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# Green Spectrofluorimetric Method for Determination of Atenolol in Pharmaceutical Tablets and Human Urine

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**Abstract**

An environment-friendly, sensitive, simple, and economic spectrofluorimetric method for the analysis of atenolol in pure powder, pharmaceutical preparations, and urine using green solvents was developed. The analysis was carried out using the solvent 0.02 N NaOH to reach the concentration range of 30-800 ng/ml, and fluorescence intensities were measured at 301±1nm after excitation at 255 nm using a slit width of 5 nm. The effects of different solvents, different pHs, and different concentrations of NaOH were studied. The method was applied successfully to pharmaceutical tablets and spiked urine samples. The proposed method was applied to investigate the urinary excretion pattern of atenolol in a healthy male volunteer after oral administration of a 50 mg single dose. Urine samples were collected at intervals for up to 24 hours. The method was validated according to the ICH guidelines, proving that it is accurate, specific, precise, and robust. So, it is suitable for quality control analysis of atenolol and for the detection of drug abuse in precision sports due to its sensitivity for the determination of atenolol on a regular basis at therapeutic urine levels. The method complies with the principles of green analytical chemistry due to its short analysis time, high sensitivity, low cost, simple instruments, small energy consumption, very little waste, and being free from harmful solvents, ensuring operator safety. The proposed method's high level of greenness was proved by its high eco-scale score (95 points) as well as by the GAPI and AGREE greenness assessments.

**Keywords:** Atenolol, NEMI , eco scale, GAPI, AGREE.

## 1. Introduction

Hypertension is a global medical concern, causing increased morbidity and mortality with an increased cardiovascular disease (CVD) risk (Mills et al, 2020). β-blockers are used to treat CVDs such as angina pectoris, cardiac arrhythmia, and hypertension.

Atenolol (ATE), a selective β1 receptor antagonist, is primarily used to treat CVD. It is treated as a doping agent in several sports. As a result, it is included in the list of drugs prohibited by the

World Anti-Doping Code of the World Anti-Doping Agency (Dvorak et al, 2014) which makes its determination in human urine very important.

ATE, with chemical name 2-[4-[2-hydroxy-3-(propan-2-ylamino) propoxy]phenyl]acetamide (Fig. 1), has less than 10% metabolism by the liver, and about 85-95% of the drug is excreted unchanged in urine. So, renal excretion is the main route of ATE elimination. Thus, performing pharmacokinetic studies and doping tests in competition sports can be done using human urine samples (Kuyper & Khan, 2014).

Several methods for ATE analysis in pure compounds, pharmaceutical preparations, and biological fluids have been reported. These include voltammetry methods for ATE analysis in pharmaceutical products (Moraes et al, 2016) and urine (Afonso et al, 2016), GC-MS method for the analysis of ATE and bisoprolol in human bone (Fernandez-Lopez et al, 2019), and UPLC–MS for the analysis of ATE and chlorthalidone in human plasma (Shah et al, 2016).

Also, different mixtures containing ATE were analyzed using HPLC methods (Anderson et al, 2017; El-Alfy et al, 2019; Elkady et al, 2020; Kannappan & Mannemala, 2016) in addition to spectrophotometry (Antakli et al, 2020; Mohammad et al, 2019; Saleem, 2019; Vaikosen et al, 2020) and capillary electrophoresis (Kuraeva et al, 2016).

Tabrizi et al. (Tabrizi & Yousefzadeh, 2019) developed a spectrofluorimetric method for the determination of ATE and carvedilol in pharmaceutical preparations, while Damiani et al. developed a spectrofluorimetric method for the determination of ATE in human urine by emission-excitation fluorescence matrices (Damiani, 2011). Basan et al. and Gorbani et al. both developed spectrofluorimetric methods for the determination of ATE in human urine using solid-phase extraction (Basan & Yarimkaya, 2014; Gorbani et al, 2017).

These methods, except spectrophotometry, have good sensitivity and selectivity, but they also require costly instrumentation, highly qualified operators, and usually sample pretreatment procedures when analyzing biological samples, while spectrophotometric methods lack sensitivity and selectivity, particularly with biological samples.

Spectrofluorimetric methods have the advantages of good sensitivity and cost-effective instrumentation and can be used for ATE determination in biological samples such as urine, as ATE has an intrinsic fluorescence.

Thus, the aim of this work is to develop an environment-friendly, sensitive, simple, and economic spectrofluorimetric method for the analysis of ATE in pure powder, pharmaceutical products, and urine using green solvents, along with validation of the method and assessment of its greenness using different metrices.

