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A GREEN HPLC METHOD FOR THE DETERMINATION OF TORASEMIDE IN PHARMACEUTICAL DOSAGE FORMS: DEVELOPMENT, VALIDATION, AND GREENNESS ASSESSMENT

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ABSTRACT. Two HPLC methods were developed for the determination of torasemide in pharmaceutical products. In the first method, a C_{18} column whose temperature was kept constant at 25 °C was used. A combination of 0.1% formic acid solution in water and acetonitrile (50/50, v/v) was used as the mobile phase, and isocratic elution was performed at a flow rate of 1.0 mL min^{-1}. Detection was carried out at 288 nm using the UV detector. Although all other conditions are the same as in the first method, the only difference in the second method is that ethanol is used instead of acetonitrile as the organic modifier in the mobile phase. HPLC methods were validated in accordance with ICH guidelines. Correlation coefficients were greater than 0.999 in the concentration range of 5-30 mg mL^{-1}. Later, HPLC methods were applied to pharmaceutical formulations. Results were compared using the student (t) test for means and the Fischer (F) test for standard deviations. No significant differences were observed between methods. Additionally, a greenness evaluation of the developed methods was carried out using AGREE software. As a result, the latter method was proposed as an excellent eco-friendly alternative for the determination of torasemide in pharmaceuticals.

KEY WORDS: Green HPLC, Analytical method, Torasemide, Pharmaceuticals

INTRODUCTION

High-performance liquid chromatography (HPLC) is widely used for drug analysis in the production and quality validation of pharmaceutical formulations [1]. HPLC methods generally use a hydrophobic stationary phase and a polar mobile phase for effective separation. Ultraviolet (UV)/Visible detector mode is generally used in quality control laboratories. For this reason, the compatibility of the mobile phase with the detector is a parameter that is frequently considered while developing a pharmaceutical analysis technique. HPLC mobile phases typically consist of a combination of water (with additions that will change the pH and ionic strength) and organic solvents such as acetonitrile/methanol. Acetonitrile and methanol are widely used organic solvents in HPLC analysis due to their low viscosity, complete miscibility with water, and low chemical reactivity with column surfaces and instrument components [2, 3]. Despite these excellent chromatographic properties, acetonitrile and methanol pose some problems in terms of analyst health and ecological impact. Acetonitrile is a volatile, flammable, and toxic chemical. Although methanol is slightly less toxic than acetonitrile, it is classified as a hazardous solvent due to the difficulty of waste disposal [4, 5]. Unfortunately, analysts are exposed to these chemicals during HPLC analysis and significant waste is generated [3]. In addition, due to technological advances, the use of HPLC is becoming increasingly common and the amount of waste is increasing. These wastes, which mostly contain acetonitrile and methanol, must be disposed of as chemical waste. This situation increases the environmental waste disposal burden

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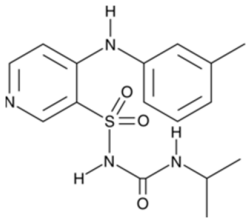
of laboratories and brings very high costs. Analytical chemists are looking for new alternatives to replace analytical methods that use chemicals that pollute the environment and adversely affect analyst health with environmentally and analyst-friendly methods. It is now imperative to eliminate the use of hazardous chemicals and develop environmentally and operator-friendly methods without compromising the performance of the analytical method [6].

All HPLC methods have the potential to be more environmentally and analyst-friendly at all stages of analysis, from sample preparation to final determination [7]. The mobile phases used in HPLC are classically a combination of organic solvents and water with additives to adjust pH and ionic strength. Since it is almost impossible to develop an HPLC method without using organic solvents, the organic modifier should be replaced with other less hazardous organic solvents than acetonitrile and methanol to make the method more environmentally friendly and minimize the negative effects on operator health [8]. Ethanol is one of the environmentally friendly organic solvents and is non-toxic like acetonitrile and methanol [9]. Having a low vapor pressure causes the ethanol to evaporate less and cause the analyst to inhale less. Ethanol is widely available and less expensive than other organic solvents [3]. Additionally, ethanol has lower disposal costs than other organic solvents. This is a big advantage for regions where chemical waste disposal is expensive. Chromatographically, it has similar properties with ethanol, acetonitrile, and methanol [10]. Adsorption mechanisms with column-filling materials are quite similar. It has similar separation mechanisms when different solvents are used. Similar peak yields were obtained in the chromatographic separation of a mixture containing basic and neutral compounds when ethanol was used instead of acetonitrile or methanol [11]. According to the organic solvent classification, ethanol is in the same group as methanol in terms of selectivity [12].

