Ultra Sensitive UPLC Method Development and Validation for the Simultaneous Estimation of Tamsulosin Hydrochloride and Tolterodine Tartrate in Bulk and Pharmaceutical Dosage Form

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Abstract
A rapid, sensitive and accurate ultra-performance reversed phase liquid chromatographic method was developed for the simultaneous determination of tamsulosin and tolterodine in pure form and pharmaceutical preparation. The developed UPLC method is superior to conventional HPLC with respect to speed, resolution, solvent consumption and cost. The separation was carried out on RP C₁₈ nucleosil (1.7 µm, 5 cm x 2 mm) using an isocratic mode in eluting Tamsulosin and Tolterodine at 1.54 min and 2.43 min respectively with a mobile phase composed of acetonitrile and 0.025N potassium phosphate buffer pH 3.50 (60%-40%), respectively. Chromatographic run time was 5 min with a flow rate 0.5 ml/min and UV detection at 220 nm. The linearity for tamsulosin and tolterodine were in the range of 2-20 µg/mL for both drugs, showed excellent recoveries for bulk and tablet dosage form with a very low LOD of 4.29 and 0.59 ng/mL for tamsulosin and tolterodine, respectively. The method has been validated for linearity, accuracy, precision, specificity, and limit of detection, limit of Quantification, robustness, and ruggedness. The method which was developed was validated as per the ICH guidelines. Finally, the method was compared statistically with reference methods indicating that there is no significant difference between them in respect of precision and accuracy.

Keywords: UPLC; Tamsulosin; Tolterodine; Isocratic; Tablets.

1. Introduction
Benign prostatic hyperplasia (BPH) is the benign growth of the prostate, generally originating in the periureteral and transition zones, with subsequent obstructive and irritative voiding symptoms. Histologically, BPH refers to the proliferation of smooth muscle, epithelium, and stromal cells within the transition zone of the prostate that surrounds the proximal urethra [1].

Medical therapies for BPH include α-adrenergic blockers, 5 α-reductase inhibitors, antimuscarinic drugs, phosphodiesterase inhibitors, and combinations of these drugs [2].

Tamsulosin (TAM), is chemically (S)-(R)-5-[2-[[2-(o-Ethoxyphenoxy) ethyl] amino] propyl]-2-methoxy benzene sulfonamide monohydrochloride (Figure 1). Tamsulosin selectivity binds and blocks the activity of alpha-1 adrenoreceptors in the human prostate and bladder neck leading to smooth muscle relaxation in the prostate and bladder neck, resulting in an improvement in urinary flow rate [3]. Some analytical methods were reported for the determination of tamsulosin by liquid chromatography [3, 4], spectrophotometrically [5-7], spectrofluorometrically [8], volumetrically [9], or by electrophoresis [10].

Tolterodine (TOL) is a competitive muscarinic receptor antagonist, used in the treatment of urogenital reductase inhibitors, antimuscarinic drugs, phosphodiesterase inhibitors, and combinations of these drugs [2].

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Tolterodine (TOL) is a competitive muscarinic receptor antagonist, used in the treatment of urogenital conditions and diseases such as urinary incontinence and prostatic hyperplasia. It is chemically 2-[(1R)-3-[di (propan-2-yl) amino]-1-phenyl propyl]-4-methyl phenol (Figure 1) [11]. Few chromatographic [12-15], spectrophotometric [16-18], spectrofluorometric [19], and electrochemical [20] methods have been reported also for determination of tolterodine either alone or in combination with other drugs other than tamsulosin.

TAM and TOL are marketed as a combined dose tablet formulation in the ratio (0.4:4mg) respectively, because overactive bladder may coexist with bladder outlet obstruction induced by benign prostatic hyperplasia. Few methods are reported for the simultaneous estimation of tamsulosin and tolterodine together [21-23], however no UPLC method was developed for such determination. Therefore, the development of a robust and selective...
analytical UPLC method would be necessary for determination of tolterodine with tamsulosin as it improves chromatographic resolution, speed and sensitivity in respect of reported methods [21, 22], and also reduces solvent consumption.