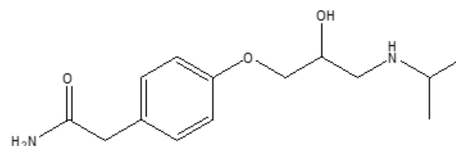


**Fig. 1 ATE chemical structure.**

# 2. Experimental:

## 2.1. Instrumentation:

Shimadzu RF-5301 PC spectrofluorophotometer, equipped with a 150 W Xenon lamp and 1 cm quartz cuvette, controlled from a PC using the Lab Solutions RF software for Windows. The excitation and emission slits were fixed at 5 nm.

A Cole Parmer pH-meter (model 59003) and a Cole Parmer 17250-00 centrifuge (USA) were used.

## 2.2. Materials and reagents:

HPLC organic solvents, ethanol, and isopropanol, were from Sigma Aldrich (Germany). Sodium acetate, acetic acid, and sodium hydroxide (NaOH) were obtained from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). The water utilized throughout the experiment was double distilled water.

A pure ATE sample was kindly supplied by the Egyptian International Pharmaceutical Industries Company, EIPICO, Egypt, and was certified to be 100.16% pure. Ateno® tablets (Batch No. 2104785, manufactured by EIPICO, Egypt), labelled to contain 50 mg ATE per tablet, were purchased from the local market.

## 2.3. Standard solutions and calibration curves:

A stock solution of ATE was prepared by dissolving 10 mg of the pure substance in 10 ml 0.02 N NaOH to obtain a concentration of 1 mg/ml. The working standard solutions were prepared by diluting the stock solution with the solvent 0.02 N NaOH to reach the concentration range of 30-800 ng/ml. All standard solutions were stored in the refrigerator at 4 °C for further use.

Fluorescence intensities were measured at 301±1 nm after excitation at 255 nm using a slit width of 5 nm. A blank experiment was carried out for the solvent mixture and the fluorescence intensities were plotted against the drug concentration to make

calibration curves.

### 2.4. Pharmaceutical sample preparations:

Ten Ateno® tablets were weighted accurately, finely powdered, and mixed thoroughly. An accurate amount equivalent to the content of one tablet (50 mg ATE) weighted, transferred to a 100 ml volumetric flask, and extracted with 70 ml 0.02 N NaOH.

The flask was swirled, sonicated for 15 min, and filtered using a 45 µm pore membrane filter into a 100 ml volumetric flask, the volume was then completed to 100 ml with 0.02 N NaOH to obtain the final solution with a concentration of 0.5 mg/ml ATE. The obtained solution was further diluted to different volumes to obtain final concentrations within the calibration range.

### 2.5. Spiked urine samples:

Aliquots (1.0 ml) from the urine were transferred into centrifugation tubes. The samples were spiked with increasing concentrations of ATE, so that the final concentration was in the range of 30-800 ng/ml. The tubes were mixed well, and 0.1 N NaOH was then added so that the final volume was equal to 5.0 ml. After vortex mixing for 3 minutes, the mixtures were centrifuged at 4000 rpm for 30 minutes. Aspiration of the upper layers was carried out carefully, and the solutions were filtered through a 45 µm disposable membrane filter. Then, 1.0 ml aliquots of the filtrates were quantitatively transferred into a series of 10 ml volumetric flasks, completing the volume with 0.02 N NaOH. A blank experiment was carried out at the same time.

### 2.6. Real human urine samples (in vivo procedure):

The proposed method was applied to investigate the urinary excretion pattern of ATE in a healthy (normal electrocardiogram, liver, and kidney functions) Egyptian male volunteer (aged 31 years, weighing 78 kg, 178 cm height) after an oral administration of 50 mg ATE (single dose Ateno® 50 mg/tablet). The volunteer was instructed to refrain from all medications for 2 weeks before administration and during the study. Also, the volunteer was informed to evacuate the urinary bladder completely just before the administration of one tablet of Ateno® (50 mg) with about 350 mL of water. The 0-h. urine sample was collected as a blank. The urine samples were collected at intervals for up to 24 hours. After each collection, the urine specimen volume was measured, recorded, and stored at -20 °C until the analysis.

