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Eco-Friendly High-Performance Thin-Layer Chromatography Method for the Determination of Tenoxicam in Commercial Formulations

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Cite This: *ACS Omega* 2023, 8, 39936−39944

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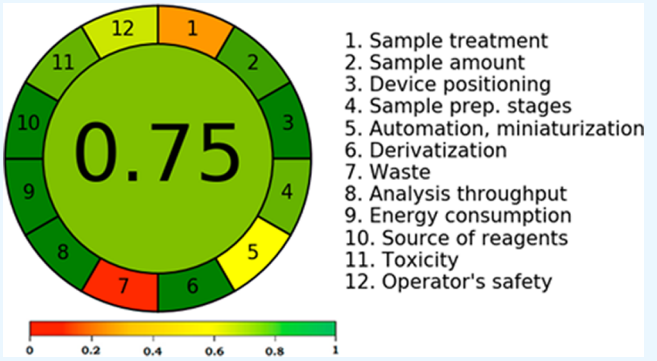
ABSTRACT: There is a dearth of information in the literature regarding environmentally benign high-performance thin-layer chromatography (HPTLC) methods to determine tenoxicam (TNX). Therefore, designing and validating an HPTLC method to detect TNX in commercial tablets and capsules was the goal of this investigation. The green mobile phase utilized was the combination of ethanol/water/ammonia solution (50:45:5 v/v/v). The TNX was quantified at a wavelength of 375 nm. The proposed method's greenness profile was established using the Analytical GREEnness (AGREE) approach. The proposed methodology for determining TNX was linear in the range of 25−1400 ng/band. The proposed methodology for measuring TNX was accurate (% recoveries = 98.24−101.48), precise (% RSD = 0.87−1.02), robust (% RSD = 0.87−0.94), sensitive (LOD = 0.98 ng/band and LOQ = 2.94 ng/band), and environmentally friendly. The AGREE scale for the present methodology was derived to be 0.75, indicating an outstanding greenness profile. TNX was found to be highly stable under acidic, base, and thermal stress conditions. However, it completely decomposed under oxidative stress conditions. Commercial tablets and capsules were found to have 98.46 and 101.24% TNX, respectively. This finding supports the validity of the current methodology for measuring TNX in commercial formulations. The outcomes of this work showed that the proposed eco-friendly HPTLC methodology can be used for the routine analysis of TNX in commercial formulations.



1. INTRODUCTION

Tenoxicam (TNX), a nonsteroidal anti-inflammatory medicine (NSAID), belongs to the oxicam class of NSAIDs.[1] Figure 1 displays the molecular structure/formula of TNX.[2] It is a nonselective cyclooxygenase-2 (COX-2) inhibitor, which inhibits the enzyme COX-2 at the site of inflammation and offers analgesic, antipyretic, and anti-inflammatory activities.[2,3] It has been suggested for the treatment of a variety of pains linked to osteoarthritis, rheumatoid arthritis, tendinitis, bursitis, and ankylosing spondylitis.[3−5] It has been found to be practically insoluble in water (H_2O), slightly soluble in some organic solvents like ethyl acetate (EA), ethylene glycol, transcutol, and polyethylene glycol-400, and very slightly soluble in ethyl alcohol (EtOH), isopropyl alcohol, propylene glycol, 1-butanol, and 2-butanol.[6,7] Its daily dose is relatively low (20 mg) compared to other NDAIDs.[3] It is commercially available in numerous dosage forms, such as tablets, capsules, and injections.[3,6] Since TNX can be found in many commercial dosage forms, it is crucial to analyze both its qualitative and quantitative contents in marketed formulations.

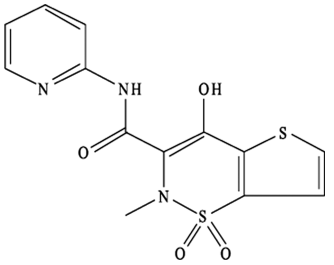


Figure 1. Molecular structure/formula of tenoxicam (TNX).

