Winter 2-22-2022

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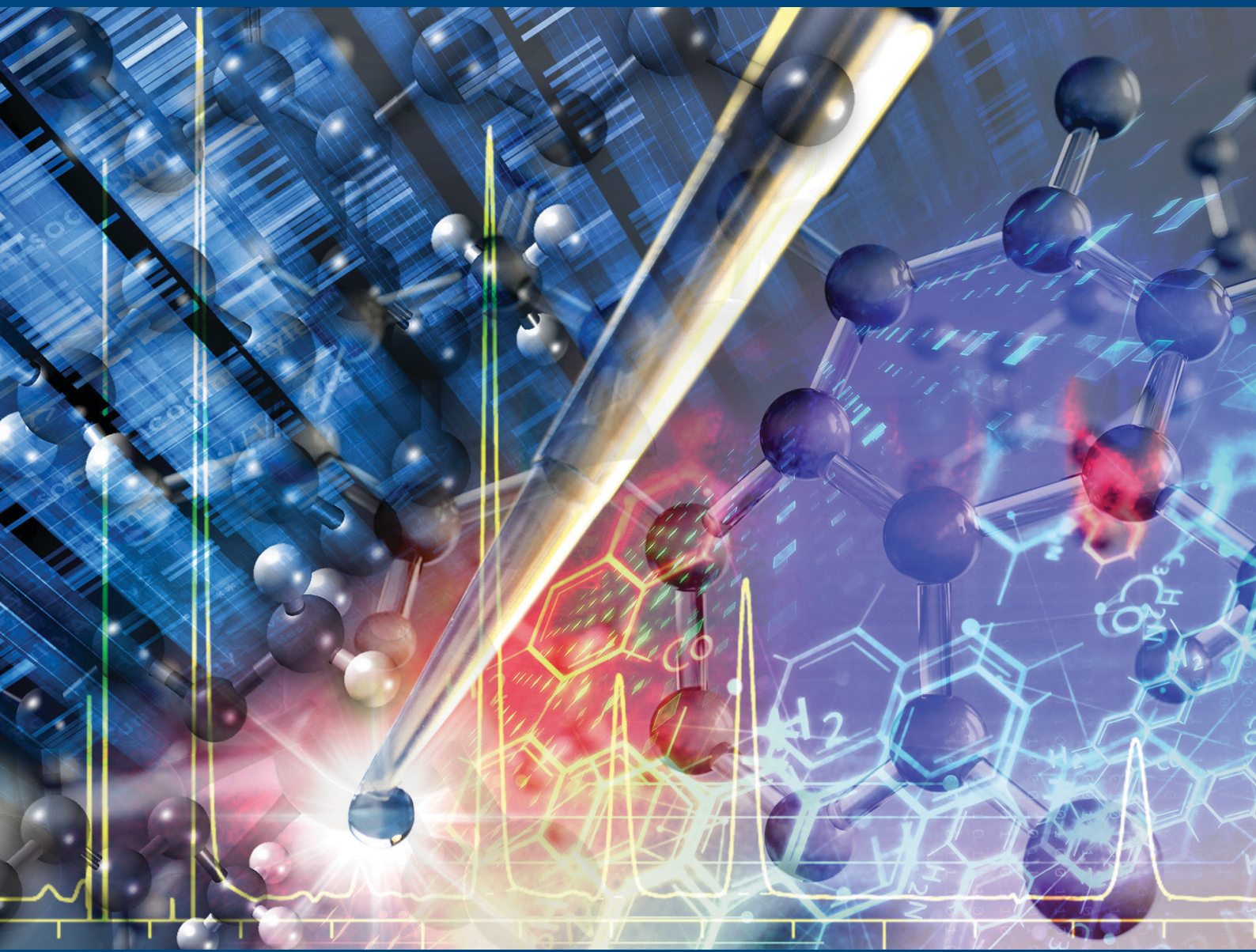
ISSN 1615-9306 · JSSCCJ 45 (4) 2022 · Vol. 45 · No. 4 · February 2022

Journal of SEPARATION SCIENCE

4|2022



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WILEY-VCH

Received: 5 September 2021 | Revised: 4 December 2021 | Accepted: 7 December 2021