A suitable volume (1 ml) from the urine specimen from each sampling point was transferred into centrifugation tubes. The tubes were mixed well, and 0.1 N NaOH was then added so that the final volume was equal to 5.0 ml. After vortex mixing for 3 min, the mixtures were centrifuged at 4000 rpm for 30 min. Aspiration of the upper layers was carried out carefully, and the solutions were filtered through a 45 µm disposable membrane filter. Then, 1.0 ml aliquots of the filtrates were quantitatively transferred into a series of 10 ml volumetric flasks, and the volume was completed with 0.02 N NaOH. Three triplicate determinations of each diluted urine sample solution were done.

Written consent was obtained from the volunteer, and the study was done according to the Egyptian Community guidelines for the use of humans in experiments. The Human Ethics Committee of the Faculty of Pharmacy, Suez Canal University approved this study (201712MH1-238).

## 3. Results and discussion:

Our aim was to develop an ecofriendly, simple, and fast spectrofluorimetric method for determination of ATE in pure form, pharmaceutical tablets and real human urine samples with high sensitivity and accuracy. The Fluorescence intensity of ATE was measured at 301±1 nm after excitation at 255 nm (**Fig. 2**).

### 3.1. Method optimization:

An intrinsic fluorescence study was carried out for ATE to develop a green, simple, accurate and highly sensitive method for its determination in pure or pharmaceutical tablets and biological fluid (urine). Different parameters affecting the intensity of emission were optimized to reach the highest possible sensitivity.

#### 3.1.1. Effect of different solvents:

A study of the diluting solvent was carried out using only some of the green solvents stated in the solvent selection guides to avoid health and environmental hazards (Prat et al, 2014). Ethanol and isopropanol were labelled green according to the guide’s three-color code, so they were chosen in addition to water as the greenest solvent (Prat et al, 2014). It was found that water gave a relatively lower sensitivity, while both propanol and ethanol gave a high blank reading (**Fig. 3**).

#### 3.1.2. Effect of pH and buffers:

The effect of pH changes on the intrinsic fluorescence of ATE was studied using acetate buffer (as environment friendly buffer) at different pH values (3.4–8). It was found that there was no obvious change in the fluorescence intensity of

ATE. Therefore, there was no need to add buffers during analysis (**Fig. 3**).

### 3.1.3. Effect of NaOH:

The effect of NaOH on the intrinsic fluorescence of ATE was carried out using 1 N NaOH, resulting in increased sensitivity with a lower blank reading, so different trails were carried out using different strengths of NaOH (1, 0.1, 0.05, and 0.02 N). It was found that the highest sensitivity was obtained using 0.02 N NaOH, while increasing the normality of NaOH resulted in lower sensitivity. Thus, the 0.02 N NaOH was the optimal choice for the calibration and determination of ATE in pure samples and pharmaceutical tablets samples (**Fig. 3**).

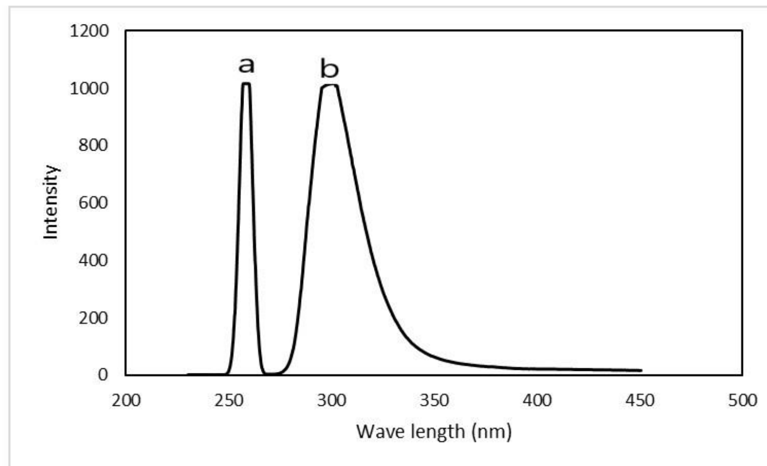


**Fig. 2 Excitation (a) and emission (b) spectra of ATE solution 800 ng/ml in 0.02 N NaOH (X is the wavelength and y axis axis represents the intensity).**