Torasemide (TSD) is a new highly efficient diuretic drug [13] that has been successfully used to treat hepatic cirrhosis [14], renal disease [15], arterial hypertension [16], and edematous conditions associated with chronic congestive heart failure [17]. The physicochemical properties of TSD are presented in Table 1 [18].

Table 1. The physicochemical properties of TSD

Property		Value
Chemical	Name	N-[[(1-methylethyl)amino]carbonyl]-4-[(3-methylphenyl)amino]-3-pyridinesulfonamide
	Formula	$C_{16}H_{20}N_4O_3S$
	Structure	
Molecular weight		348.42
Melting point		163-164 ºC
Log P		1.76
pK_a	Strongest acidic	5.92
	Strongest basic	4.20
Solubility		Soluble in dimethyl sulfoxide and dimethyl formamide. Insoluble in water.

Analytical procedures previously published in the literature were scanned to determine the amount of TSD in bulk and pharmaceutical dosage forms and biological fluids.

Spectrophotometric [19-26], voltammetric [27], capillary electrophoresis [28], high-performance liquid chromatographic [29-40], liquid chromatographic coupled with tandem mass spectrometry (LC-MS/MS) [40, 41], thermal [42], and high-performance thin-layer chromatography [43–46] methods were reported for determination of TSD in bulk and pharmaceutical dosage forms and biological fluids were developed.

Most of these methods are highly complex and require expensive equipment, toxic organic solvents, and specialized chemicals. In the literature search, a green HPLC method for the determination of TSD in pharmaceutical formulations was not found. Therefore, the present study aimed to develop and validate an environmental and analyst-friendly liquid chromatography method using ethanol as a mobile phase organic solvent for the quantification of TSD in pharmaceutical products by a simple extraction procedure. Ethanol is considered as an ecological alternative to acetonitrile and methanol. This study also shows how easy it is to replace standard mobile phases with less hazardous chemicals and "greener" solvents with satisfactory performance.

EXPERIMENTAL

Instruments

An Agilent 1260 series HPLC system equipped with a degasser, quaternary pump, autoinjector, ultraviolet detector, and Chemstation software was used for chromatographic analysis. An Agilent C_{18} (250 x 4.6 mm, 5 µm) column was used and the column oven temperature was maintained at 30 ºC. A Millipore Milli-Q water purification system was used to prepare ultrapure water. A Mettler Toledo pH meter equipped with a glass electrode was used for pH measurement.

Reagents

TSD United States Pharmacopeia (USP) Reference Standard, ethanol (≥98.0%), acetonitrile (≥99.9%), and formic acid (≥99.0%) were purchased from Sigma-Aldrich Chemie GmbH (Istanbul, Turkey). All other chemicals and reagents were of the analytical grade. TSD tablets (Sutril Neo, 10 mg) used in this study were purchased from a local pharmacy (Afyonkarahisar, Turkey). Ultra-pure water (conductivity <0.10 µS cm^{-1}) was produced using the Milli-Q Water purification system and used in these studies.

Standard solutions

A mass (25 mg) of TSD reference standard was precisely weighed and transferred to a 50 mL volumetric flask and mixed with 30 mL methanol, dissolved in an ultrasonic bath until a clear solution was obtained, balanced to room temperature (25 ºC), and the volume was completed to 50 mL with methanol. This was used as the primary stock solution of TSD. The working standard solutions of TSD at six concentrations from 5 to 30 µg mL^{-1} were prepared for analysis.

Sample solutions

Ten TSD-containing tablets were precisely weighed. It was ground into a fine powder in a dryand clean mortar. The tablet powder equivalent to 25 mg of TSD was then transferred into a 50 mL volumetric flask. About 30 mL of ethanol was added and shaken in a rotaryshaker for 20 min to ensure complete dissolution. The volume was completed with ultra-pure water. The mixture was sonicated for 10 min and then filtered through a 0.45 mm membrane filter. The sample solution was diluted with methanol from the prepared stock sample solution and prepared at a concentration of 20 µg mL^{-1}.