DOI: 10.1002/jssc.202100711

JOURNAL OF SEPARATION SCIENCE

RESEARCH ARTICLE

Development of a multivariate model with desirability-based optimization for determination of atenolol and hydrochlorothiazide by eco-friendly HPLC method with fluorescence detection

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Determination of a widely used antihypertensive combination of atenolol and hydrochlorothiazide was achieved by rapid and eco-friendly high-performance liquid chromatography method combined with fluorescence detection. The response surface methodology is conducive to the complete separation of the two drugs in a shorter analysis time. The separation of the mixture was achieved using an Inertsil C18 analytical column (150 × 4.6 mm, 5 μ). The mobile phase used was ethanol: potassium dihydrogen phosphate at pH 3 (65:35 v/v) and the flow rate was 0.7 mL/min. The fluorescence detector operated at excitation and emission wavelengths of 230 and 310 nm (atenolol) and 270 and 375 nm (hydrochlorothiazide). The linearity of the developed method covered a concentration of atenolol of 0.05–5 μg/mL and a concentration of hydrochlorothiazide of 0.02–1 μg/mL. The greenness of the developed method was evaluated by analytical eco-scale and the recently reported evaluation method, that is, green analytical procedure index, and it was found to be an excellent, sensitive, and green alternative to the reported methods. The developed method was validated according to the ICH guidelines and compared with the reference method. No significant difference was found in terms of accuracy.

KEYWORDS
atenolol, fluorescence, green, hydrochlorothiazide, response surface methodology

1 | INTRODUCTION

One of the risk factors for atherosclerosis and ischemic heart disease is hypertension. Although modern and effective antihypertensive drugs can be used, most patients have poor blood pressure control. This is why doctors prescribe a combination of antihypertensive drugs to control high blood pressure. In order to achieve treatment goals, most patients need at least two antihypertensive drugs [1]. The latest report from the Joint National Committee (JNC VI) and the World Health Organization recommends the combination of beta-blockers and diuretics as first-line treatment for uncomplicated essential hypertension [2,3].

Atenolol (Figure S1) is a ß1 selective adrenergic blocker. Due to its additive effect, it can be used simultaneously with diuretics. Hydrochlorothiazide (Figure S1) is a thiazide diuretic, which can reduce the reabsorption of electrolytes in the renal tubules, thus increasing the excretion

Article Related Abbreviations: GAPI, green analytical procedure index; FLD, fluorescence detection; RSM, response surface methodology

of sodium and chloride, and also the excretion of water. In some preliminary experiments, potassium ions were also lost as adverse side effects [4].

Differences in the solubility, log P, pK_a, and absorption spectra of these drug combinations make their separation and determination difficult, especially when green solvents are used in method development [5].

Literature survey revealed different reported methods for determination of atenolol and hydrochlorothiazide including UV-spectrophotometry [6–8], HPLC [9, 10], and CE [11]. Unfortunately, most of the solvents used may be harmful to the environment and human health [12,13]. They also did not provide sufficient sensitivity for pharmacokinetic research applications and were developed by changing One Factor At a Time (OFAT), which has proven to be time consuming, costly, does not resolve errors, and can produce unpredictable responses [14]. The analysis time of all reported chromatographic methods is at least 7 min, which is considered a long time from the perspective of green analytical chemistry as it will result in large amount of waste.

For solving all the shortcomings of the reported methods, the following was adopted:

1. Ethanol is not harmful to the environment and is used as an organic modifier in the mobile phase and an extraction solvent for pharmaceutical preparations [15, 16].
2. Due to the high sensitivity of the fluorescence detector, HPLC with fluorescence detection (FLD) is used [17–19].
3. Response surface methodology (RSM) is used to reduce the number of preliminary experiments and help separate analytes in the shortest possible run time.

Therefore, the objective of this work was to develop a new environmentally friendly, sensitive, and rapid HPLC-FLD method for the determination of the antihypertensive combination of atenolol and hydrochlorothiazide in bulk and pharmaceutical preparations. The developed method was evaluated by analytical eco-scale and the green analytical procedure index (GAPI) assessment tool and has proven to be an excellent eco-friendly alternative to the reported method [20, 21].

2 | MATERIALS AND METHODS

2.1 | Materials and solvents

Atenolol and hydrochlorothiazide reference standards were kindly supplied from National Organization for Drug Control and Research (NODCAR, Cairo, Egypt). The purity of the standards was certified to be 99.5 and 99.7%, respectively.