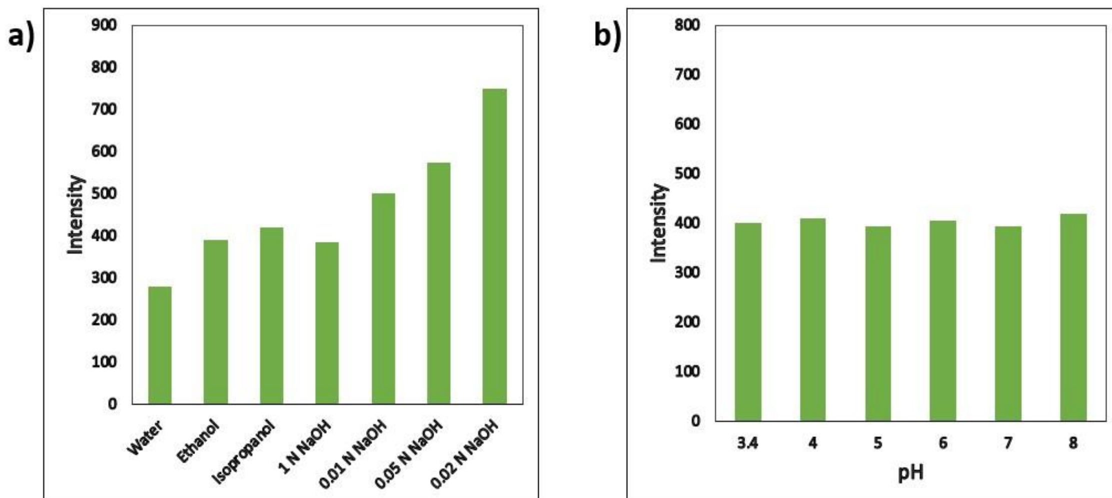


**Fig. 3 Optimization results: a) effect of different solvents b) effect of acetate buffer at different pH values (X axis represents the different conditions and y axis represents the intensity).**

#### 3.1.4. Optimization of procedures for urine samples:

For spiked urine samples with ATE standard solution, the first trial was simply diluting the spiked sample with different solvents (water, ethanol, and isopropanol), but they did not reveal any fluorescence peaks, which may be due to interference from the content of the urine matrix.

Thus, different agents were used (ethanol, isopropanol, ethyl acetate, and NaOH) to overcome the interference of the urine contents with the analysis, followed by centrifugation and filtration.

It was found that all the experiments failed to reveal the fluorescence of ATE except NaOH, so different concentrations and volumes of NaOH were used in the trails to reach the highest possible sensitivity. The previously mentioned procedures in Section 2.5 had the best sensitivity.

### 3.2. Method validation:

To test the performance of the developed spectrofluorimetric method for the determination of ATE in pure solutions, pharmaceutical tablets, and human urine, analytical parameters including linearity, limit of detection (LOD), limit of quantification (LOQ), intraday (repeatability) precision, interday (reproducibility) precision, and accuracy were evaluated according to the ICH guidelines (Guideline, 2005).

#### 3.2.1. Linearity and range:

The linearity and range of the proposed method were assessed by analyzing set of ATE (n = 7) for establishing the calibration curve for standard solutions and urine samples. The calibration curve was obtained by plotting the concentrations versus the corresponding fluorescence intensities. The regression plot showed a linearity range of 30-800 ng/ml with a high value for the resulting correlation coefficient of 0.9998. The characteristics of the proposed method are summarized in Table 1.

#### 3.2.2. Precision:

The method's precision was assessed by evaluating intraday and inter-day precision expressed as relative standard deviation (RSD%). Three concentration levels of ATE covering low, medium, and high levels along its range were used. and each concentration was replicated three times on the same day (repeatability) and over a period of three successive days (intermediate precision).

During precision studies, blank human urine samples were spiked with low, medium, and high concentrations of ATE, and analyzed using the proposed spectrofluorimetric method on the same day (repeatability) and over three consecutive days (intermediate precision).

Table **2** summarizes the results of the precision studies, showing that the calculated RSD% was lower than 2%, which indicates very good precision of the proposed method in terms of repeatability and intermediate precision.

#### 3.2.3. Accuracy:

The standard addition method was applied to a commercial pharmaceutical formulation containing ATE. The mean percentage recovery and the standard deviation for the proposed method for six concentrations (each was repeated three times) were calculated Table 3). Good accuracy was observed for this method. According to the obtained results, the analysis of ATE in its pharmaceutical dose form is unaffected by excipients in the pharmaceutical dosage form (Fig. 4).

Recovery studies on spiked urine samples were also performed (

Table **2**). Relatively high recovery values confirmed the accuracy of the method. Thus, very high accuracy and low LOD and LOQ values were achieved for the determination of trace amounts of ATE in human urine.