Determination of the wavelength

Spectrophotometric analyses were performed on a Shimadzu UV 1800 double beam (Shimadzu, Japan) spectrophotometer, with UV-Probe software. Absorbance values of solutions were determined using a 1.00 cm quartz cell against a blank sample. The working standard solutions of TSD at six concentrations from 5 to 30 µg mL^{-1} were scanned in a UV spectrophotometer (Shimadzu UV-1800 spectrophotometer) device at a wavelength range of 200-400 nm.

Method development

Chromatographic conditions were optimized to obtain good peak parameters such as a good peak shape, a good tailing factor, a short retention time, and a high theoretical plate number. Mobile phases consisting of several buffer systems were investigated at the beginning of the study, but the required system compatibility characteristics were not achieved. Different typesof columns with different lengths were tested. However, system suitability parameters were found to be poor. Good peak parameters were obtained with an Agilent C_{18} (250 mm x 4.6 mm, 5 µm) column. Different ratios of water/methanol, water/acetonitrile, and methanol/acetonitrile mixtures were tested as mobile phases.

Initially, acetonitrile and ultrapure water (20/80, v/v) were used as mobile phase, resulting in a very long analysis time. The water component of the mobile phase was then acidified with formic acid (pH 2.0). In these conditions, the sample solution was injected to detect both impurities that could interfere with the TSD peak and the presence of drug matrix componentsthat could remain longer on the column under the indicated conditions. In addition, sample solutions were injected sequentially into the system with an analysis period of 10 min and it was observed that no impurities passed from one analysis to the next. Therefore, the analysis time was determined as 5 min. In addition, the column temperature was chosen as 25 °C due to its many advantages such as high column efficiency, low column pressure,and favorable peak shape as well as cost-effectiveness.

Method validation

Chromatographic methods were validated in accordance with the International Conference on Harmonization recommendations on the validation of analytical procedures [47, 48].Validation parameters such as selectivity, linearity, system suitability, accuracy, specificity, precision, the limit of detection (LOD), the limit of quantification (LOQ), and robustnesswere investigated. Based on these evaluations, a concentration range of (5-30 µg mL^{-1}) was selected for the validation procedure.

Selectivity

Standard, sample, and mobile phase solutions were injected into the chromatographic system to evaluate the selectivity of the chromatographic methods. The chromatograms were compared and analyzed for interference peaks in the retention time region of the TSD.

Linearity

The linearity of the chromatographic methods was assessed by injecting six standard solutions with a concentration range of 5 to 30 µg mL^{-1} into the HPLC system at λ_{max} of TSD. Three replicate analyses were performed on three different days. A calibration curve was constructed by plotting the analyte concentration against the mean peak area. The slope and intercept of the

regression equation were calculated using linear regression analysis based on the least squares method. The linearity of the method was assessed by the absolute mean recovery, RSD, and R^2 of the resulting calibration curve.

System suitability

The standard solution at a concentration of 20 µg mL^{-1} was injected six times into the chromatographic system to determine the suitability of the system. Peak area, retention time, tailing factor, and the theoretical number of plates were recorded from the chromatograms. The relative standard deviation values of peak areas and retention times for the six injections were calculated.

Detection (LOD) and quantification (LOQ) limits

The limit of detection is the lowest amount of analyte that the chromatographic method can reliably distinguish from background noise levels, while the limit of quantification is the lowest concentration on the linearity curve that can be accurately and precisely measured.The limit of detection was determined using the equation LOD = 3.3xσ⁄S and the limit of quantification was determined using the equation LOQ = 10xσ⁄S. In these equations, σ is the standard deviation of the y-axis; S is the slope of the calibration plot.

Accuracy

The accuracy of the analytical methods was determined by adding three different amounts of TSD standard to the sample solution. The standard was added to the sample solution (20 µg mL^{-1}) at 50%, 100%, and 150% of TSD content. The resulting solutions were injected into the chromatographic system. The % recovery values of the added standard amount were calculated. Three replicate tests were performed for each concentration.