- Ethanol (HPLC grade; Fisher Scientific, UK).
- Water (HPLC grade; Fisher Scientific).
- Aten-H 25 tablets manufactured by Zydus Cadila Healthcare, India, and labeled to contain 50 mg atenolol and 25 mg hydrochlorothiazide.
- Potassium dihydrogen orthophosphate (Fisher Scientific, Pittsburgh, PA, USA).

Preparation of 25 mM buffer: 3.4 g was dissolved in 250 mL water, then the pH was adjusted to 3 using 0.1 N HCl. Then the volume was completed to 1000 mL with water.

2.2 | Instrument and software

The separation was achieved using a chromatographic system Schimadzu LC-2040C 3D PLUS nexera – i (Kyoto, Japan) equipped with Fluorescence detector (RF-20AXs), LC-2040 pump and four-line degasser. The separation was achieved using Inertsil C-18 (150 × 4.6 mm, 5 μ) analytical column. Data acquisition was performed with LabSolutions software. JMP 13.2.1 (Copyright 2012, SAS Institute, Cary, NC, USA) was used for experimental response surface design and interpretation of statistical data.

2.3 | Procedures

2.3.1 | Solutions

Preparation of standard stock solutions

Atenolol and hydrochlorothiazide standard stock solutions were prepared by dissolving 10 mg standard powder in a minimum amount of ethanol in a 100 mL volumetric flask and bringing to the mark with water. The standard stock solution of the analyte is then diluted with the mobile phase to prepare a standard working solution (50 μg/mL).

2.3.2 | Chromatographic conditions

Separation of atenolol and hydrochlorothiazide on an Inertsil C18 column (150 × 4.6 mm, 5 μ). The RSM was applied using a three-level two-factor experimental design to optimize ethanol percentage and flow rate. The 10 random runs generated are shown in Table S1. Optimum performance was obtained using ethanol-potassium dihydrogen orthophosphate buffer with pH 3 adjusted by 0.1 N HCl (65:35 v/v). The fluorescence detector operates at excitation and emission wavelengths of 230 and 310 nm

(atenolol) and 270 and 375 nm (hydrochlorothiazide). The flow rate was 0.7 mL/min and the injection volume was 10 µL.

2.3.3 | Validation

Linearity and range

Working standard solutions of atenolol and hydrochlorothiazide were used to prepare calibration standards for both drugs. The samples were chromatographed in triplicates. The peak area of the analytes was plotted versus the corresponding concentrations. Then, the regression equations were calculated.

Accuracy

Three replicates of six concentrations of both atenolol and hydrochlorothiazide were determined to check method accuracy. The percentage recoveries were calculated from the corresponding regression equation.

Precision repeatability

Different concentrations of atenolol and hydrochlorothiazide were injected three times on the same day under the same experimental conditions. The relative standard deviations were calculated.

Intermediate precision

The previous procedures were repeated interdaily on three different days for the analysis of three previously chosen concentrations. The relative standard deviations were calculated.

Limit of quantitation and limit of detection

According to ICH recommendations, S/N is used to determine LOQ and LOD in chromatographic methods. A S/N between 3:1 is generally considered acceptable for estimating the detection limit where ratio 10:1 is used to estimate the quantitation limit.

2.3.4 | Application to pharmaceutical preparation

Ten tablets of Aten-H 25 formulation were accurately weighed, finely powdered, and homogeneously mixed. An accurately weighed portion of the homogenized powder equivalent to 25 mg of atenolol and 12.5 mg hydrochlorothiazide was dissolved in 60 mL of the mobile phase using an ultrasonic bath for 30 min. The solution was further diluted to 100 mL with the same mobile phase and then filtered using a 0.45 µm nylon membrane filter disc before use. Then further dilutions of the above solution were prepared to achieve concentrations within the linearity range. The concentration of each drug was calculated using the specified regression equation.

3 | RESULTS AND DISCUSSION

Chromatographic methods developed for the simultaneous determination of drug combinations that do not require pretreatment are very important for quality control laboratories. In this study, a green, rapid, and sensitive HPLC-FLD method was developed and validated for the determination of the antihypertensive combination atenolol and hydrochlorothiazide in bulk and pharmaceutical preparations.