#### 3.2.4. Limit of detection (LOD) and limit of quantitation (LOQ):

To evaluate method sensitivity, The LODs and LOQs were calculated based on the following equations: LOD = 3.3 σ/S and LOQ = 10 σ/S, in which (S) is the slope of the calibration curve. The resulted values were 0.037 and 0.121 ng/ml for LODs and LOQs, respectively, indicating good sensitivity, as shown in Table 1.

#### 3.2.5. Selectivity:

There was no interference from common additives found in tablet formulations, indicating that the proposed analytical method has high selectivity as it produced excellent results from analysis of ATE tablet formulations (Table 4).

These additives include colloidal silicon dioxide, magnesium stearate, microcrystalline cellulose, povidone, and sodium starch glycolate as stated by the manufacturer in the package's brochure.

Also, high recovery percents of ATE from the spiked urine samples indicate high selectivity of the proposed method.

#### 3.2.6. Robustness:

To assess the robustness of the proposed method, we measured fluorescence constancy with slight intentional variations in the analysis conditions such as solvent strength and excitation wavelength (255± 2 nm). These little alterations might occur during the practical experiments but did not affect the emission intensity of ATE. The stability of the ATE fluorescence readings during analysis made

this method the one of choice for spectrofluorimetric analysis.

**3.2.7. Stability of standard solutions:**

The stability of ATE standard solutions in 0.02 N NaOH (1 mg/ml) was evaluated by leaving the standard solutions in tightly capped volumetric flasks, protected from light, at room temperature (25°C) and in the refrigerator (4 °C). The standard solution was stable for 6 h. at room temperature and stable for 10 days in the refrigerator.

**Table 1 Characteristic parameters of the calibration equation for the proposed spectrofluorimetric method for determination of ATE solution.**

| Parameters | ATE |
|---|---|
| Calibration range (ng /ml) | 30-800 |
| Detection limit (ng /ml) | 0.04 |
| Quantitation limit (ng /ml) | 0.12 |
| Regression equation (Y)[a]:<br>Slope (b) | 1.30 |
| Standard deviation of the slope ($S_b$) | 0.02 |
| Relative standard deviation of the slope (%) | 1.65 |
| Confidence limit of the slope [b] | 1.28:1.32 |
| Intercept (a) | -31.28 |
| Standard deviation of the intercept ($S_a$) | 8.91 |
| Confidence limit of the intercept [b] | -39.94:-22.63 |
| Correlation coefficient (r) | 0.9998 |

[a]Y = a + bC, where C is the concentration of compound in ng/ml and Y is the fluorescence intensity.
[b] 95% confidence limit.

**Table 2 Precision of the proposed spectrofluorimetric method for analysis of ATE in pure solution and spiked urine samples.**

| Matrix | conc (ng/ml) | Intra-day | | Inter-day | |
|---|---|---|---|---|---|
| | | Recovery%[a]±SD[b] | RSD% | Recovery%[a]±SD[b] | RSD% |
| Pure solution | 40 | 99.91±0.71 | 0.71 | 99.86±0.81 | 0.81 |
| | 300 | 100.06±0.94 | 0.94 | 99.76±1.04 | 1.04 |
| | 700 | 100.03±1.06 | 1.06 | 100.09±0.79 | 0.79 |
| Urine | 40 | 100.12±1.09 | 1.09 | 99.15±1.03 | 1.04 |
| | 300 | 99.27±0.96 | 0.97 | 101.02±0.93 | 0.92 |
| | 700 | 99.18±1.03 | 1.04 | 98.97±0.83 | 0.84 |

a: mean of three measurements.
b standard deviation.

**Table 3 Application of standard addition technique to Ateno® tablets solution using the proposed spectrofluorimetric method for analysis of ATE.**

| Claimed conc. (ng/ml) | Added conc. (ng/ml) | % Recovery of added[a] |
|---|---|---|
| 40 | 40 | 100.5 |
| 60 | 50 | 99.81 |
| 100 | 100 | 100.19 |
| 300 | 50 | 101.09 |
| 400 | 150 | 98.93 |
| 600 | 100 | 100.15 |
| **Mean** | | 100.11 |
| **± SD** | | 0.72 |

[a] mean of three determinations.

