WS}}{DS} \times \frac{\text{DT}}{WT} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{LC} \times 100$$

Where, AS: Average peak area due to standard preparation, AT: Peak area due to assay preparation, WS: Standard Weight of IVACAFTOR/ LUMACAFTOR in mg, WT: Weight of sample in assay preparation, DT: Dilution of assay preparation,DS: Dilution of standard preparation, P: Purity of QUINAPRIL /TOLCAPONE, AV: Average weight of tablets in mg, LC: Labelled claim of QUINAPRIL/TOLCAPONE

## RESULTS AND DISCUSSIONS:

**Method Optimization:** The developed method was optimized after many trials. The optimized method developed on PhenomenexCyano(50x2.1 ID) 1.8µm as column using acetate buffer and Acetonitrile in the ratio 60:40 %v/v as mobile phase. The column temperature was maintained constantly at 30ºC. Mobile phase pumped with a flow rate of 0.5ml/min and injection volume is 20µl.

**System suitability:** All system suitability parameters were passed which include the theoretical plates, tailing factor, resolution for Quinapril and Tolcapone respectively. Table No.01 and Table No.02

**Linearity:** The best fit line was obtained with regression coefficient between the peak area vs concentration. In Table No.04 and Table No.05 and Fig No.1 and 2

**Specificity:** It was evaluated by injecting blank and placebo along with drug product, no interference was found at the components respective retention timings. Chromatogram depicted below Fig No. 3 and 4.

**Method Precision:** The Precision of the method was determined by injecting Tolcapone and Quinapril with sample solution 6 times respectively. Method precision was expressed in terms of % RSD. Results are given in table no.08.

**Accuracy:** Prepared accuracy at 3 levels in triplicate at 50% , 100% and 150% with matrix and achieved satisfactory results and at each level of recovery was calculated. Results are given in table No.08.

## CONCLUSION

Another exact, exact fast technique has been produced for the concurrent estimation of Quinapril and Tolcapone in pharmaceutical measurement shape by RP-UHPLC. The ideal wavelength for the assurance of Quinapril and Tolcapone was chose at 270 nm based on isobestic point. A few preliminaries were performed with unique portable stages in divergent proportions, yet finally Ammonium acetic acid derivation Buffer pH 3.2: Acetonitrile (60:40) %v/v) was chosen as great pinnacle symmetry and goals between the pinnacles was observed. The Retention time of Quinapril and Tolcaponewere observed to be 1.323 and 2.360 min separately. The adjustment bend was gotten by plotting top region versus the focus over the scope of 50-100 µg/mL For Tolcapone and25-75 µg/mL for Quinapril. From linearity the relationship coefficient R2 esteem was observed to be 0.999for ICF and 0.999 for LMF. The %RSD in the pinnacle region of medication was observed to be under 2%. The quantity of hypothetical plates was observed to be more than 2000, which demonstrates proficient execution of the segment. The level of recuperation of Quinapril and Tolcapone were observed to be 99.9 and 99.8respectivelyshows that the proposed technique is exceptionally exact. Subsequently the proposed strategy is exceptionally exact, delicate and exact and it effectively connected for the evaluation of API content in the business plans of Quinapril and Tolcapone in Educational foundations and Quality control research centers.

## REFERENCES:


1. Chatwal, R. G.; Anand, K. S. Elite fluid chromatography. Instrumental techniques for synthetic examination, 5thed.; Himalaya distributers: Mumbai, 2010; 2.570-2.629.
2. Sharma, B. K. Elite fluid chromatography. Instrumental techniques for synthetic examination, 24th ed.; Goelpublishers: Meerut, 2005; 295 - 300.
3. Dong,W. M. HPLC instrumentation and patterns. Current HPLC for rehearsing researchers, USA, 2006; 5-10, 78-110.
4. Swartz, M. E.; Ira Krull, S, Analytical technique improvement. Investigative technique improvement and approval, first ed.; Marcel Dekker, Inc: New York, 2009; 17-80.
5. Satinder, A.; Dong, M. W. Technique improvement and approval. Pharmaceutical examination by HPLC, fifteenth ed.; New York, 2005; 16-70.
6. Snyder, R. L.; Kirkland, J. J.; Glajch, L. J. Beginning. Functional HPLC Method Development, second ed.; New York, 1997; 30-100.
7. http://www.sigmaaldrich.com/and soforth/medialib/docs/Aldrich/General_Information/labbasics_pg144.Par.0001.File.tmp/labbasics_pg144.pdf.
8. ICH, Text on Validation of Analytical Procedures, ICH – Q2A, International Conference on Harmonization, IFPMA, Geneva, 1995, 2-3, A– 1 to A– 3.
9. ICH, Validation of Analytical Procedures: Methodology, ICH – Q2B, International Conference on Harmonization, 1996, 1-3.

10. ICH Guidelines, Q2 (R1) - Validation of Analytical Procedures: Text and Methodology,2005, 1-6.
11. Quinapril medication profile - https://www.drugbank.ca/drugs/DB00881
12. Tolcapone medication profile - https://www.drugbank.ca/drugs/DB00323
13. Girija and Bhavaret. al.Quinapril hydrochloride and hydrochlorothiazide were at the same time dictated by HPTLC in pharmaceutical
14. Sujitha Rani and Mohan Gandhi et. al another rp-hplc strategy for concurrent estimation of quinapril and hydrochlorothiazide in pharmaceutical measurements shapes
15. Krishna and Shyamalaet. al A Novel approved steadiness showing technique for estimation of quinapril and tolcapone by RP-HPLC strategy.