Literature survey revealed that all the reported methods for determination of this combination used environmentally toxic solvents like methanol (MeOH) and/or acetonitrile (ACN). MeOH and ACN are classified as hazardous solvents by the United States Environmental Protection Agency (EPA) due to their inherent toxicity [22]. In addition, the conditions used in the reported chromatographic methods cannot achieve the separation of the mixture in less than 7 min, which is considered time consuming. It was found that previously reported methods were not sensitive enough, which made their application difficult in certain applications, such as pharmacokinetic studies. Therefore, RSM experimental design provides a viable solution to overcome the mentioned shortcomings in reported methods.

Screening tests showed that pH 3 was the most suitable for separation, where pH 5 and 7 caused overlapping of the two peaks or very broad peaks of atenolol as shown in Figure S2. The retention time of hydrochlorothiazide is not affected by pH changes, because the pK_a of hydrochlorothiazide is 7.9, while the pK_a of atenolol is 9.6 [23]. Screening experiments showed that the ethanol percentage and the flow rate have the greatest impact on the separation of the analytes' peaks. Two-factor three-level experimental design was established by JMP software that created 10 runs for developing the model as shown in Table S1. All the samples were chromatographed, and the statistical data revealed the following:

$$Y_{Rs} = 4.924 - 0.5268\,(x_1) - 0.5885\,(x_2) - 0.0622\,(x_1)\,(x_2) - 0.4333x_1^2 + 0.0137x_2^2 \quad (1)$$

$$Y_{N1} = 1772.357 + 287.1667\,(x_1) - 455\,(x_2) - 156.75\,(x_1)\,(x_2) - 195.7143x_1^2 + 15.7857x_2^2 \quad (2)$$

$$Y_{N2} = 3350.7143 + 743.3333\,(x_1) - 594.1667\,(x_2) - 255\,(x_1)\,(x_2) - 784.4286x_1^2 + 3.0714x_2^2 \quad (3)$$

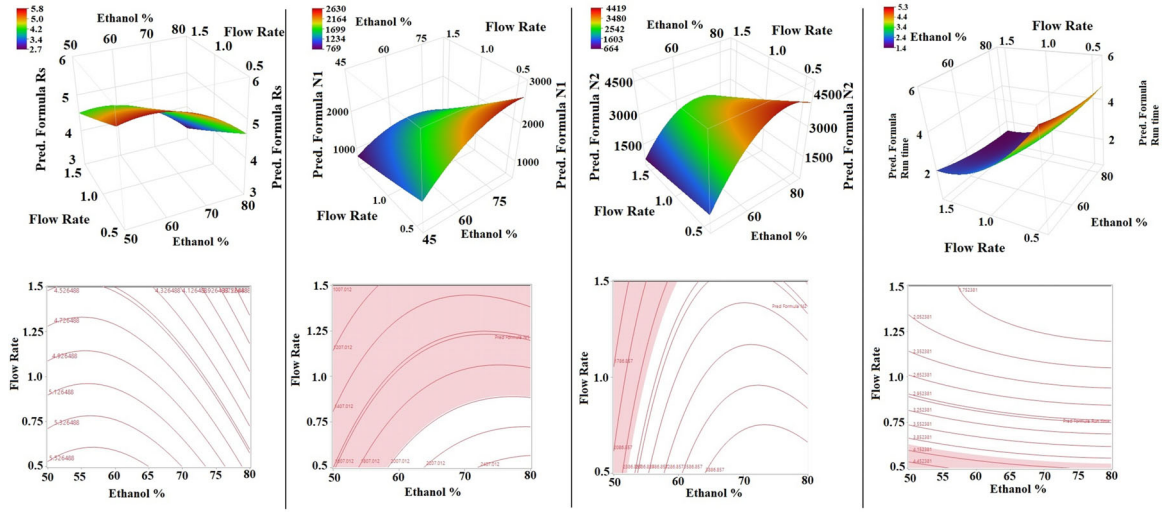

FIGURE 1 Surface plots (top) and contour profilers (bottom) for the effect of ethanol percentage and flow rate on the studied responses

$$Y_{\text{Run}} = 2.3214 - 0.2500\,(x_1) - 1.3333\,(x_2) + 0.1071x_1^2 + 0.6071x_2^2 \quad (4)$$

where (x_1) is the ethanol percentage and (x_2) is the flow rate.