Ultra Performance Liquid Chromatography (UPLC) system is an innovative technique that brought revolution in high performance liquid chromatography by outperforming conventional HPLC. UPLC decreases sample run times up to a factor of 10, uses up to 95% less solvent and significantly improves productivity in the lab as compared to HPLC. The sub-2-μm hybrid particle chemistry, which offers significant benefits over today's HPLC systems, equipped with standard 5-μm particle chemistries. UPLC achieves the speed by using novel sub 2-μm particles that reduce chromatographic run times and improve resolution. UPLC was designed as a total system to leverage both ultra-high pressure and small particle separation attributes that result in uniquely superior performance with significant improvements in resolution, sensitivity and speed. UPLC system allows chromatographers to work at higher efficiencies with a much wider range of linear velocities, flow rates and backpressures [24]. The present study was conducted to quantify tamsulosin hydrochloride and tolterodine tartrate pharmaceutical dosage form by using RP-UPLC technique. The proposed method was found to be greener in terms of usage of hazardous chemicals and solvents, energy consumption, and production of waste. The proposed method can be safely used for the routine analysis of the studied pharmaceutical mixture with a minimal detrimental impact on human health and the environment.

Figure 1. Chemical structures of tamsulosin (TAM) and tolterodine (TOL)

2. Experimental

2.1. Materials and Reagents

2.1.1. Pharmaceutical Grade Tamsulosin and Tolterodine

Were kindly supplied by Adwia Pharmaceuticals Co., EGYPT. A tablet Bapter® (Dr. Reddy’s) which contains 0.4 mg tamsulosin and 4mg tolterodine was used. The used chemicals in all experiments were of analytical HPLC grade. Methanol and acetonitrile (POCH S.A., Poland), potassium dihydrogen phosphate, and orthophosphoric acid 85% (Merck, USA) were used. Fresh double distilled water was used throughout the whole experiment.

2.1.2. Preparation of 0.025M Dipotassium Hydrogen Phosphate Buffer

0.2017g of dipotassium hydrogen phosphate was dissolved in 250 mL of HPLC water in a 1000 mL beaker and the volume was made up to 1000 mL with HPLC water, then the pH was adjusted to 3.45-3.55.

2.1.3. Preparation of Mobile Phase

A combination of acetonitrile (60%) and 0.025 N dipotassium hydrogen phosphate buffer pH 3.5 (40%) was mixed and degassed in a fast clean ultra sonicator for 5 minutes. Finally, the mixture was filtered through 0.45μ membrane filter. This prepared solution was used as diluent.

2.1.4. Instrumentation and Chromatographic Conditions

Agilent 1290 series (USA) system consists of Binary pump, Auto sampler, column heater and Variable Wave length Detector. The pH of the mobile phase was adjusted using a Metrohm 744 pH meter (Herisau, Switzerland). UPLC separation of tamsulosin and tolterodine was achieved using reversed phase C18 nucleosil column (50 mm x 2 mm), 1.7 μm particle size (phenomenex, USA). The elution was isocratic and the mobile phase consists of 60% acetonitrile and 40% phosphate buffer (0.025 N potassium dihydrogen phosphate, adjusted to pH 3.5 by using orthophosphoric acid). The injection volume was 3.0 μL, the temperature of column was kept at 40°C. Flow rate was
adjusted at 0.5 ml/min and detection was achieved at 220 nm. Prior to use, the mobile phase was degassed and filtered by passing through a 0.2 µm pore size membrane filter (Millipore, Milford, MA, USA).

2.2. General Procedure
2.2.1. Preparation of Standard Solutions
4 mg of tamsulosin and 40 mg of tolterodine were weighted and transferred into a 100 mL volumetric flask, methanol was added as a solvent and sonicated for 20 minutes and then diluent was added up to the final volume. From this stock solution, 1 mL was pipetted out into a 10 mL volumetric flask where the diluent was added up to the final volume to get concentrations of 4 µg/mL and 40 µg/mL for tamsulosin and tolterodine, respectively.

