RESEARCH ARTICLE

ISSN: 2455-8095
doi: https://doi.org/10.21477/ijapsr.7.3.04

# Development and Validation of Analytical Method for Simultaneous Estimation of Triamterene and Benzthiazide by RP-HPLC

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**ABSTRACT**

In the process of reversed-phase high-performance liquid chromatography (RP-HPLC), compounds are separated according to their hydrophobicity. The solute particle from the solvent system binds to the adsorbed hydrophobic ligands coupled to the sorbent in a hydrophobic manner, which results in their separation. In the present study, Triamterene (TMT) and Benzthiazide (BZT) were quantified simultaneously using a unique RP-HPLC technology that has been developed and validated. The technique is carried out on Symmetry C18 (4.6 x 150 mm, 5 m, manufactured: Waters). Methanol: Buffer: ACN (60:30:10) serves as the mobile phase, injected at a flow rate of 1.0 mL/min, to perform the separation. The study is carried out with the column fixed at 40°C. The overall run duration is around 6 minutes, and the injecting volume is fixed at 10 µL. According to ICH specifications, the method has been validated for linearity, ruggedness, precision, and specificity, robustness, and system suitability. The approach is precise and linear for the determination of TMT at 15-75 µg/mL and BZT at 10-50 µg/mL. Additionally, positive responses are also obtained concerning robustness, intra-day and inter-day precision, and mean % recovery (100.0% for TMT and 100.3% for BZT). The outcomes show that the approach is appropriate for regular quality control assessment of commercial tablet dosage forms.

**Keywords:** Accuracy, Benzthiazide, Precision, Quantification, RP-HPLC, Triamterene.

## 1. INTRODUCTION

HPLC, also called high-performance liquid chromatography, is an analytical method for separating, characterizing, or estimating every element in a composition. In reversed-phase high-performance liquid chromatography, compounds are separated according to their hydrophobicity.[1] The solute particle from the solvent system binds to the adsorbed hydrophobic ligands coupled to the sorbent in a hydrophobic manner, which results in their separation.[2,3] In the presence of water-based buffers, the dissolved solute is first loaded to the stationary phase; the solutes are then extracted by adding a solvent to the mobile phase. Either gradient setting, in which the quantity of organic phase is raised gradually over time, or isocratic elution, in which the quantity of organic phase is fixed, can be used to elute.[4,5]

A potassium-retaining diuretic, triamterene (2,4,7-triamino-6-phenylpteridine) (TMT) (Figure 1) is utilized to treat high blood pressure.[6,7] It functions by increasing $Na^+$ ion and water output but reducing potassium efflux in the distal tubule of the nephrons by acting on the lumen.[8] Triamterene is thought to possess low antihypertensive potency because it operates on the distal tubule, in which only a tiny portion of $Na^+$ ion reabsorption takes place.[9] It is linked to a higher likelihood of causing hyperkalemia because of its impact on elevated serum potassium levels.[10] TMT is a photosensitizing medication and a mild inhibitor of folic acid.[11]

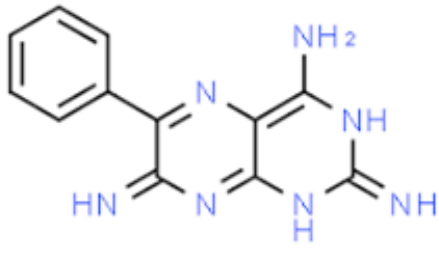


Figure 1: Structure of Triamterene

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**Received:** 18-04-2022 **Accepted:** 12-05-2022 **Available Online:** 01-07-2022