Precision

The precision of the chromatographic methods was assessed based on the intra-day repeatability and inter-day reproducibility of the methods. Intraday repeatability was evaluated by determining the relative standard deviation of peak areas obtained from three injections of the standard solution (20 µg mL^{-1}) on the same day. For inter-day reproducibility, the same standard solution was injected three times on three consecutive days. The relative standard deviation of the areas obtained was determined and evaluated.

Robustness

Small deliberate changes were made to the method conditions to assess the robustness of the chromatographic methods. Small changes were made in the flow rate of the mobile phase (±0.1 mL min^{-1}), in the organic solvent content in the mobile phase (±4%), and in the detection wavelength (±5 nm) and the effect of these changes on the system suitabilityparameters was observed. These effects were investigated by ternary analysis of the standard solution for a concentration of 20 µg mL^{-1}.

Solution stability

The stability of the standard solution was evaluated by storage under different conditions suchas ambient conditions (25 °C) for 12, 24, 36, and 48 hours and refrigerator conditions (4 °C) for

10 days. At the end of each storage period, the solutions were injected into the HPLC system and the results were compared with freshly prepared sample solutions. Stability studies were performed using an analytical solution at a concentration of 20 µg mL^{-1}.

Evaluation of the greenness of chromatographic methods

The greenness of both chromatographic methods was evaluated using the AGREE-Analytical GREEnness metric software. AGREE is a metric system for assessing the greenness ofanalytical procedures based on important principles. AGREE is an easy-to-implement program with user-friendly software that has been extended with the inclusion of 12 basic principles in greenness assessment, allows flexible working by allowing weight assignment, includes easily interpretable color pictogram output showing strengths and weaknesses, and is easy to interpret. The Analytical Greenness score is the weighted average of the benchmark scores. It is shown in the center of the graph, rounded to two decimal places, and its value ranges from 0.0 (lowest score) to 1.0 (perfect score). The graph is a visual representation of the score itself, the benchmark scores, and the benchmark weights [49, 50].

RESULTS AND DISCUSSION

Method development

Although the solubility of TSD in water is quite low, it is easily soluble in methanol. Therefore, standard solutions were prepared using ethanol. By scanning the standard solutions on a spectrophotometer device, λ_{max} was determined to be 288 nm (Figure 1).

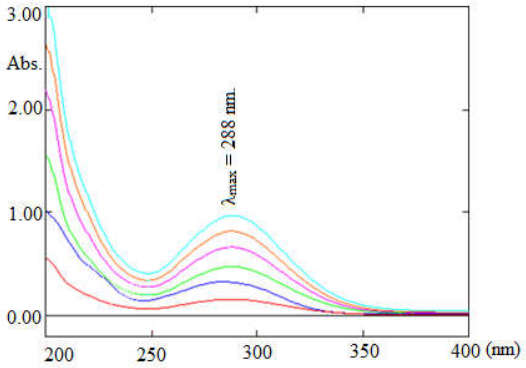


Figure 1. Overlaid spectra of TSD standard solutions (5-30 µg mL^{-1}).

Conditions of chromatographic methods

Chromatographic conditions were optimized to obtain good peak parameters such as a good peak shape, a good tailing factor, a short retention time, and a high theoretical plate number.

Method I (Acetonitrile): An Extend C_{18} (250 x 4.6 mm, 5 µm) column was used as the stationary phase and the temperature was kept constant at 25 ºC. A combination of formic acid solution (pH 2.00, 0.1% aqueous solution) and acetonitrile (50/50, v/v) was used as the mobile phase, and isocratic elution was performed at a flow rate of 1.0 mL min^{-1}. Detection was performed at 288 nm using a UV detector.

Method II (Ethanol): Although all other conditions are the same as method 1, the only difference in method II is that ethanol is used instead of acetonitrile as the organic modifier in the mobile phase. A combination of formic acid solution (pH 2.00, 0.1% aqueous solution) and ethanol (50/50, v/v) was used as the mobile phase, and isocratic elution was performed at a flow rate of 1.0 mL min^{-1}. Chromatograms obtained using method I and method II are presented in Figure 2.