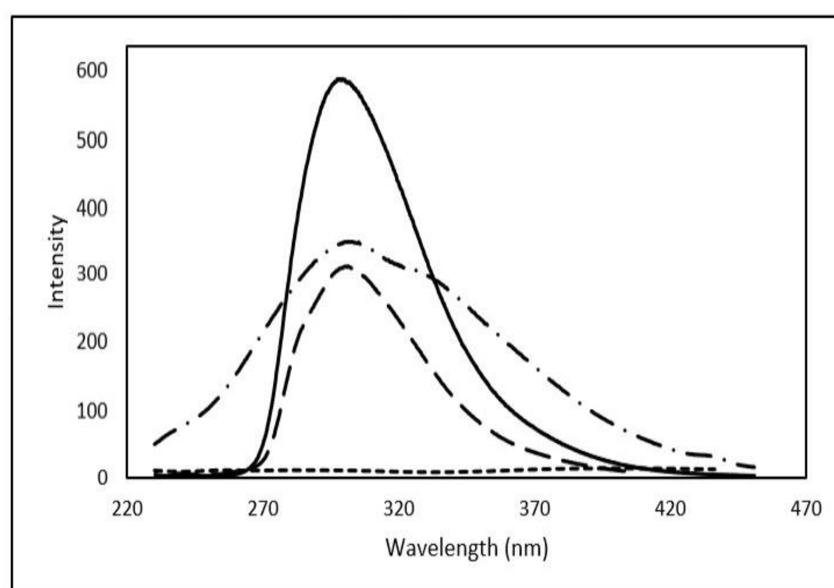


**Fig. 4 Emission spectra of pure ATE 500 ng/ml (———), Ateno® solution containing 250 ng/ml ATE (— — —), urine sample containing 300 ng/ml (— - —— -) and blank urine (--------) (X axis is the wavelength and y axis represents the intensity).**

### 3.3. Analysis of pharmaceutical products:

The proposed method was applied for the determination of ATE in its pharmaceutical preparation, Ateno® tablets (Table 4). Five replicate determinations were made, and satisfactory results were obtained for ATE in good agreement with label claims. The results showed no interference with tablet excipients from the preparation, which indicates high selectivity of the proposed method (Fig. 4).

The official British Pharmacopoeia spectrophotometric method (2019) was applied for determination of ATE in tablets. A statistical analysis of the results obtained by the proposed method and the official method (2019) was made using the Student's t-test and variance ratio F-test. The results presented in Table 4 show that the calculated t and F values are lower than the theoretical ones at 95% confidence level, indicating that there is no significant difference between the two methods with respect to accuracy and precision.

### 3.4. Urinary excretion pattern of ATE:

To check the clinical applicability of the proposed spectrofluorimetric method, the urinary excretion pattern of ATE was studied in a healthy male volunteer after a single oral administration of 50 mg of ATE. The urinary excretion pattern of ATE during 24h and the cumulative excretion pattern are presented in **Fig. 5**.

Following oral administration, it was noticed that approximately 44% of the administered drug was excreted unchanged within 24 h. These ATE urine excretion data were in agreement with the literature (Wan et al, 1979) proving the applicability of the proposed method for the analysis of ATE in human urine samples.

**Table 4 Determination of ATE in Ateno® tablets solution using the proposed spectrofluorimetric method.**

| No. | Proposed method | | Official method (2019) | |
|---|---|---|---|---|
| | ATE Conc.ng/ml | % Recovery | ATE Conc.ng/ml | % Recovery |
| 1 | 40 | 99.03 | 50 | 98.91 |
| 2 | 100 | 100.87 | 100 | 99.62 |
| 3 | 250 | 99.53 | 200 | 100.31 |
| 4 | 500 | 100.96 | 300 | 100.19 |
| 5 | 700 | 98.81 | 500 | 99.37 |
| | **Mean** | 99.84 | **Mean** | 99.68 |
| | **SD** | 0.91 | **SD** | 0.58 |
| | **Student t-test [a]** | 0.31 | | |
| | **F-test [a]** | 3.06 | | |

a the tabulated t value and F value are 2.78 and 6.39, respectively at 95% confidence level (P = 0.05, n =5).

**4. Assessment of method greenness:**

Different metrices were used to assess the proposed method's environmental and health impacts to ensure its greenness.

NEMI was used for the preliminary judgment, followed by AGREE, GAPI, and eco-scale assessments to obtain a comprehensive greenness evaluation with more information regarding the greenness of each step.

**4.1. National environmental methods index (NEMI):**

According to the National environmental methods index (NEMI), 4 quarters circle is drawn and each quarter is colored green or left blank indicating these criteria : PBT (persistent, bio accumulative and toxic), corrosive (2<pH<12), hazardous and waste (Plotka-Wasylka, 2018).