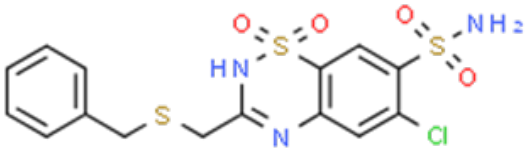

**Figure 2:** Structure of Benzthiazide

Benzthiazide (BZT) is employed to treat inflammation and high blood pressure. Similar to many other thiazides, it encourages the body to lose fluids (diuretics).[12] They prevent the distal tubule from reabsorbing Na+/Cl-. Additionally, potassium deficiency and a rise in uric acid levels are side effects of thiazides.[13] The NaCl co-transporter is blocked by the BZT at the initial distal convoluted tubule, resulting in a rise in the body's elimination of Na, Cl, and water.[14] Additionally, the medication interacts with the thiazide-sensitive Na-Cl transporter and blocks the movement of Na+ across the glomerular membrane.[15,16] This triggers the Na-K interchange pathway, which raises potassium elimination. Additionally, it may exert its effects through the carbonic anhydrase or KCa channel. Figure 2 depicts the molecular structure of benzthiazide.

The goal of the current work was to devise a new, uncomplicated, accurate, and economically viable quantitative technique for the simultaneous quantification of triamterene and benzthiazide in pharmaceutical formulations.

## 2. MATERIALS AND METHODS

### Chemicals

Triamterene and benzthiazide were purchased from Sura labs, Hyderabad, Telanagna. Water, methanol, and acetonitrile (ACN) for HPLC were obtained from Merck, USA. Anhydrous di hydrogen phosphate and citric acid were procured from Finar chemicals, Hyderabad, Telnagana.

### Equipment used

HPLC (WATERS, software: Empower 2, 2695 separation module. 996 PDA detector), UV/Vis spectrophotometer (LABINDIA UV), pH meter (LabIndia), weighing machine (Sartorius), pipettes, burettes and beakers (Borosil) and digital ultra sonicator (Labman).

## 2.1 Method development

### *2.1.1 Preparation of Mobile Phase and Stock Solutions*

#### *Preparation of Phosphate buffer(pH-3.6)*

1000 mL of solution was prepared by dissolving 0.9 grams of anhydrous di hydrogen phosphate with 1.298 g of citric acid monohydrate.

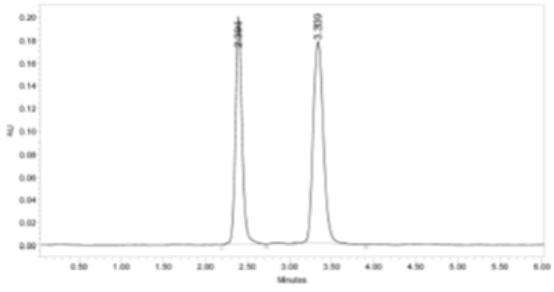

**Figure 3:** Chromatogram for Optimized Chromatogram of Standard Solutions

#### *Preparation of mobile phase*

In an electronic ultrasonicator for 10 min, 100 mL (10%) of HPLC ACN was combined with 600 mL (60%) of methanol (MeOH) plus 300 mL (30%) of phosphate buffer before being strained using a 0.45 µ filter under vacuum filtration.

#### *Diluent Preparation*

The diluent (dilutant) used was the mobile phase.

#### *Preparation of Standard Stock Solutions of Triamterene and Benzthiazide*

10 mg each of TMT and BZT working standards were weighed accurately and transferred to 2 separate volumetric flasks. Approximately 7 mL of diluent was added to both flasks, and the mixture was sonicated to dissolve it. Next, enough diluent was added to each flask to bring the volume up to the required level. Pipette 0.45 mL and 0.3 mL of these respective TMT and BZT stock solutions into a 10 mL volumetric flask and dissolve with diluents to the required concentration. These solutions were labeled as standard TMT stock solution and standard BZT stock solution, respectively, and preserved for further use.

#### *Preparation of Sample Stock Solution*

Ten combination tablets each containing TMT and BZT were weighed, crumbled in a mortar and pestle, and the amount equivalent to each compound—10 mg of TMT and 10 mg of BZT (in a marketed formulation)—was poured into separate volumetric flasks. 7mL of diluents were added to both flasks, then sonicated to dissolve before adding further volume until it reached the mark. Pipette 0.45 mL and 0.3 mL of these respective TMT and BZT stock solutions into a 10 mL volumetric flask and dissolve with diluents to the required concentration. These solutions were labeled as sample TMT stock solution and sample BZT stock solution, respectively and preserved for further use.