There have been several published analytical techniques for detecting TNX in biological samples and dosage forms. Spectrophotometric,[8,9] derivative spectrophotometric,[10] infrared spectrophotometric,[11] colorimetric,[12] and spectrofluoro-

Received: September 20, 2023
Accepted: September 28, 2023
Published: October 12, 2023

metric methods[13] have been reported to determine TNX in pharmaceutical dosage forms. Some high-performance liquid chromatography (HPLC) approaches were utilized to determine TNX in pure and dosage forms.[14−16] In the transdermal therapeutic system, TNX and meloxicam were also determined using an HPLC approach.[17] A HPLC approach has also been used to measure potential genotoxic impurity, 5-amino-2-chloropyrine in TNX.[18] Numerous HPLC assays were also used for the determination of TNX in the plasma samples of human subjects.[19−23] The liquid-chromatography mass-spectrometry (LC-MS) and micro-HPLC methodologies were utilized to identify TNX in conjunction with other NSAIDs in pharmaceutical preparations and biological materials, including blood, plasma, and erythrocytes.[24] For the purpose of determining TNX in pharmaceutical formulations, a few high-performance thin-layer chromatography (HPTLC) methods were also published.[14,25,26] An HPTLC method has also been reported to determine TNX in combination with piroxicam, celecoxib, and rofecoxib in the whole human blood and urine.[27] Some voltammetry techniques were also used to detect TNX in dosage forms.[28,29] Some other approaches such as potentiometric,[30] polarographic,[31] indirect flow-injection spectrophotometry,[32] reverse flow-injection method,[33] fluorescence probe,[34] colloidal gold strip sensor,[35] and quantum dots as fluorescence probe[36] have also been utilized to determine TNX, either alone or in combination with other NSAIDs in pharmaceutical preparations.

There are several analytical approaches for TNX measurement in pharmaceutical preparations and biological samples. However, eco-friendly liquid chromatographic approaches are lacking in the literature. Additionally, the greenness profile was not reported for any of the reported methods of TNX analysis. Utilizing alternative environmentally acceptable eluents to reduce the harmful impacts of toxic solvents on the ecosystem is one of the 12 principles of green analytical chemistry (GAC), which is a method of chemical analysis.[37] A literature search found an exponential rise in the usage of environmentally friendly solvents over the past few decades.[38−40] The literature has described a number of approaches to assess the analytical methodologies' greenness profiles.[41−46] To measure greenness profiles, only the Analytical GREEnness (AGREE) technique applies all 12 GAC principles.[37,46] As a result, the AGREE approach was used to calculate the greenness profiles of the current method.[46] In comparison to traditional liquid chromatographic techniques, the HPTLC method has a number of benefits, including ease of use, little pretreatment, sensitivity, efficiency, simultaneous analysis of many samples, a nondestructive mode of detection, low solvent consumption, short analysis time, and low cost.[38,39] Based on these details and findings, the current approach aims to design and evaluate an environmentally friendly reversed-phase HPTLC strategy for the measurement of TNX in procured tablets and capsules. Enhancing the greenness index of analytical procedures with good sensitivity, accuracy, precision, robustness, and environmental friendliness is a good approach to reducing the toxic effects of conventional liquid chromatographic methods. As a result, the proposed method was used in this study.[37−39] Using the International Council for Harmonization (ICH)-Q2-R1 guidelines, the proposed approach for TNX analysis was validated.[47]

2. RESULTS AND DISCUSSION

2.1. Analytical Method Development. In order to develop the present TNX analytical method, different binary and ternary mixtures, such as EtOH−H_2O, acetone (Ace)−H_2O, cyclohexane (CYH)−EA, EtOH−H_2O−ammonia (NH_3), Ace−H_2O−NH_3, and CYH−EA−NH_3 in different proportions, were investigated as the eco-friendly solvent systems. The examined solvents (EtOH, H_2O, Ace, EA, and CYH) are categorized as eco-friendly solvents since they are safe from an environmental point of view.[48,49] All solvent systems were created under saturated chamber conditions. In accordance with the best solvent systems, Figure 2 shows a representative TLC picture for solutions of standard TNX, commercial formulations, and degradation-study solutions. Table 1 lists the components of several binary and ternary eco-friendly solvent mixtures as well as the measured chromatographic characteristics. It was noticed that when various combinations such as Ace−H_2O (50:50 v/v), Ace−H_2O (55:45 v/v), Ace−H_2O−NH_3 (50:45:5 v/v/v), CYH−EA (50:50 v/v), CYH−EA (55:45 v/v), and CYH−EA−NH_3 (50:45:5 v/v/v) were investigated, the unreliable TNX signals with larger tailing factor (As) (As = 1.29−1.39) and less number of theoretical plates per meter (N/m) (N/m = 1641−1998) were recorded.