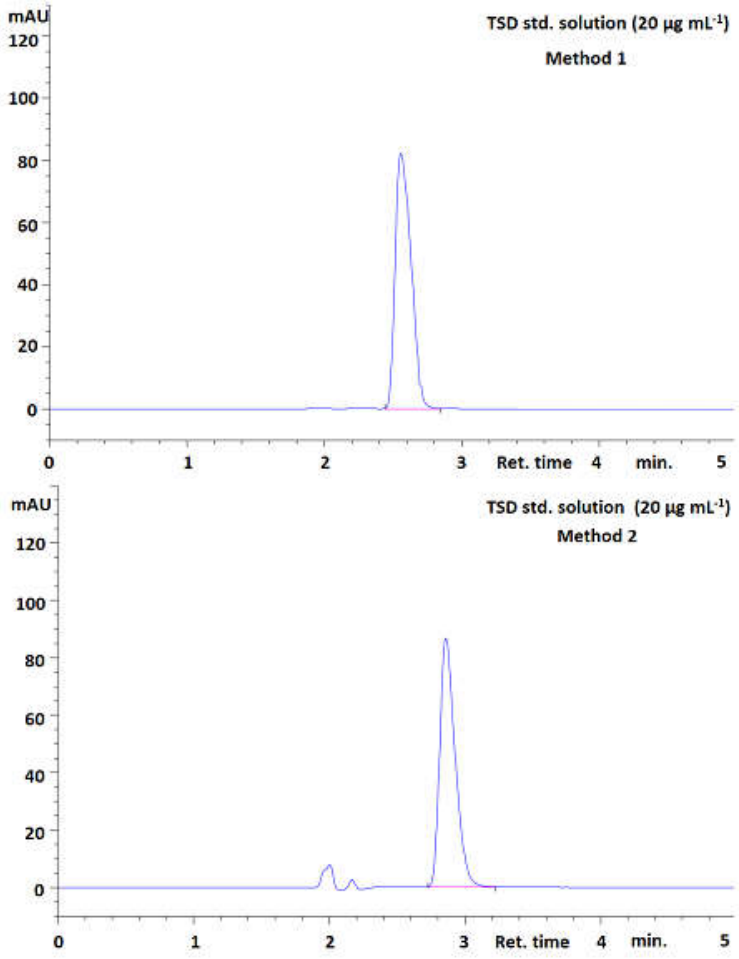


Figure 2. Chromatograms of standard solution (Method I and Method II, 20 µg mL^{-1}).

Selectivity

Standard, sample, and mobile phase solutions were injected into the chromatographic systemto evaluate the selectivity of chromatographic methods. The chromatograms were compared and examined for interfering peak(s) around the analyte peak. No peak interfering with the TSD retention time was observed in either method. Figure 3 shows the chromatograms of the standard

and sample solution using the developed chromatographic method II. The analyte is well-eluted, and a short retention time (2.86 min) and a good peak symmetry are obtained.

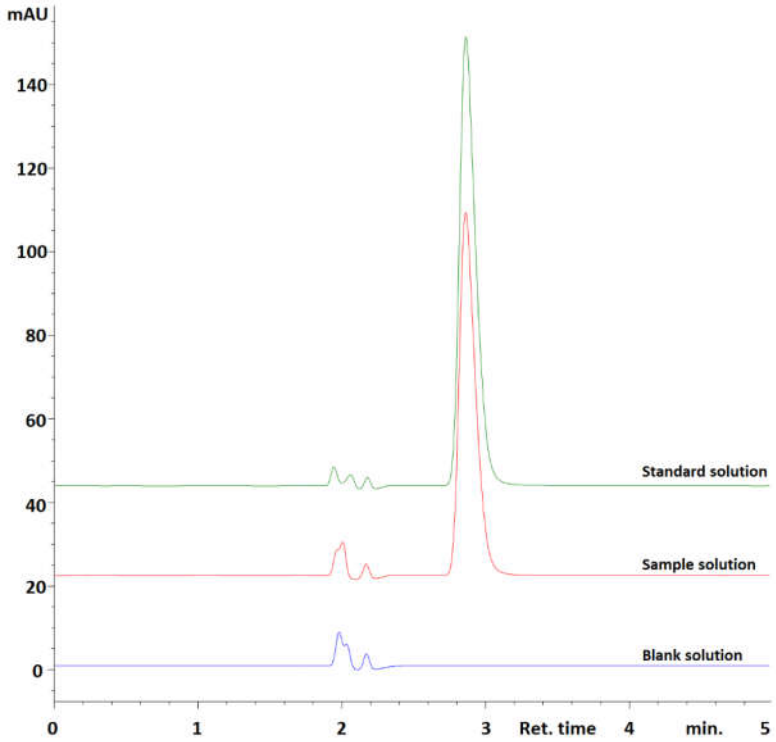


Figure 3. Chromatograms showing the selectivity of method II.