**Table 1:** Peak Results for Optimized Chromatogram of Standard Solutions

| S. No | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | TMT | 2.395 | 1242388 | 197332 | | 1.1 | 4741 |
| 2 | BZT | 3.339 | 1494848 | 177825 | 5.2 | 1.2 | 3793 |

**Table 2:** Results of System Suitability Parameters for Triamterene and Benzthiazide

| S. No | Name | Retention time(min) | Area (µV sec) | Height (µV) | USP Resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | TMT | 2.395 | 1242388 | 197332 | | 1.1 | 4741 |
| 2 | BZT | 3.339 | 1494848 | 177825 | 5.2 | 1.2 | 3793 |

**Table 3:** Peak Purity Results

| S. No | Name of compound | Label claim (mg) | Amount Found (mg) | % purity/% Assay |
|---|---|---|---|---|
| 1 | TMT | 10 | 9.99 | 99.90 |
| 2 | BZT | 10 | 9.95 | 99.5 |

**Table 4:** Results of Method Precision for Triamterene

| S. No | Name | Retention time | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | TMT | 2.264 | 1010585 | 191522 | 1.0 | 3802 |
| 2 | TMT | 2.246 | 1011075 | 192935 | 1.1 | 3546 |
| 3 | TMT | 2.264 | 1011924 | 191560 | 1.4 | 4633 |
| 4 | TMT | 2.246 | 1014299 | 193027 | 1.1 | 4812 |
| 5 | TMT | 2.280 | 1022159 | 191578 | 1.0 | 3802 |
| Mean | | | 1014008.4 | | | |
| Std. Dev. | | | 4774.60.5 | | | |
| %RSD | | | 0.5 | | | |

**Table 5:** Results of Method Precision for Benzthiazide

| S. No | Name | Retention time | Area (µV*) | Height (µV) | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | BZT | 3.132 | 1513391 | 192372 | 1.2 | 4759 |
| 2 | BZT | 3.132 | 1513391 | 192372 | 1.1 | 3695 |
| 3 | BZT | 3.129 | 1526673 | 212569 | 1.1 | 4741 |
| 4 | BZT | 3.113 | 1560819 | 209700 | 1.2 | 3793 |
| 5 | BZT | 3.113 | 1560819 | 209700 | 1.1 | 4741 |
| Mean | | | 1535018.7 | | | |
| Std. Dev. | | | 24168.8 | | | |
| %RSD | | | 1.6 | | | |

### *2.1.2 Optimization of mobile phase and column*

#### *Mobile Phase Optimization*

At first, different ratios of methanol: water and methanol: phosphate buffer were applied as the mobile phase. The mobile phase was subsequently adjusted to comprise phosphate buffer (pH 3.6), methanol, and ACN in the ratios of 30:60:10 v/v, accordingly.

#### *Optimization of Column*

The technique was carried out using a variety of columns, including the C8, C18, X-bridge, and Xterra columns. It was observed that Symmetry C18 (4.6 x 150mm, 5m, manufactured: Waters) was appropriate since it showed excellent peak shape and resolution at 1-mL/min flow rate.