The proposed method has 4 quarters green circle as all the reagents used are not hazardous, PBT or corrosive with minimal waste (**Fig. 6**).

**4.2. The Analytical GREEnness Metric (AGREE):**

AGREE is based on the twelve principles of GAC. It is a clock-shaped graph divided around its perimeter into 12 parts, each representing one GAC principle. Their color code ranges between red, yellow, and green, representing high, medium, and low impacts. The center of its graph introduces a numerical figure (excellent green equals 1) with different colors to represent an overall assessment of the method's greenness (Pena-Pereira et al, 2020). The proposed method was found to be excellent green with a score of 0.96 (**Error! Reference source not found.**).

**4.3. GAPI (Green Analytical Procedure Index):**

GAPI offers a comprehensive ecological evaluation of the entire technique, from the first step of sample preparation all the way through to the last step. It is shown as a colorful pictogram made up of 15 portions, each corresponding to one of the 15 evaluated factors (Plotka-Wasylka, 2018).

These sections refer to sample collection, preservation, transportation, usage of solvents and reagents, energy use, waste production, and other instrumental aspects. Each part is colored green, yellow, or red to reflect a low, medium, or high environmental effect (Plotka-Wasylka, 2018).

The proposed method was found to be highly green after visual analysis of the resulting GAPI pictogram, since it produced 11 green zones, 3 yellow zones, and only 1 red zone (**Fig. 8**).

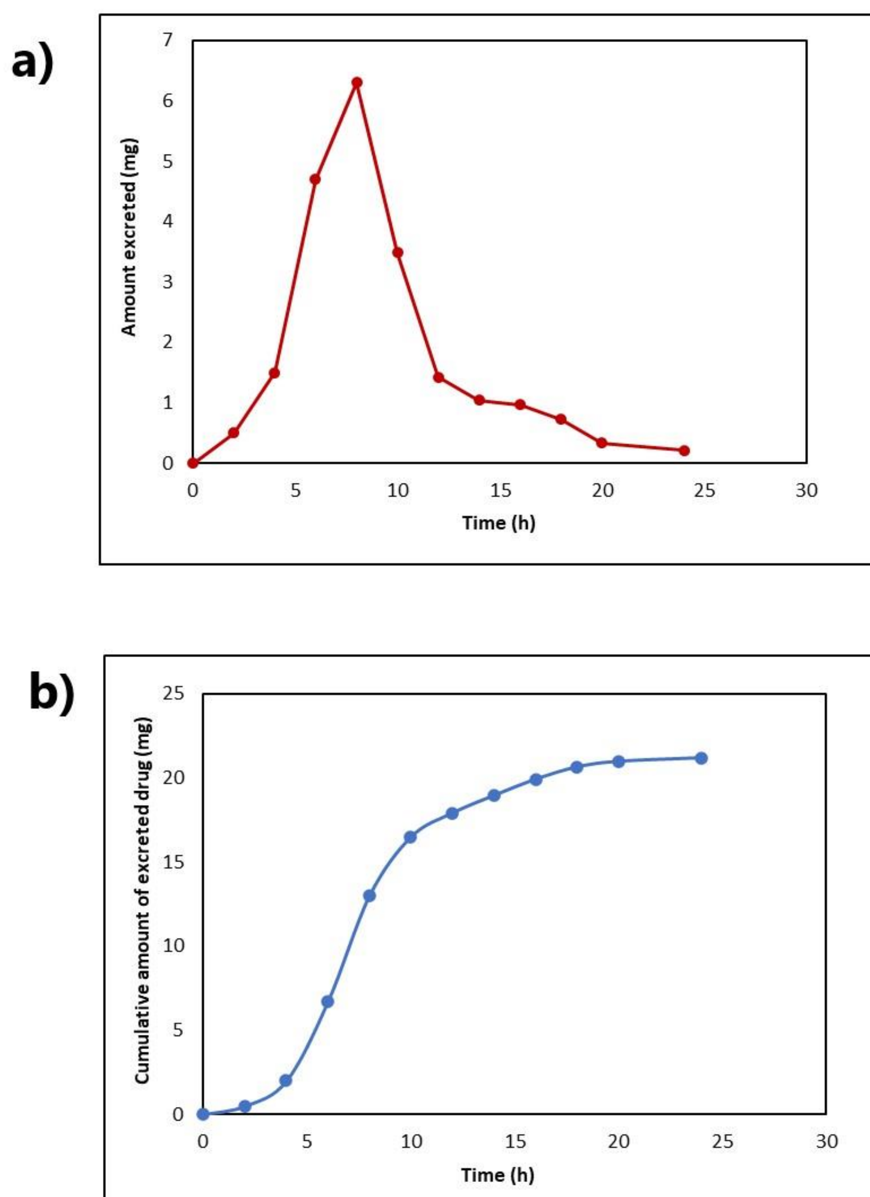


**Fig. 5 a) Urinary excretion pattern of ATE b) cumulative urinary excretion of ATE after oral dose of Ateno® tablets (50 mg) (X axis is the time (h) and y axis represents the amount (mg) of excreted ATE for (a) and cumulative amount (mg) of excreted ATE for (b)).**