As shown in the previous equations, quadratic models were suggested for all the responses.

According to regression (1), lower ethanol percentage and flow rate will increase the resolution. While regressions (2) and (3) showed that increasing ethanol percentage and decreasing flow rate are required to increase column efficiency. Regression (4) suggested that increasing ethanol percentage and flow rate are required to shorten the run time.

The influence of factors on the responses can be fully understood through the surface and the contour profilers, as shown in Figure 1. Table 1 lists the estimated values of the predictive model parameters, the main, and interactions effects of the factors on each of the studied responses. Desirability-based optimization of the factors was utilized in the prediction profiler tool in JMP software, where, resolution, N1, and N2 were set to maximum and run time was set to minimum as shown in Figure 2. Note that 65% ethanol and flow rate of 0.7 ml/min were suggested by the prediction profiler tool to give the ideal responses.

Table 2 shows all the system suitability of the developed method.

3.1 | Validation

ICH guidelines were followed for the validation of the developed method [24]. The proposed method was found to be accurate and precise as presented in Table 3.

3.1.1 | Linearity and range

Six different concentrations covering the range 0.05–5 μg/mL for atenolol and 0.02–1 μg/mL for hydrochlorothiazide were used to assess method linearity. Correlation coefficients were 0.9998 for atenolol and hydrochlorothiazide. Regression equation of atenolol calibration was ($Y = 0.5274x + 1.2841$), while the regression equation of hydrochlorothiazide was ($Y = 0.8534x + 1.2823$).

3.1.2 | Accuracy and precision

Accuracy of the method for determination of both drugs was evaluated and the recoveries were above 98%. The precision was evaluated in terms of precision repeatability and intermediate precision where the RSD% was below 1.4%.

3.1.3 | Limit of quantitation and limit of detection

S/N was used to determine LOQ and LOD in chromatographic methods. LOQ values were 0.01 and 0.02 μg/mL for atenolol and hydrochlorothiazide, respectively, where LOD values were 0.003 and 0.007 for atenolol and hydrochlorothiazide, respectively.

3.2 | Greenness assessment

Due to the number and diversity of analytical methods and the special analysis criteria that must be considered (such as precision, accuracy, and LOD), it is not easy to

TABLE 1 Parameter estimates of studied factors

Response	Factor	Estimate	SE	*t* Ratio	Prob > |t|
Resolution	Ethanol(50,80)	−0.527	0.041	−13.000	0.0002
	Flow rate(0.5,1.5)	−0.589	0.041	−14.520	0.0001
	Ethanol*Flow rate	−0.062	0.050	−1.250	0.2781
	Ethanol*Ethanol	−0.433	0.065	−6.670	0.0026
	Flow rate*Flow rate	0.014	0.065	0.210	0.8432
N1	Ethanol(50,80)	287.167	29.309	9.800	0.0006
	Flow rate(0.5,1.5)	−455.000	29.309	−15.520	0.0001
	Ethanol*Flow rate	−156.750	35.895	−4.370	0.0120
	Ethanol*Ethanol	−195.714	46.998	−4.160	0.0141
	Flow rate*Flow rate	15.786	46.998	0.340	0.7538
N2	Ethanol(50,80)	743.333	52.900	14.050	0.0001
	Flow rate(0.5,1.5)	−594.167	52.900	−11.230	0.0004
	Ethanol*Flow rate	−255.000	64.789	−3.940	0.0170
	Ethanol*Ethanol	−784.429	84.828	−9.250	0.0008
	Flow rate*Flow rate	3.071	84.828	0.040	0.9729
Run time	Ethanol(50,80)	−0.250	0.070	−3.550	0.0238
	Flow rate(0.5,1.5)	−1.333	0.070	−18.930	<.0001
	Ethanol*Flow rate	0.000	0.086	0.000	1.0000
	Ethanol*Ethanol	0.107	0.113	0.950	0.3965
	Flow rate*Flow rate	0.607	0.113	5.380	0.0058

*Significant values at $p = 0.05$.