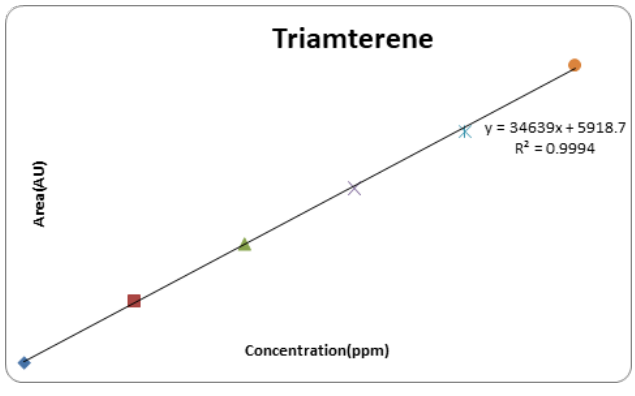


**Figure 4:** Calibration curve of Triamterene

**Table 6:** Results of Ruggedness for Triamterene

| S. No. | Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | TMT | 2.538 | 1015722 | 193604 | 3478 | 1.0 |
| 2 | TMT | 2.403 | 1016087 | 193857 | 4894 | 0.9 |
| 3 | TMT | 2.285 | 1018135 | 192177 | 3908 | 0.9 |
| 4 | TMT | 2.403 | 1019549 | 194085 | 3998 | 1.2 |
| 5 | TMT | 2.538 | 1032335 | 194582 | 4284 | 1.1 |
| Mean | | | 1020365.5 | | | |
| Std. Dev. | | | 6869.6 | | | |
| % RSD | | | 0.7 | | | |

**Table 7:** Results of Method Ruggedness for BZT

| S. No. | Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | BZT | 3.469 | 1496209 | 177051 | 3778 | 1.0 |
| 2 | BZT | 3.469 | 1507963 | 177285 | 5894 | 0.9 |
| 3 | BZT | 3.344 | 1521163 | 183178 | 5908 | 0.9 |
| 4 | BZT | 3.344 | 1522810 | 183224 | 5998 | 1.2 |
| 5 | BZT | 3.135 | 1528916 | 202095 | 5284 | 1.1 |
| Mean | | | 1515412.0 | | | |
| Std. Dev. | | | 13175.7 | | | |
| % RSD | | | 0.9 | | | |

**Table 8:** Results of Accuracy for Standard

| S. No. | Name | RT | Area | Height | USP Resolution | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|
| 1 | TMT | 1.819 | 262534 | 46767 | | 1.19 | 2571 |
| 2 | BZT | 4.424 | 319878 | 14317 | 8.60 | 1.58 | 2254 |
| 3 | TMT | 1.821 | 256968 | 46254 | | 1.17 | 2611 |
| 4 | BZT | 4.429 | 317792 | 14396 | 8.59 | 1.59 | 2442 |
| 5 | TMT | 1.818 | 260969 | 46265 | | 1.21 | 2541 |
| 6 | BZT | 4.438 | 320487 | 14410 | 8.68 | 1.59 | 2280 |

**Table 9:** The Accuracy Results for Triamterene

| %Concentration(At specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 605652.5 | 7.5 | 7.48 | 98.1% | |
| 100% | 1246314 | 15 | 15.2 | 101.0% | 100.0% |
| 150% | 1869868 | 22.5 | 22.51 | 101.0% | |

**Table 10:** The Accuracy Results for Benzthiazide

| %Concentration (At specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 774787.7 | 5 | 5.0 | 101.3% | 100.3% |
| 100% | 1537580 | 10 | 10.0 | 100.3% | |
| 150% | 2285575 | 15 | 14.9 | 99.4% | |

**Table 11:** Results of method linearity for Triamterene

| S. No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | TMT | 2.332 | 374052 | 70110 | 1.0 | 3987 | 1 |
| 2 | TMT | 2.236 | 682802 | 104981 | 0.9 | 3897 | 2 |
| 3 | TMT | 2.294 | 1012619 | 194128 | 0.9 | 3487 | 3 |
| 4 | TMT | 2.219 | 1324938 | 207248 | 1.0 | 3789 | 4 |
| 5 | TMT | 2.313 | 1708316 | 323848 | 0.9 | 3866 | 5 |

**Table 12:** Results of method linearity for benzthiazide

| S. No. | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | BZT | 3.345 | 545062 | 58346 | 0.9 | 4273 | 1 |
| 2 | BZT | 3.116 | 1052605 | 145124 | 0.9 | 4478 | 2 |
| 3 | BZT | 3.200 | 1545948 | 207648 | 1.0 | 4356 | 3 |
| 4 | BZT | 3.170 | 2050561 | 280917 | 0.9 | 4398 | 4 |
| 5 | BZT | 3.160 | 2635141 | 374459 | 1.0 | 4298 | 5 |