**Fig. 6 NEMI assessment of greenness of the proposed spectrofluorimetric method.**

**Fig. 7 AGREE greenness assessment of the proposed spectrofluorimetric method.**

### 4.4. Eco-scale assessment:

It is a good, semiquantitative tool that considers all the steps of the analytical process, the devices, and the waste. Analysis with no or minimal use of reagents, lower energy consumption, and no waste is considered ideal green analysis with a score of 100 points.

If any of these parameters deviate from the ideal green analysis, penalty points are assigned, and the total number of penalty points is subtracted. The analysis with an Eco-Scale value >75 is considered excellent green; if the value <75- >50 it is acceptable, and <50 is inadequate green analysis (Gałuszka et al, 2012).

The eco scale of the proposed method proves it is excellent green with a score of 95 points (**Table 5**).

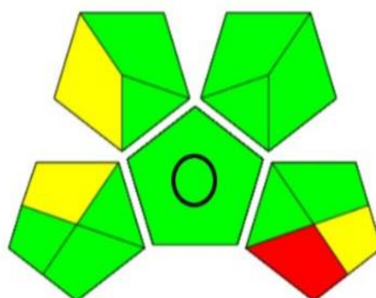


**Fig. 8 GAPI greenness assessment of the proposed spectrofluorimetric method.**

## 5. Comparison with other methods:

The proposed method was compared to two previously published spectrofluorimetric methods for ATE determination in urine(Basan & Yarimkaya, 2014; Gorbani et al, 2017). The proposed method has the advantages of high sensitivity, low cost, short time, simplicity of procedures, and using environmentally friendly solvents.

Our method revealed itself to be more sensitive when compared to the reported methods(Basan & Yarimkaya, 2014; Gorbani et al, 2017) due to lower LOD and LOQ and a higher correlation coefficient.

Basan et al. (Basan & Yarimkaya, 2014) used a solid phase extraction method, whereas Gorbani et al sorbent, which both required a longer time and relatively more expensive materials than our purposed method.

The two methods were applied to spiked urine samples only (Basan et al.'s method was applied to pharmaceutical tablets too), while our method was applied for analysis of ATE in pharmaceutical tablets and in both spiked and real human urine samples in addition to elucidation of the cumulative excretion pattern of ATE in urine after a volunteer took a 50 mg oral dose.

The proposed method used green solvents and simple procedures, so it is considered an eco-friendly substitute for the determination of ATE in quality control laboratories and in real urine

al.(Gorbani et al, 2017) used a high affinity samples.

**Table 5 Eco-scale assessment for the greenness of the proposed spectrofluorimetric method.**

| Parameters | Penalty points |
|---|---|
| Reagents | |
| – Water | 0 |
| – NaOH | 2 |
| Energy consumption | 0 |
| Occupational hazards | 0 |
| Waste | 3 |
| Total penalty points | ∑5 |
| **Analytical Eco-scale score** | **95** |

## 6. Conclusion:

An environment friendly, simple, economic, and fast spectrofluorimetric method for ATE determination in either its pure form, its pharmaceutical tablets or real human urine samples with high sensitivity and accuracy.

The method was validated according to the ICH guidelines, proving that it is accurate, specific, precise, and robust. So, it is suitable for quality control analysis of ATE because of its simplicity, high sensitivity, and independence from expensive instrumentation. It's also suitable for the detection of drug abuse in precision sports because it is sensitive enough to analyze ATE on a regular basis at therapeutic urine levels.

The method complies with the principles of GAC as it takes a very short time for the analysis of ATE with high sensitivity, low cost and simple instruments, small energy consumption, very little waste, and is free from harmful solvents, ensuring operator safety.

The proposed method's high level of greenness was proved by its high eco-scale score (95 points) as well as by the NEMI, GAPI and AGREE greenness assessments.

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