System suitability

To assess the suitability of the system, the standard solution with a concentration of (20 µg mL^{-1}) was injected six times into the chromatographic system and the primary parameters were determined from the resulting chromatograms. For Method I, the peak symmetry was determined as 0.880, the relative standard deviation of the peak areas was 0.350, the relative standard deviation of the retention times was 0.097, the tailing factor was 1.150, and the theoretical number of plates was 4391. For Method II, peak symmetry was determined as 0.750, the relative standard deviation of peak areas as 0.250, the relative standard deviation of retention times as 0.033, the tailing factor as 1.310, and the theoretical plate number as 3651. Although the system suitability parameters for both methods were within the acceptance criteria, the system suitability parameters of Method II were excellent. The correlation coefficients of the calibration curve for both methods were above 0.999, indicating that the methods are suitable for samples with simple or complex matrices.

Linearity

Standard solutions (5, 10, 15, 20, 25, and 30 µg mL^{-1}) were prepared by diluting the stock standard solution (500 µg mL^{-1}) with ultrapure water. These standard solutions were injected into the

chromatographic system and the peak areas and retention times of the analyte were recorded. This process was repeated for three consecutive days. Peak areas and retentiontimes were recorded. Average peak areas were calculated for each concentration level. A calibration graph was plotted with peak area values versus peak area versus standard solution concentration. Linearity data of the chromatographic methods were evaluated by regression analysis. The regression equation, slope, and intercept were calculated using linear regression analysis based on the least squares method. The linearity of the method was quantified by the absolute mean recovery, RSD, and R^2 of the resulting calibration curve. All linearity data are given in Table 2.

Table 2. Regression analysis results of chromatographic methods.

Parameter	Method I (Acetonitrile)	Method II (Ethanol)
Concentration range [μg mL^{-1}] [n = 6]	5–30	5–30
The slope of the regression equation	33.856	33.613
The intercept of the regression equation	4.2829	2.9447
Correlation coefficient	0.9999	0.9997
Retention time [min]	2.552	2.860
Detection limit [μg mL^{-1}]	0.50	0.60
Quantification limit [μg mL^{-1}]	1.40	1.90
Recovery % [n = 3]	99.69–100.48	99.20–100.30

Table 3. Accuracy data of chromatographic methods.

Method	Spiked Level %	Amount added (μg mL^{-1})	Amount recovered (μg mL^{-1})	Recovery, %	Average, %	SD	RSD, %
Method I (Acetonitrile)	50	10	9.90	99.00	99.27	0.252	0.254
		10	9.95	99.50			
		10	9.93	99.30			
	100	20	19.96	99.80	99.62	0.202	0.203
		20	19.93	99.65			
		20	19.88	99.40			
	150	30	29.97	99.90	99.82	0.107	0.107
		30	29.91	99.70			
		30	29.96	99.87			
Method II (Ethanol)	50	10	9.92	99.20	99.37	0.208	0.209
		10	9.96	99.60			
		10	9.93	99.30			
	100	20	19.93	99.65	99.68	0.058	0.058
		20	19.93	99.65			
		20	19.95	99.75			
	150	30	24.97	99.90	99.90	0.033	0.033
		30	24.98	99.93			
		30	24.96	99.87			

Accuracy and recovery

The accuracy of the chromatographic methods was determined by spiking three different amounts of TSD standard to the sample solutions. Standard was added to the sample solution (20 mg mL^{-1}) at 50%, 100%, and 150% of the TSD content. A standard of 50%, 100%, and 150% of TSD content was added to the sample solution (20 mg mL^{-1}). These solutions were injected into the chromatographic system. The % recovery values of the amount of standard added were calculated. Triplicate tests were performed for each concentration. Recovery percentages ranged from

99.27% to 99.62% for method I and from 99.37% to 99.90% for method II. Relative standard deviation values were determined as a maximum of 0.254 in the method I and a maximum of 0.209 in method II. The results of the recovery studies are presented in Table 3.