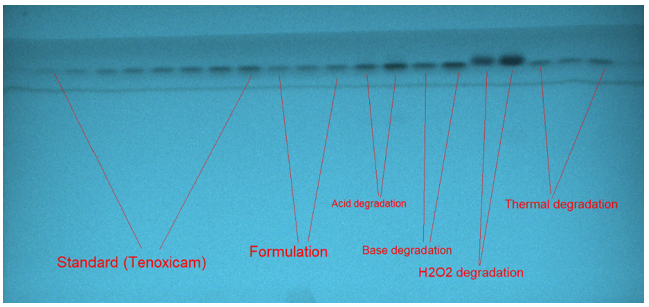


Figure 2. Representative TLC image for standard TNX, marketed formulations, and forced-degradation solutions recorded using an eco-friendly EtOH−H_2O−NH_3 (50:45:5 v/v/v) solvent system for the present method.

Table 1. Tenoxicam (TNX) Chromatographic Parameters and Environmentally Friendly Solvent System Optimization for the Present Method (Mean ± SD; n = 3)[a]

solvent system	As	N/m	R_f
EtOH−H_2O (50:50 v/v)	1.28 ± 0.03	3241 ± 2.61	0.88 ± 0.02
EtOH−H_2O (55:45 v/v)	1.30 ± 0.04	3184 ± 2.57	0.90 ± 0.03
EtOH−H_2O−NH_3 (50:45:5 v/v/v)	1.07 ± 0.02	4971 ± 3.13	0.85 ± 0.01
Ace−H_2O (50:50 v/v)	1.32 ± 0.05	1942 ± 1.63	0.82 ± 0.03
Ace−H_2O (55:45 v/v)	1.35 ± 0.06	1874 ± 1.43	0.83 ± 0.02
Ace−H_2O−NH_3 (50:45:5 v/v/v)	1.29 ± 0.04	1998 ± 1.81	0.80 ± 0.02
CYH−EA (50:50 v/v)	1.37 ± 0.06	1784 ± 1.38	0.78 ± 0.03
CYH−EA (55:45 v/v)	1.39 ± 0.07	1641 ± 1.32	0.79 ± 0.04
CYH−EA−NH_3 (50:45:5 v/v/v)	1.31 ± 0.05	1816 ± 1.40	0.77 ± 0.02

[a]EtOH: ethanol; H_2O: water; Ace: acetone; EA: ethyl acetate; CYH: cyclohexane; R_f: retardation factor; As: asymmetry factor; and N/m: theoretical plates number per meter.

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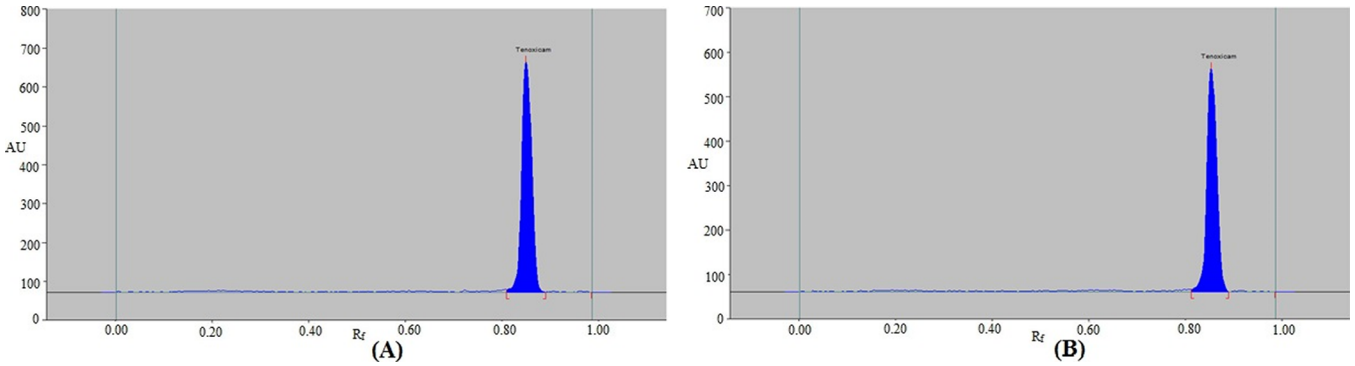