**Table 13:** Results for Variation in Flow

| S. No. | Drug name | Flow (mL/min) | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | TMT | Less (0.9) | 1104154 | 189779 | 4479 | 0.9 |
| | | Actual (1) | 1245977 | 191456 | 4759 | 0.9 |
| | | More (1.1) | 1408920 | 180921 | 3072 | 0.9 |
| 2 | BZT | Less (0.9) | 2104921 | 206420 | 4508 | 0.9 |
| | | Actual (1) | 1517199 | 175740 | 3695 | 0.9 |
| | | More (1.1) | 1408920 | 180921 | 3072 | 1.0 |

**Table 14:** Results for Variation in Mobile Phase Composition

| S. No. | Drug name | Organic (mL/min) | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1. | TMT | Less (50%) | 1012763 | 137954 | 2028 | 0.9 |
| | | Actual | 1245977 | 191456 | 4759 | 0.9 |
| | | More (70%) | 912635 | 181569 | 3002 | 1.0 |
| 2. | BZT | Less (50%) | 1501336 | 146804 | 3035 | 1.0 |
| | | Actual | 1517199 | 175740 | 3695 | 0.9 |
| | | More (70%) | 1415632 | 181569 | 3002 | 1.0 |

## 2.2 Validation parameters

### 2.2.1 System suitability

6 replicate administrations of prepared standard solutions of TMT and BZT were applied. The area, USP plates, retention time (RT), and tailing factor (TF) were computed for every injection to verify the method's system suitability.

### 2.2.2 Method Precision

The peak areas were obtained when the sample and standard solutions of 30 µg/mL were inserted into the column five times each. Using peak areas, the mean and % RSD was calculated.

### 2.2.3 Ruggedness

To assess the technique's ruggedness, precision tests were carried out on various days utilizing various brand columns with identical sizes. In the HPLC column, the standard solution was introduced five times, with the peak area being recorded every time. The %RSD for the obtained peak areas of 5 injections was reported to fall within the prescribed ranges.

### 2.2.4 Accuracy

*Preparation Sample solutions*

*For the preparation of 50% solution (For target Assay concentration)*

5 mg each of TMT and BZT working standards were poured into two separate 10 mL volumetric flasks, followed by the addition of approximately 7 mL of dilutants to both flasks. The solutions were sonicated to thoroughly mix the components, and then the volume in both of them was brought up to the mark using the dilutant.

0.45 mL of TMT and 0.3 mL of BZT solutions were taken in a 10 mL flask, and the volume was brought up to the mark by adding dilutants.

*For the preparation of 100% solution (For target assay concentration)*

For accuracy, 100% calculation, previously prepared standard stock solutions of TMT and BZT were used

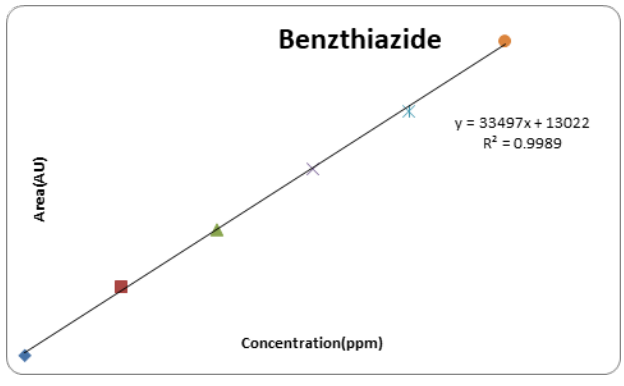


**Figure 5:** Calibration curve of benzthiazide

*For the preparation of 150% solution (For target assay concentration)*

15 mg each of TMT and BZT working standards were poured into two separate 10 mL volumetric flasks, followed by the addition of approximately 7 mL of dilutants to both flasks. The solutions were sonicated to thoroughly mix the components, and then the volume in both of them was brought up to the mark using the dilutant.

0.45 mL of TMT and 0.3 mL of BZT solutions were taken in a 10 mL flask, and the volume was brought up to the mark by adding dilutants.

*Procedure*

To determine the accuracy, the prepared solutions of TMT and BZT (50%, 100% & 150%) were inserted into the column, and amounts of drugs found and amounts of drugs added were noted. Mean recovery was calculated using individual recovery values. The solutions were passed through a 0.45-µ filter membrane before each concentration was run through the column three times at optimal settings. The chromatograms were acquired, and peak values were noted.