2.2.2. Preparation of Sample Solutions
About 10 tablets were crushed in a mortar and the weight of drug equivalent to (0.4 mg tamsulosin and 4 mg tolterodine) was transferred to a 10 mL volumetric flask containing diluent. After sonication and filtration through 0.45µ membrane filter, 1mL was transferred from this solution into another 10mL volumetric flask to get the same concentrations of standard solutions.

2.2.3. Calibration Curves
Aliquots of standard solution of tamsulosin and tolterodine were taken and adjusted with methanol to obtain their final concentration in the range of 2-20 µg/mL for both. Calibration curves were constructed by plotting absorbance against the corresponding injected concentrations for each drug.

3. Results and Discussion
3.1. Optimization of Chromatographic Conditions
This method was optimized after studying many variables. Many columns were used to obtain the optimum separation and shortest run time. Reversed phase C18 nucleosil column (50 mm x 2 mm), 1.7 µm particle size (phenomenex, USA) was used. In order to obtain sharp, separated peaks without tailing or distortion, further optimization was needed. As such, different wavelengths and percentages of mobile phase contents were used. Mobile phase composed of acetonitrile and 0.025N potassium phosphate buffer with pH 3.5 (60%:40%) respectively gave the best results with wavelength at 220 nm. Under these conditions, TAM and TOL in pure form can be separated and eluted at 1.54 min and 2.43 minutes, respectively as illustrated in Figure 2.

Figure 2. Typical chromatogram of tamsulosin hydrochloride (TAM) and tolterodine tartrate (TOL) using RP C18 nucleosil (1.7 µm, 5 cm x 2 mm) and a mobile phase of acetonitrile and 0.025N potassium phosphate buffer pH 3.50 (60%:40%). Other chromatographic conditions are stated in Results and Discussion section 3.1.

3.2. Method Validation
Method Validation of the developed method was carried out according to International Conference on Harmonization guidelines [24] for its linearity, accuracy, precision, specificity, robustness, ruggedness, limit of detection and limit of quantification.

3.2.1. System Suitability
The column efficiency, resolution, and peak symmetry were calculated for the standard solutions (n=6). The values obtained demonstrated the suitability of the system for the analysis of this drug combination (Table 1).
optimum mobile phase showed symmetrical peaks (0.84 < T < 0.90), capacity factor (1 < k < 10), resolution > 2 and theoretical plates > 2000 as per required by the center for drug evaluation and research [25].

Table-1. System suitability parameters for tamsulosin (TAM) and tolterodine (TOL) in pure forms using the proposed UPLC method

| Parameter                        | TAM        | TOL        | Reference values [29]          |
|----------------------------------|------------|------------|-------------------------------|
| Capacity factor, k'              | 1.07       | 2.44       | Accepted value (1-10)         |
| Symmetry factor (T)              | 0.90       | 0.84       | Accepted value ≤ 2            |
| Resolution (Rs)                  | ---        | 5.15       | Accepted value > 2            |
| USP Plate count (N)              | 12216      | 12350      | Accepted value > 2000         |
| RSD%                             | 0.99       | 0.85       | Accepted value ≤ 1            |
| Selectivity (Separation factor, α)| ---        | 2.28       |                               |

3.2.2. Linearity
Linearity of this method was studied by preparing ten different concentrations of the standard solutions of TAM and TOL. Absorbance of these solutions were measured and the calibration curves were obtained by plotting absorbance vs. concentration of the drugs showing linearity in the concentration range of 2-20 µg/mL (Table 2). Linear regression equations were found to be y = 6.3274x + 3.8558, and y = 47.038x + 14.973, while the regression coefficient values (r) were 0.9993 and 0.9994 for TAM and TOL, respectively, indicating a high degree of linearity (Figure S1).