Figure 3. Typical chromatograms of (A) pure TNX and (B) marketed tablets for the proposed method.

The TNX chromatographic signals were somewhat enhanced with the least As (As = 1.07−1.30), and high N/m data (N/m = 3184−4971) when different combinations of EtOH−H_2O (50:50 v/v), EtOH−H_2O (55:45 v/v), and EtOH−H_2O−NH_3 (50:45:5 v/v/v) were explored (Table 1). The environmentally friendly EtOH−H_2O−NH_3 (50:45:5 v/v/v) solvent system stood out among these combinations by displaying a clear and unbroken TNX peak at retardation-factor (R_f) = 0.85 ± 0.01 (Figure 3A). Furthermore, it was found that TNX had a suitable As value of 1.07 for TNX analysis. EtOH−H_2O−NH_3 (50:45:5 v/v/v) was subsequently chosen as the most environmentally acceptable solvent composition for the proposed method of TNX quantification. When spectral bands of TNX were evaluated in absorbance mode, it was observed that 375 nm was the wavelength that produced the greatest chromatographic response. As a result, the analysis of TNX for the entire TNX measurement was carried out at 375 nm.

2.2. Validation Studies. *2.2.1. System Suitability.* Several validation factors for TNX detection were established using the ICH-Q2-R1 recommendations.[47] The system suitability criteria for the current methodology are listed in Table 2. The present approach's R_f, As, and N/m for TNX detection were derived to be 0.85, 1.07, and 4971, respectively, which were acceptable for TNX detection.

Table 2. Parameters of System Suitability of TNX for the Current Methodology (Mean ± SD; *n* = 3)

parameters	values
R_f	0.85 ± 0.01
As	1.07 ± 0.02
N/m	4971 ± 3.13

2.2.2. Linearity. Table 3 displays the outcomes of the linearity evaluation for the TNX calibration plot using the proposed technique. The current method's TNX calibration plot was found to be linear in the range of 25−1400 ng/band. The TNX's determination coefficient (R^2) and regression coefficient (R) for the current method were, respectively, 0.9977 and 0.9988. These observations demonstrated a significant relationship between TNX concentrations and the recorded peak response. The linear range of TNX for three HPTLC methods has been reported as 0.25−6.0 μg/band,[14] 35−1820 mg/mL,[25] and 100−400 ng/band,[26] respectively. Three reported HPTLC approaches had substantially inferior TNX linear ranges than the current method.[14,25,26] These results presented the linearity of the method presented here for TNX analysis.

Table 3. Results of the TNX Linearity Measurement for the Present Methodology (Mean ± SD; *n* = 6)

parameters	value
linear range (ng/band)	25−1400
regression equation	y = 15.757x + 1081.9
R^2	0.9977
R	0.9988
SE of slope	0.25
SE of intercept	1.91
CI of slope[a]	14.65−16.86
CI of intercept[a]	1073.65−1090.14
LOD ± SD (ng/band)	0.98 ± 0.02
LOQ ± SD (ng/band)	2.94 ± 0.06

[a]95% confidence interval; y: TNX peak response; x: TNX concentration; SE: standard error; LOD: limit of detection; and LOQ: limit of quantification.