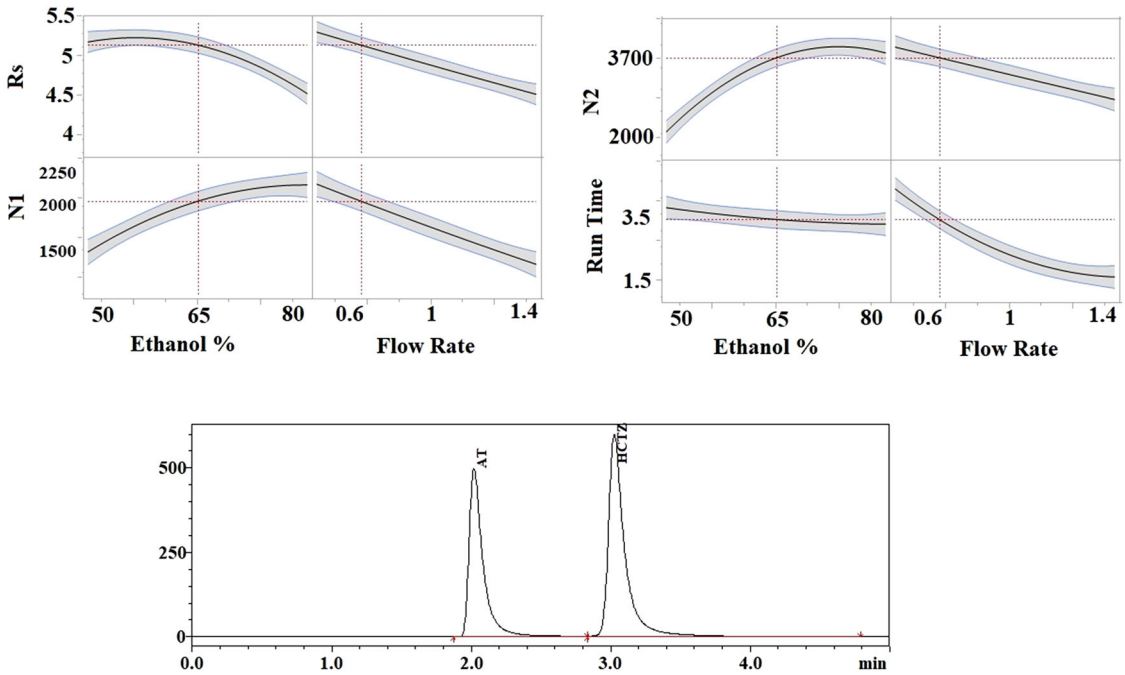

FIGURE 2 Prediction profilers showing the effect of each factor on the responses, desirability set to maximum, and complete resolution of the analytes by the proposed HPLC method

TABLE 2 System suitability parameters of the developed method

Drug	Parameters	C18 column 150 x 4.6 mm
Atenolol	Retention time (min)	2.02
	NTP*	2334.00
	HETP (μm)**	64.27
	Resolution***	5.65
	Symmetry factor	1.25
Hydrochlorothiazi	Retention time (min)	3.02
	NTP	4104.00
	HETP (μm)	36.55
	Resolution****	–
	Symmetry factor	1.15

*Number of theoretical plates.
**Height equivalent to theoretical plates.
***Resolution relative to the next peak.

evaluate the greenness of the developed analysis methods [20]. Evaluation of greenness extent of the developed method was performed by the following methods.

3.2.1 | Analytical eco-scale

It depends on subtracting penalty points from the developed method. The penalty points depend on the reagent used, amount, exposure of the analyst, instruments, and the amount and treatment of waste [20]. Methods succeeded to score more than 75 points are considered excellent green methods. Table S2 shows the score of the developed HPLC-FLD method, which is considered excellent green method according to eco-scale.

3.2.2 | Green analytical procedure index

GAPI consists of five pentagrams, which represent the environmental impact of the method developed. It is colored in three different colors: red, yellow, and green, corresponding to high, medium, and low impacts. By visually inspecting the pentagrams, the environmental impact of the developed method and other methods can be evaluated and compared. When comparing the developed method with the reported chromatographic methods [9,10], it was found to be a successful eco-friendly alternative method, as shown in Figure 3.

3.3 | Statistical comparison with other reported methods

Accuracy results of the developed method were statistically compared with reported method [10] and no significant differences were found as shown in Table S3.