### *2.2.5 Linearity*

*Preparation of Level – I (15 ppm of Triamterene & 10 ppm of benzthiazide)*

In a 10 mL volumetric flask, 0.15 mL and 0.1 mL of TMT and BZT were added, and the volume was brought up to the mark by adding dilutant.

*Preparation of Level – II (30 ppm of Triamterene &20 ppm of benzthiazide)*

In a 10 mL volumetric flask, 0.3 mL and 0.2 mL of TMT and BZT were added, and the volume was brought up to the mark by adding dilutant.

*Preparation of Level – III (45 ppm of Triamterene & 30 ppm of benzthiazide)*

In a 10 mL volumetric flask, 0.45 mL and 0.3 mL of TMT and BZT, respectively, were added and the volume was brought up to the mark by adding dilutant.

*Preparation of Level – IV (60ppm of Triamterene &40ppm of benzthiazide):*

In a 10 mL volumetric flask, 0.6 mL and 0.4 mL of TMT and BZT, respectively, were added, and the volume was brought up to the mark by adding dilutant.

*Preparation of Level – V (75ppm of Triamterene &50ppm of benzthiazide)*

In a 10 mL volumetric flask, 0.75 mL and 0.5 mL of TMT and BZT, respectively, were added, and the volume was brought up to the mark by adding dilutant.

### *Procedure*

Each of the above-prepared level solutions was injected into the column, and peak areas were noted. A graph was plotted with a concentration on X-axis against the peak area on Y-axis, and the correlation coefficient was determined.

### *2.2.6 Robustness*

Rather than employing a flow rate of 1.0 mL/min, it was maintained at 0.9 mL/min and 1.1 mL/min, and its influence was examined to assess the robustness of the developed technique. In place of (Methanol: Buffer: ACN = 60:30:10), the proportions of the mobile phase were altered to 65:20:15 and 55:40:5, and its influence was examined to determine the robustness of the developed technique. In every instance, the %RSD of robustness analysis under such circumstances was determined.

## 3. RESULTS

### 3.1 Optimized chromatogram

| | | |
|---|---|---|
| Column | : | Symmetry C18 (4.6 x 150mm, 5µm, Make: waters) |
| Column temperature | : | 40°C |
| Wavelength | : | 230 nm |
| Mobile phase ratio | : | Methanol:Phosphate buffer 3.6 pH: ACN (60:30:10) |
| Flow rate | : | 1-mL/min |
| Autosampler temperature | : | Ambient |
| Injection volume | : | 10 µL |
| Run time | : | 6 minutes |

The optimized chromatogram obtained for the standard solution is shown in Figure 3. Table 1 shows peak results obtained for optimized chromatographic conditions

### *Observation*

More theoretical plates (TP), fewer tailing factors (TF), and adequate resolution are all evident in both peaks in this assay which indicate that the method meets the system suitability standards. Therefore, the chromatogram has been optimized.

The chromatogram above revealed that the sample peaks for TMT and BZT are distinct.

The retention time of TMT – 2.395min

The retention time of BZT – 3.339 min

### 3.2 Method validation

### *3.2.1 System Suitability*

The results obtained for system suitability studies

are shown in Table 2.

*Acceptance criteria*

- Resolution between two medications must be at least 2,
- TP must not fall below 2000.
- The TF must be between 0.9 and 2, but not higher.

The data stated above indicated that each of the system suitability parameters for the proposed technique was within the acceptable ranges.

### 3.2.2 Peak purity analysis of TMT and BZT

The results for % purity of TMT and BZT in marketed dosage forms are shown in Table 3.

Pharmaceutical formulations of TMT and BZT were reported to be 99.90% and 99.5% pure, correspondingly.

### 3.2.3 Precision

Tables 4 and 5 show the results for the precision of TMT and BZT respectively.

*Acceptance criteria:*

- The %RSD for the sample must be below 2.

The standard solution's %RSD is less than 2, which falls within the acceptable range, indicating that the proposed technique is precise.