Precision

Intra-day precision was assessed by determining the relative standard deviations of the retention times and areas of TSD peaks obtained from three injections of the standard solution (20 µg mL^{-1}) on the same day. The relative standard deviation values of the peak areas and retention times were determined below 1.00% in chromatographic methods. For inter-day precision, the same standard solution was injected three times a day for three consecutive days. The relative standard deviations of the retention times and the areas of the obtained peaks were determined and evaluated. The relative standard deviation values of the retention times and peak areas of TSD peaks were determined below 1.00% in chromatographic methods. The intra-day and inter-day precision results are given in Table 4. Our data show that the methods are suitable for validation requirements.

Table 4. Intra-day and inter-day precision results of chromatographic methods.

Precision	Value	Method I (Acetonitrile)			Method II (Ethanol)		
		Retention time, min	Peak area	Assay, %	Retention time, min	Peak area	Assay, %
Intra-day	Average	2.552	682.53	100.00	2.860	675.50	100.00
	SD	0.003	0.2499	0.037	0.002	0.4809	0.071
	RSD, %	0.118	0.0366	0.037	0.070	0.0712	0.071
Inter-day	Average	2.550	681.65	100.00	2.860	675.33	100.00
	SD	0.002	0.8303	0.122	0.002	0.9411	0.1393
	RSD, %	0.083	0.1218	0.122	0.063	0.1393	0.1393

Robustness

The results of the robustness study showed that the linearity, accuracy, and recovery of the chromatographic methods were not affected by small changes in critical method parameters such as the flow rate of the mobile phase, column temperature, and organic solvent content of the mobile phase. The results of the robustness study are presented in Table 5. The average recovery for all tests ranged from 99.69% to 100.35% and the RSD% level was less than 0.85.

Table 5. The results of robustness tests.

Method	Parameters	Values	Average recovery, %	RSD, %
Method I (Acetonitrile)	The flow rate of the mobile phase	0.90 mL min^{-1}	100.35	0.24
		1.10 mL min^{-1}	99.69	0.18
	Column temperature	20 °C	99.87	0.20
		30 °C	100.03	0.30
	Acetonitrile content of the mobile phase	48%	99.89	0.82
		52%	100.31	0.56
Method II (Ethanol)	The flow rate of the mobile phase	0.90 mL min^{-1}	100.25	0.27
		1.10 mL min^{-1}	99.74	0.20
	Column temperature	20 °C	99.91	0.22
		30 °C	99.80	0.34
	The ethanol content of the mobile phase	48%	99.89	0.85
		52%	100.33	0.60

Application of chromatographic methods to pharmaceutical formulations and comparison of results

TSD-containing tablets (Sutril Neo, 10 mg) were analyzed by chromatographic methods. The results obtained by both chromatographic methods and average, standard deviation and relative standard deviation values calculated over 6 replications are given in Table 6. Comparisons of the results obtained by both chromatographic methods in terms of meanswere made using Student's (t) test and comparisons in terms of standard deviations were madeusing Fischer (F) test. When the results in the table were examined, it was determined that there was no significant difference between the two methods developed in terms of accuracy and precision, since the 95% confidence interval and the t and F values calculated for 6 trials were lower than the values reported in the relevant tables.

Table 6. Statistical evaluation of the analysis results of tablets (Sutril Neo, 10 mg)

Sample	Method I (Acetonitrile)		Method II (Ethanol)	
	mg in tablet	Assay, %	mg in tablet	Assay, %
1	9.949	99.50	10.024	100.32
2	9.945	99.46	9.978	99.85
3	10.037	100.39	10.044	100.52
4	10.028	100.30	9.992	99.99
5	9.978	99.79	10.003	100.11
6	10.054	100.56	9.914	99.21
Average	9.999	100.00	9.993	100.00
SD	0.0473	0.4726	0.0450	0.4504
RSD. %	0.4726	0.4726	0.4504	0.4504
t_{value}/t_{table}	0.1961/2.5706			
F_{value}/F_{table}	1.1025/5.0503			

Stability of the solution

No stability-related problems were observed when the standard solution was kept under different conditions. The standard solution was able to stay stable at laboratory conditions (25 °C) for 48 hours and at refrigerator temperature (4 °C) for 10 days without degradation.