So, the proposed HPLC-FLD method is considered to be the first eco-friendly method for determination of atenolol and hydrochlorothiazide combination in short analysis time with high sensitivity.

4 | CONCLUDING REMARKS

A green HPLC-FLD method was developed and validated for determination of atenolol and hydrochlorothiazide. Ethanol was used as the organic modifier as it is environmentally nontoxic. The method is highly sensitive because of the fluorescence detector. RSM experimental design was utilized to completely separate the mixture in

TABLE 3 Validation parameters and assay of pharmaceutical formulation by the proposed method

Parameter	Atenolol	Hydrochlorothiazide
Range μg/mL	0.05–5	0.02–1
Regression Equation	$y = 0.5274x + 1.2841$	$y = 0.8534x + 1.2823$
Correlation coefficient (r)	0.9998	0.9998
Accuracy[a]	98.68 ± 0.81	99.81 ± 0.428
Repeatability[b]	101.42	99.57
RSD%	0.982	1.351
Intermediate precision[c]	101.55	98.57
RSD%	1.217	0.974
LOQ (μg/mL)	0.01	0.02
LOD (μg/mL)	0.003	0.007
Recovery of pharmaceutical preparation[d]	98.47 ± 1.712	99.73 ± 1.628

[a]Six concentrations of each analyte covering the range (0.08–4 μg/mL) for atenolol, and (0.05–0.8 μg/mL) for hydrochlorothiazide.
[b]Intraday ($n = 3$), average of three concentrations of the analytes repeated three times within the same day.
[c]Interday ($n = 3$), average of three concentrations of the analytes repeated three times in three consecutive days.
[d]Aten-H25 tablets labeled to contain 50 mg atenolol and 25 mg hydrochlorothiazide.

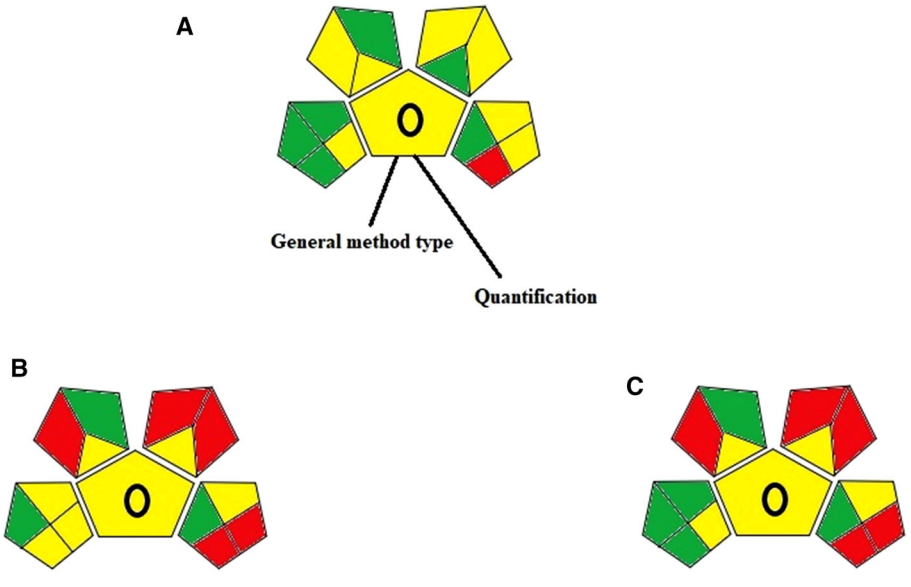

FIGURE 3 Green analytical procedure index (GAPI) assessment tool for the proposed method (a) in comparison with reported methods (b) and (c)

short run time. The developed method was compared with other reported methods and found to be more sensitive and rapid. GAPI assessment tool was used to evaluate the method greenness and found to be excellent green alternative to reported chromatographic methods. The developed method is the first green HPLC-FLD method for the mentioned combination using response surface methodology.

FUNDING INFORMATION

None.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Hemdan A, Al-Tannak NF, Mohamed EH. Development of a multivariate model with desirability-based optimization for determination of atenolol and hydrochlorothiazide by eco-friendly HPLC method with fluorescence detection. J Sep Sci. 2022;45:824–831. https://doi.org/10.1002/jssc.202100711