2.2.3. Accuracy. The two degrees of accuracy for the present TNX analytical method were attained by applying a spiking methodology. The results of the % recovery using the current strategy are shown in Table 4. Using the current method, the intra-assay % recoveries of TNX at three different quality-control (QC) samples were calculated to be 98.87−101.40%. The TNX interassay % recoveries for the proposed approach were observed to range from 98.24 to 101.48% at three different QC levels. Two HPTLC methods have reported TNX recovery rates of 99.51−102.63%[14] and 98.90−101.99%,[26] respectively. The first reported HPTLC method's TNX % recovery was inferior to the present method,[14] and the second reported HPTLC method's TNX % recovery was

Table 4. Accuracy Outcomes of TNX for the Present Methodology (Mean ± SD; *n* = 6)

conc. (ng/band)	conc. found (ng/band) ±SD	recovery (%)	RSD (%)
	intraday accuracy		
300	303.21 ± 3.76	101.07	1.24
400	405.61 ± 4.94	101.40	1.21
500	494.35 ± 5.31	98.87	1.07
	interday accuracy		
300	294.72 ± 3.71	98.24	1.25
400	396.81 ± 4.91	99.20	1.23
500	507.41 ± 5.29	101.48	1.04

https://doi.org/10.1021/acsomega.3c07252
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Table 5. Precision Evaluation Results of TNX for the Present Methodology (Mean ± SD; n = 6)[a]

conc. (ng/band)	intraday precision			interday precision		
	conc. (ng/band) ± SD	SE	RSD (%)	conc. (ng/band) ± SD	SE	RSD (%)
300	302.51 ± 3.02	1.23	0.99	304.13 ± 3.1	1.26	1.02
400	404.13 ± 3.64	1.48	0.90	392.54 ± 3.66	1.49	0.93
500	496.12 ± 4.32	1.76	0.87	502.36 ± 4.61	1.88	0.91

[a]SE: standard error; RSD: relative standard deviation.

comparable to proposed method.[26] The derived results showed that the proposed technique was accurate for detecting TNX.

2.2.4. Precision. The findings for TNX precision are presented as a percentage of relative standard deviation (% RSD), and the intra- and interday precision of the current approach was highlighted. The results are given in Table 5. The intraday precision RSD of TNX for the current approach was discovered to be between 0.87 and 0.99%. It was noted that the RSD of TNX for the current method's interday precision is between 0.91 and 1.02%. The precision of TNX for three HPTLC methods has been reported as 1.33%,[14] < 2.0%,[25] and 0.96−1.64%,[26] respectively. The precision of TNX for three reported HPTLC methods was similar to the current method.[14,25,26] These results demonstrate how precise the method currently being used to analyze TNX is.

2.2.5. Robustness. The planned solvent system's intended composition was purposefully changed for the assessment of the proposed method's robustness. The findings are displayed in Table 6. The calculated TNX %RSD for the suggested approach was 0.87−0.94%. The TNX R_f values for the current method ranged from 0.84 to 0.86. These findings showed how robust the TNX analysis method was.

Table 6. Results of TNX Robustness for the Current Methodology (Mean ± SD; n = 6)[a]

conc. (ng/band)	eco-friendly mobile phase (EtOH−H_2O−NH_3)			results		
	original	used	level	conc. (ng/band) ± SD	RSD (%)	R_f
400	50:45:5	52:43:5	+2.0	391.41 ± 3.41	0.87	0.84
		50:45:5	0.0	397.84 ± 3.67	0.92	0.85
		48:47:5	−2.0	404.62 ± 3.82	0.94	0.86

[a]R_f: retardation factor.

2.2.6. Sensitivity. The limit of detection (LOD) and limit of quantification (LOQ) were established in order to assess the sensitivity of the currently used TNX analytical approach. The obtained LOD and LOQ values of TNX for the current technique are listed in Table 3. According to Table 3's calculations, the LOD and LOQ of TNX are, respectively, 0.98 ± 0.02 and 2.94 ± 0.06 ng/band. According to reports, the first HPTLC technique's LOD and LOQ of TNX were 0.40 and 1.36 μg/band, respectively.[14] For the second HPTLC technique, the LOD and LOQ of TNX have been reported as 0.86 and 2.30 mg/band, respectively.[25] The third HPTLC technique's LOD and LOQ of TNX, according to reports, were 25 and 50 ng/band, respectively.[26] Three published HPTLC approaches had substantially lower LOD and LOQ of TNX than the current method.[14,25,26] As a result, it has been concluded that the present method is highly sensitive compared to all of the reported HPTLC methods for TNX measurement.[14,25,26] The outcomes demonstrated the excellent sensitivity of the current TNX measuring technology.