Table-2. Linearity Data of tamsulosin (TAM) and tolterodine (TOL) in pure forms using the proposed UPLC method.

| Taken Conc. (µg/mL) | TAM        | TOL        | Recovery % | Accuracy % | Taken Conc. (µg/mL) | TOL        | Recovery % | Accuracy % |
|---------------------|------------|------------|------------|------------|---------------------|------------|------------|------------|
| 2                   | 1.99       | 99.59      | -0.40      | 98.74      | 2                   | 1.97       | 99.74      | -1.26      |
| 4                   | 3.92       | 98.16      | -1.83      | 98.06      | 4                   | 3.92       | 98.06      | -1.94      |
| 6                   | 5.84       | 97.41      | -2.58      | 100.60     | 6                   | 6.03       | 99.64      | -0.36      |
| 8                   | 7.94       | 99.28      | -0.71      | 99.64      | 8                   | 7.97       | 99.64      | -0.36      |
| 10                  | 10.10      | 101.00     | 1.00       | 100.20     | 10                  | 10.02      | 100.20     | 0.18       |
| 12                  | 12.26      | 102.20     | 2.20       | 100.80     | 12                  | 12.10      | 100.80     | 0.80       |
| 14                  | 14.08      | 100.61     | 0.61       | 101.10     | 14                  | 14.16      | 101.10     | 1.14       |
| 16                  | 16.14      | 100.90     | 0.90       | 100.80     | 16                  | 16.12      | 100.80     | 0.75       |
| 18                  | 17.98      | 99.90      | -0.09      | 99.57      | 18                  | 17.92      | 99.57      | -0.43      |
| 20                  | 19.71      | 98.57      | -1.42      | 98.97      | 20                  | 19.79      | 98.97      | -1.03      |
| Mean                |            | 99.76      | -0.23      | 99.84      |                     |            | 99.84      | -0.16      |
| SD                  | 1.46       | 1.02       |            |            |                     |            |            |            |
| RSD                 | 1.47       | 1.02       |            |            |                     |            |            |            |
| SE                  | 0.46       | 0.32       |            |            |                     |            |            |            |
| Variance            | 2.14       | 1.04       |            |            |                     |            |            |            |
| Slope (b)           | 6.32       | 47.03      |            |            |                     |            |            |            |
| Intercept (a)       | 3.85       | 14.97      |            |            |                     |            |            |            |
| Correlation coefficient (r²) | 0.9993      | 0.9994      |            |            |                     |            |            |            |
| LOD (µg/mL)         | 0.004291   | 0.00059    |            |            |                     |            |            |            |
| LOQ (µg/mL)         | 0.014303   | 0.001968   |            |            |                     |            |            |            |

3.2.3. Accuracy
Accuracy was determined by spiking the solution containing 0.4 µg/mL of TAM and 4.0 µg/mL of TOL with four different concentrations of the drug i.e. these solutions were then diluted with methanol to yield solutions; having concentrations in the calibrations range. These concentrations represent the lower part of calibration curve (6 µg/mL of TAM and 8 µg/mL of TOL) then the middle part (10 & 14 µg/mL of TAM and 12 & 16 µg/mL of TOL) and the higher concentration part (18 µg/mL of TAM and 20 µg/mL of TOL). Mean recoveries were reported to be 100.80 and 100.10 while RSD% were calculated to be 0.99 and 0.85 TAM and TOL, respectively, indicating a high degree of the method accuracy (Table 3).
3.2.4. Precision

Precision was calculated in terms of method precision and intermediate precision [24]. Method precision (% repeatability) of the proposed UPLC method was determined by 6 replicate injections of different homogenous samples of TAM and TOL in the same day, while intermediate precision (day to day precision) was determined by 3 replicate injections of TAM and TOL per day table. However, the % recovery was well within 98 to 102 % and RSD of these injections does not exceed 2% as reported in Table 4, indicating that the developed method can accurately quantify TAM and TOL simultaneously in pharmaceutical formulation.