### 3.2.4 Ruggedness

The results of the ruggedness of TMT and BZT are tabulated in Tables 6 and 7, respectively.

*Acceptance criteria*

- % RSD for five different test solutions must not be greater than 2.

The estimated %RSD falls within the acceptable range; therefore, the technique is rugged.

### 3.2.5 Accuracy

The accuracy results for TMT and BZT standard solutions are shown in Table 8, 9 and 10 show accuracy results for TMT and BZT sample solutions, respectively.

*Acceptance Criteria*

- %Recovery for a given sample must be more than 97% and less than 103%

%Recovery for TMT was found to be within the acceptable limit, indicating that the method is accurate.

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- %Recovery for a given sample must be more than 97% and less than 103%

%Recovery for TMT was found to be within the acceptable limit, indicating that the method is accurate.

### 3.2.6 Linearity

Linearity curves for TMT and BZT are shown in Figures 4 and 5, respectively.

The results for the linearity studies of TMT and BZT are shown in Tables 11 and 12, respectively.

The linearity assay was carried out for concentrations varying between 15–75 ppm for TMT and 10–50 ppm for BZT, and the correlation coefficient ($r^2$) was reported to be 0.999 and 0.998, respectively ($r^2 \geq 0.998$).

### 3.2.7 Limit of Detection (LOD)

The least quantity of analyte contained in a sample that may be observed but cannot be determined as actual values is the limit of detection (LOD) of a specific analytical technique.

$$LOD = 3.3 \times \sigma/s$$

Where **σ = Standard** deviation of the response, S = Slope of the calibration curve

TMT =3.3 × 30909.5/34639 =2.9 **μg/m**L

BZT =3.3 ×23949.8/33497 =2.3 **μg/m**L

### 3.2.8 Limit of Quantitation (LOQ)

The least concentration of analyte that can be numerically quantified in a given sample is a specific analytical method's limit of quantitation (LoQ).

$$LOQ = 10 \times \sigma/S$$

Where **σ = Standard** deviation of the response, S = Slope of the calibration curve

TMT =10×30909.5/34639 = 8.9 **μg/m**L

BZT =10 ×23949.8/33497 = 7.1 **μg/m**L

### 3.2.9 Robustness

The results obtained when the flow rate was altered are tabulated in Tables 13, and 14 show the results obtained when the composition of the mobile phase was changed.

*Acceptance criteria*

- Percentage RSD should not exceed 2.
- The % RSD recorded by adjusting the flow rate and altering the mobility phase was reported to be less than 1, which is within limits. This indicates that the technique is robust.

## 4. DISCUSSION

High-performance liquid chromatography is currently among the analysis techniques that offer the most advanced features. TMT and BZT concentrations were quantified by employing RP-HPLC. The mobile phase was modified with methanol: phosphate buffer: ACN (60:30:10) and C18 column (4.6 x 150mm, 5 m, Make: Waters). Chromatography of the solutions was performed at a fixed flow rate of 1 mL/min. Triamterene and benzthiazide were reported to have linearity ranges of 15–75

μg/mL and 10–50 μg/mL, correspondingly. The linear resolution efficiency was not greater than 0.998, 0.999. The RSD values are below 2%, demonstrating the accuracy and precision of the technique. The % of Benzthiazide and Triamterene recovered ranges between 99.90 and 99.5%. LOD and LOQ were reported to fall within the acceptable range.

## 5. CONCLUSION

The validation parameter values were all in compliance with ICH and USP standards. It implied that the technique is uncomplicated, reliable, efficient, and linear. The technique was discovered to be appropriate for routine analysis offering a significant level of precision and accuracy.

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**How to cite this article:** Srinija, G., Kondra, S. Development and Validation of Analytical Method for Simultaneous Estimation of Triamterene and Benzthiazide by RP-HPLC. Int. J. Appl. Pharm. Sci. Res. (2022);7(3): 52-59. doi: https://doi.org/10.21477/ijapsr.7.3.04

**Source of Support:** Nil.

**Conflict of Support:** None declared.