2.2.7. Specificity. The specificity of the current approach for detecting TNX concentrations could be assessed by comparing the R_f values and UV-absorption spectra of TNX in commercial tablets (formulation A) and capsules (formulation B) to those of standard TNX. Figure 4 indicates the overlay UV-absorption spectra of standard TNX with TNX present in formulations A and B. The peak regions of formulations A and B, and standard TNX were measured at a wavelength of 375 nm. The fact that formulations A and B and standard TNX had identical UV spectra, R_f values, and detection wavelengths showed how specific the current method is for identifying TNX is. Overall, it has been concluded that the proposed methodology for TNX analysis is more linear and sensitive than literature HPTLC methods.[14,25,26]

2.3. Forced-Degradation Evaluation. Forced-degradation of the proposed methodology was studied under four distinct stress conditions. Table 7 and Figure 5 present the findings. TNX was found to remain at 100.00% under the acid-, base-, and thermal-degradation stress settings, and hence 0.0% of it decomposed (Table 7). As a result, it was discovered that TNX was extremely stable under stress conditions involving acid, base, and thermal deterioration. The R_f value

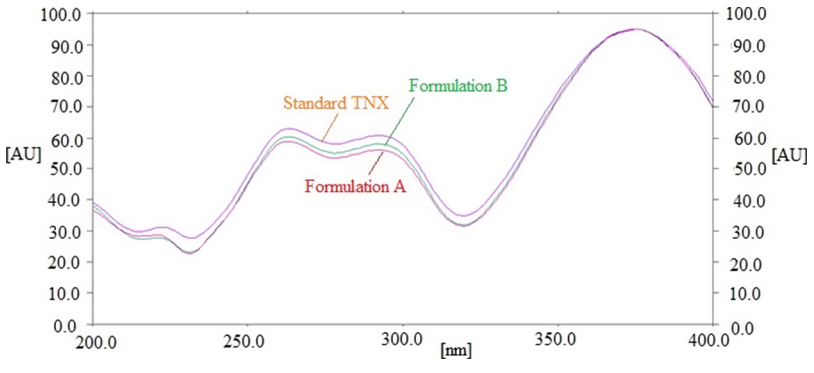

Figure 4. Overlaid UV-absorption spectra of standard TNX and marketed formulations A and B.

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Table 7. Outcomes of Forced-Degradation Assessment of TNX Using Four Different Stress Settings for the Current Method (Mean ± SD; n = 3)[a]

stress setting	number of degradation products	TNX R_f	TNX remained (ng/band)	TNX recovered (%)
1 M HCl	0	0.85	400.00	100.0 ± 0.00
1 M NaOH	0	0.85	400.00	100.00 ± 0.00
30% H_2O_2	ND	ND	ND	ND
thermal	0	0.85	400.00	100.00 ± 0.00

[a]ND: not detected.

of TNX under the acid (Figure 5A), base (Figure 5B), and thermal-degradation (Figure 5D) stress settings was found to be unaltered (R_f = 0.85). No TNX or breakdown products were found during the oxidative degradation (Table 7 and Figure 5C). Since no TNX peak was found, it was assumed that the whole amount of TNX had undergone oxidative destruction.

2.4. Application of the Current Methodology in the Assay of TNX in Marketed Tablets and Capsules. The current method was employed to analyze TNX in commercial tablets (formulation A) and capsules (formulation B) as a substitute for traditional liquid chromatographic methodologies. The chromatogram of TNX from the procured formulations was confirmed by contrasting the TLC band at R_f = 0.85 ± 0.01 for TNX with standard TNX utilizing the proposed method. When evaluated utilizing the proposed method, TNX in formulations A and B had identical chromatograms as the reference TNX. It was demonstrated that there was no interaction between the constituents of the formulations and TNX by the absence of excipient peaks in formulations A and B (Figure 3B). The amount of TNX in formulations A (label claim = 20 mg of TNX) and B (label claim = 20 mg of TNX) was calculated by the calibration curve of TNX. The current methodology revealed that formulations A and B contained 98.46 ± 0.98 and 101.24 ± 1.08% of TNX, respectively, with respect to their label claims in formulations A and B. As per ICH protocol, the % amount of active ingredient in the pharmaceutical formulations must be in the magnitude of 100 ± 2%.[47] The recorded values of TNX in commercial tablets and capsules were within the magnitude of ICH protocol and hence acceptable for the pharmaceutical assay of TNX.[47] These results suggested that the existing technique was suitable for TNX pharmaceutical analysis.