Table 3. Accuracy results for tamsulosin (TAM) and tolterodine (TOL) in pure forms using the proposed UPLC method

| Sample No. | Nominal conc. (µg/mL) | Found conc. (µg/mL) | Recovery % | Nominal conc. (µg/mL) | Found conc. (µg/mL) | Recovery % |
|------------|----------------------|---------------------|------------|----------------------|---------------------|------------|
| 1          | 6                    | 6.05                | 100.88     | 8                    | 7.99                | 99.93      |
| 2          | 10                   | 10.21               | 102.00     | 12                   | 12.10               | 100.85     |
| 3          | 14                   | 14.10               | 100.78     | 16                   | 16.10               | 100.67     |
| 4          | 18                   | 17.91               | 99.54      | 20                   | 19.79               | 98.96      |
| Mean       |                      |                     |            |                      |                     | 100.80     |
| SD         |                      |                     |            |                      |                     | 1.00       |
| RSD        |                      |                     |            |                      |                     | 0.99       |

Table 4. Repeatability and Intermediate precision of tamsulosin (TAM) and tolterodine (TOL) using the proposed UPLC method

|                              | TAM Mean ± RSD | TOL Mean ± RSD |
|------------------------------|---------------|--------------|
| Method precision (Repeatability) (n=6) | 101.29 ± 0.06 | 100.87 ± 1.50 |
| Intermediate precision (n=3)     | 101.22 ± 1.89 | 100.67 ± 1.97 |

3.2.5. Limit of Detection and Limit of Quantification

The limit of detection (LOD) was calculated from the linearity curve using the formula LOD = 3.3 ×SD/Slope while the limit of Quantification (LOQ) was calculated from the linearity curve using the formula LOQ = 10 × SD/Slope. The LOD for TAM was confirmed to be 4.29 ng/mL and that for TOL was 0.59 ng/mL. On the other hand, The LOQ for TAM was calculated to be 14.30 ng/mL and 1.96 ng/mL for TOL (Table 2). These results indeed show that the proposed method is highly sensitive and applicable for pharmaceutical studies even if detection of small concentrations in the nanogram range is required.

3.2.6. Specificity

The specificity of the analytical method was checked in whereas, the chromatogram of the tablet illustrates that there is no interference of the peaks of excipients in the determination of TAM and TOL. The chromatograms are being illustrated in Figures S2-S7 and the results for specificity were tabulated in Table S1.

3.2.8. Ruggedness and Robustness

Ruggedness of the method was determined by carrying out the experiment on agilent 1090 series by different operators using the same chromatographic conditions. Robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. No significant changes in the chromatograms were observed, where all SD values were less than 2 (Table 5), proving that the developed method is highly rugged and robust.

3.2.9. Analysis of Tablet Formulations

Bapter® (Dr. Reddy’s) which contains 0.4 mg TAM and 4mg TOL has been successfully analyzed by the proposed UPLC method. Excipients and impurities did not show interference indicating high specificity of the method. Results obtained were compared to those obtained by applying reference methods [15, 26] where Student’s t-test and F-test were performed for comparison. Results shown in Table 6 indicated that calculated t and F values were less than tabulated ones for TAM and TOL which in turn indicate that there is no significant difference between proposed method and reference ones relative to precision and accuracy.
Table 5. Results of the robustness for the determination of 10 µg/mL tamsulosin (TAM) and tolterodine (TOL) using the proposed UPLC method