Assessment of the greenness of chromatographic methods

The greenness evaluation pictograms of the chromatographic methods are given in Figure 5. The greenness score of chromatographic method I (using acetonitrile as the mobile phase) is 0.67, while the score of chromatographic method II (using ethanol as the mobile phase) is 0.78. In the AGREE pictogram of the chromatographic method I, the performance for principles 1, 8, and 11 of green analytical chemistry is very poor, while the performance for principles 2, 4, 6, and 9 is excellent (Figure 4A). The corresponding color scale for the reference in the AGREE pictogram is presented in Figure 4B. In the AGREE pictogram of chromatographic method II, the performance for principles 1 and 8 of green analytical chemistry is rather poor, while the performance for principles 2, 4, 6, 9, 10, and 11 is excellent (Figure 4C). The second chromatographic method (using ethanol in the mobile phase) can be said to be greener than the other method.

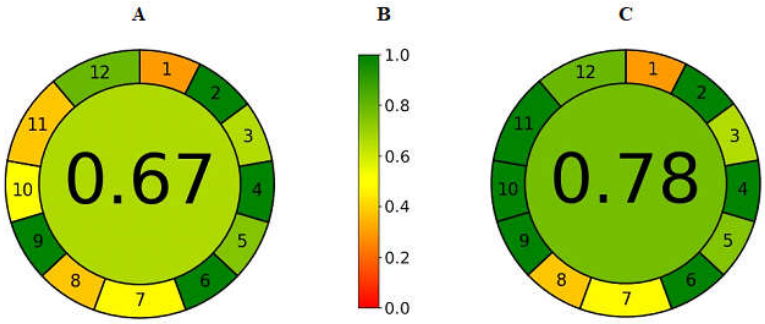


Figure 4. **A**: AGREE pictogram of the chromatographic method I, **B**: corresponding colorscale for reference, **C**: AGREE pictogram of the chromatographic method II.

This study aims to evaluate the chromatographic behavior of TSD using an environment and operator-friendly mobile phase. Contrary to conventional chromatographic components, the use of ethanol in the mobile phase has brought an alternative perspective to environmentally friendly analyses. The greenness of the analytical methods is evaluated from the sample preparation stage to the detection stage.

An important result of this study is that it produces non-toxic waste. TSD was selectively determined with high sensitivity, accuracy, linearity, repeatability, and robustness by the chromatographic method we developed. The detection and quantification limits of the developed chromatographic method were quite low. Additionally, the system suitability parameters showed that the chromatographic performance was not lacking. The purpose ofthis study was achieved by meeting all the needs of the validation process without compromising the quality of the chromatographic performance. As the HPLC technique is widely used in the pharmaceutical industry, this greening effort is very important to minimize toxicity during the analysis stage.

The results of our study showed that ethanol and water-based mobile phases can be successfully applied in pharmaceutical analysis. Such chromatographic analyses will encourage analysts who want to develop more environmentally friendly analysis methods in their laboratories. When our chromatographic method was compared with other reportedmethods, detection and quantification limits values, and improved greening aspects are better. The findings showed that the green quantification of TSD in pharmaceutical products was performed without losing chromatographic quality thanks to the reduced hazardous effects.

The developed chromatographic method was compared with other previously published methods. LOD and LOQ values and improved foliage aspects are better than studies reported in the literature. The findings showed that the green pharmaceutical analysis for TSD in this study was performed without losing chromatographic quality thanks to the reduced hazardous effects caused by the analytical methodology.

CONCLUSION

In a world where clean water resources are rapidly decreasing, air pollution is a major problem, and the effects of global warming and climate change are becoming more evident, preventing environmental pollution, reducing energy consumption, and developing environmentally friendly methods for waste management have become even more critical for the future of humanity. It is thought that the method developed with this in mind can be considered an environmentally friendly alternative to the methods currently used in the quantification of TSD in pharmaceutical products. The developed liquid chromatographic method contains safe and economical organic

solvents such as ethanol for the determination of TSD in pharmaceutical products. The greenness profile score of the developed method was found to be higher than published chromatographic methods for TSD determination. In the literature review, no method using a mobile phase containing less toxic solvents for TSD determination was found. Therefore, the proposed method can be considered anadvantageous and innovative method in the application of green analytical chemistry, being an alternative ecologically safe and correct to be used in routine quality control analysis.

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