2.5. Greenness Determination. There are several methodologies that can be utilized to measure the greenness attributes of analytical procedures according to the literature.[41−46] The AGREE metric approach is the only one that can precisely forecast the greenness profile utilizing all 12 GAC criteria.[46] Accordingly, the greener profile of the proposed methodology was predicted by utilizing the AGREE-metric technique. An illustration of the current method's AGREE scale is shown in Figure 6. According to the AGREE methodology, the AGREE score for each GAC principle is assigned from 0 to 1. For the present method, score 1 was derived for the GAC principles 3, 6, 8, 9, and 10. The score 0.08 was derived for the GAC principle's 7. The score of 0.8 was derived for the GAC principles 4 and 11, respectively. The score 0.88 was derived for the GAC principle's 2. The AGREE scores 0.3, 0.5, and 0.6 were recorded for the GAC principles 1, 5, and 12, respectively. According to the AGREE approach, the AGREE score of greater than 0.75 indicated excellent greenness for the analytical method. However, the AGREE score of 0.50 is considered an acceptable score for drug analysis. The AGREE score of more than 0.50 and less than

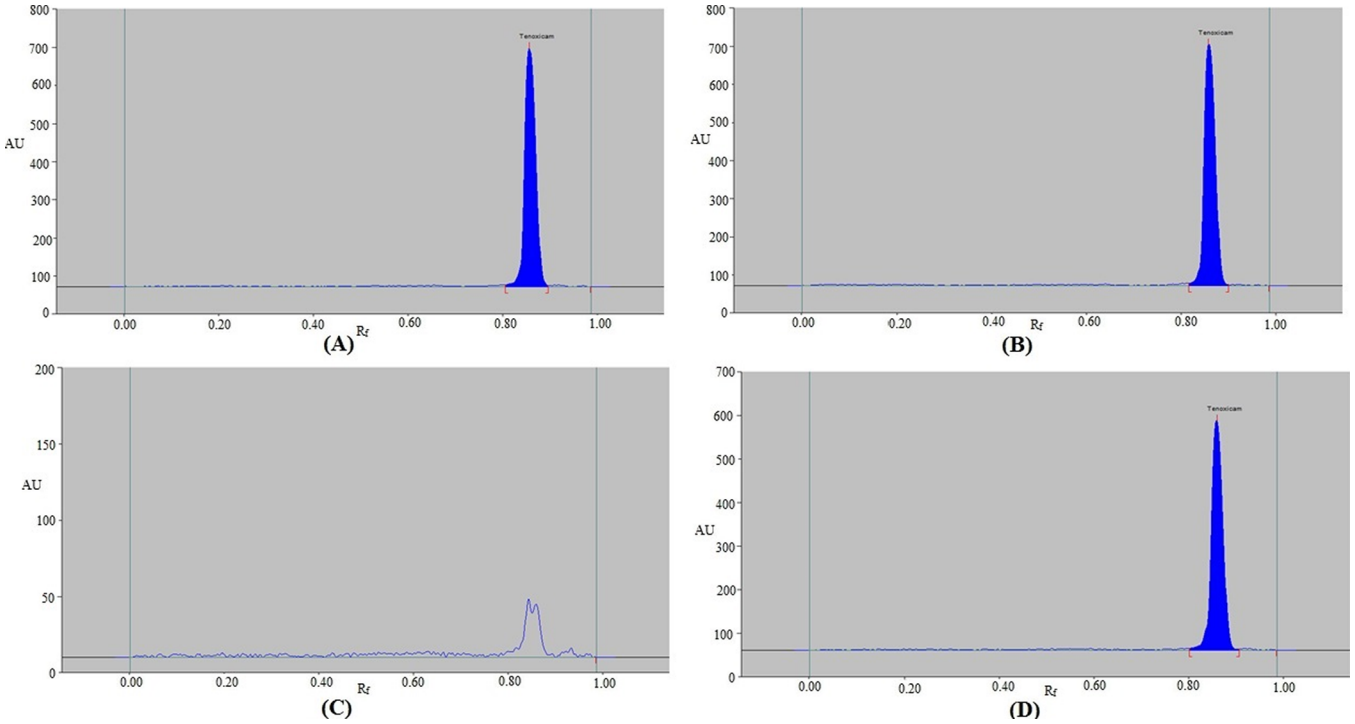

Figure 5. Representative chromatograms of TNX recorded under (A) acid, (B) base, (C) oxidative, and (D) thermal stress degradation conditions of TNX.

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ACS Omega 2023, 8, 39936−39944

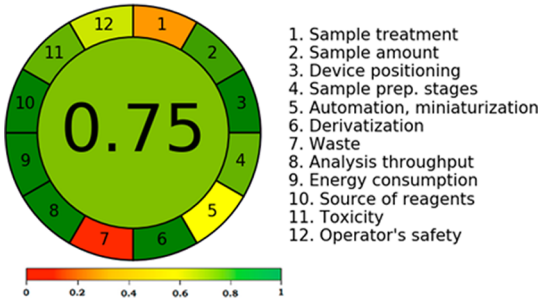

Figure 6. Typical image of the AGREE score obtained using the AGREE methodology for the current technique.

0.75 is considered a good score for pharmaceutical analysis. Accordingly, the AGREE score of less than 0.50 indicated an unacceptable score for pharmaceutical analysis.[46] With an overall AGREE score of 0.75, the current approach demonstrated an excellent greenness profile for the TNX measurement.

3. CONCLUSIONS