| Changing Parameter | Actual Parameter | Modification | TAM Mean ± SD | TOL Mean ± RSD |
|--------------------|------------------|--------------|---------------|---------------|
| Mobile phase       | 60 : 40          | 55 : 45      | 101.29 ± 1.70 | 100.87 ± 1.57 |
|                    |                  | 65 : 35      | 101.41 ± 1.61 | 100.83 ± 1.65 |
| pH                 | 3.5              | 3.45         | 100.15 ± 1.87 | 100.47 ± 0.88 |
|                    |                  | 3.55         | 100.58 ± 1.53 | 100.62 ± 1.69 |
| Flow rate (mL/min) | 0.5              | 0.45         | 100.89 ± 1.67 | 100.95 ± 0.85 |
|                    |                  | 0.55         | 100.29 ± 1.38 | 100.62 ± 1.73 |
| Wavelength (nm)    | 220              | 225          | 100.18 ± 1.72 | 100.51 ± 1.66 |
|                    |                  | 215          | 100.76 ± 1.21 | 100.00 ± 0.35 |
| Temperature (°C)   | 40.0             | +5.0         | 100.80 ± 1.59 | 100.25 ± 0.70 |
|                    |                  | -5.0         | 100.01 ± 0.45 | 100.82 ± 1.61 |

Table 6. Statistical analysis of results obtained by the proposed HPLC method applied on Bapter® tablet compared with reference methods (n=3)

|       | Proposed Method Mean ± SD | Reference Methods [5, 17] Mean ± SD | Calculated t-values | Calculated F-values |
|-------|---------------------------|-------------------------------------|---------------------|---------------------|
| TAM   | 100.50 ± 0.57             | 100.20 ± 0.21                       | 0.41 (2.13)*        | 7.48 (19.00)*       |
| TOL   | 100.25 ± 0.43             | 99.27 ± 0.31                        | 1.83                | 1.85                |

* and * are the Theoretical Student t-values and F-ratios at p=0.05.

4. Conclusion
In this study, an accurate, rapid, simple and sensitive reversed-phase UPLC method with UV detection was developed for the assay of tamsulosin and tolterodine. The method was fully validated and applied successfully to quantify the drug in pharmaceutical dosage form. The complete separation of the analytes was achieved within short chromatographic run time (3 min) with no interfering peaks where there is no need for traditional HPLC methods with complex mobile phase mixtures, long chromatographic run times and more solvent consumed methods. The developed UPLC technique will eliminate significant time and cost per sample from analytical process while improving the quality of results. This method is not hazardous to human or to the environment and is more economic because a large number of samples can be analyzed within a short period of time.

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The authors declare that there is no conflict of interests regarding the publication of this paper.

Compliance with ethical standards

Conflict of interest
The authors declare that there is no conflict of interest in the manuscript.

Ethical Approval
This manuscript does not include any studies on human or animals.

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Figure S1. Calibration curves for authentic mixture of tamsulosin (TAM) and tolterodine (TOL) using the proposed UPLC method.

\[
y = 47.038x + 14.973 \\
R^2 = 0.9994
\]

\[
y = 6.3274x + 3.8558 \\
R^2 = 0.9993
\]

Figure S2. Chromatogram for specificity - blank

Figure S3. Chromatogram for specificity – placebo
Figure-S4. Chromatogram of tamsulosin tartrate - Standard

Figure-S5. Chromatogram of tolterodine hydrochloride - Standard

Figure-S6. Chromatogram of specificity – tamsulosin hydrochloride and tolterodine tartrate - Standard
Figure-S7. Chromatogram of specificity – tamsulosin hydrochloride and tolterodine tartrate - Sample

Table-S1. Specificity results for tamsulosin (TAM) and tolterodine (TOL) using the proposed UPLC method

| S. No. | Sample name      | Rt  | Peak area   |
|-------|------------------|-----|-------------|
| 1     | Blank            | ----| ----        |
| 2     | Tamsulosin Standard | 1.45| 125.24      |
| 3     | Tolterodine Standard | 2.41| 9075.32     |
| 4     | Mixed Standard   | 1.45| 126.24      |
|       | TOL              | 1.45| 9073.32     |
|       | TOL              | 2.41| 9125.52     |
| 5     | Placebo          | ----| ----        |
| 6     | Sample           | 1.45| 125.29      |
|       | TOL              | 1.45| 9125.52     |
|       | TOL              | 2.41| 9125.52     |