The eco-friendly HPTLC and other liquid chromatography methods for the determination of TNX are lacking in the literature. In order to develop and validate an eco-friendly HPTLC approach for TNX quantification in procured tablets and capsules, this research work was performed. The proposed TNX analytical methodology is simple, precise, robust, highly sensitive, and eco-friendly. The AGREE evaluation indicated that the present method has superb greenness characteristics. Under acid, basic, and heat degradation conditions, TNX was discovered to be the most stable; however, when exposed to oxidative hydrolysis, it completely broke down. It has been proven that the proposed methodology is linear and sensitive to the literature on HPTLC techniques. The derived outcomes revealed that TNX in procured tablets and capsules could be routinely assayed by the current HPTLC method. The use of the method reported in this work could be an alternative approach for TNX analysis in the pharmaceutical industry with good sensitivity, accuracy, precision, robustness, and environmental friendliness compared to conventional liquid chromatographic methods. For future studies, the proposed HPTLC method can be used for the determination of TNX in plasma samples and its pharmacokinetic evaluation in suitable animal models.

4. MATERIALS AND METHODS

4.1. Materials. Reference TNX (purity: 99.2%) and NH_3 solution (25% for HPLC) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Liquid chromatography-grade green eluents, such as EtOH, Ace, CYH, and EA, were acquired from E-Merck (Darmstadt, Germany). Utilizing a Milli-Q (Milli-Q, Lyon, France) unit, liquid chromatography-grade H_2O was procured. Pharmaceutical stores in Riyadh, Saudi Arabia, provided commercial TNX tablets (formulation A) and capsules (formulation B), each containing 20 mg of TNX. The grades of all other materials were analytical.

4.2. Instrumentation and Analytical Procedures. TNX in commercial tablets and capsules was quantified by utilizing the HPTLC CAMAG technology (Muttenz, Switzerland). A CAMAG Automatic TLC Sampler 4 (ATS4) Applicator (Muttenz, Switzerland) was used to apply the samples in the form of 6 mm bands. The TLC silica gel 60 RP-18F254S plates (E-Merck, Darmstadt, Germany) were employed as the stationary phase for TNX detection. The CAMAG microliter syringe (Hamilton, Bonaduz, Switzerland) was connected to the sample applicator. The application rate for TNX was set to 150 nL/s for all measurements. The TLC plates were arranged at an 80 mm distance inside a CAMAG automated development chamber 2 (ADC2) (Muttenz, Switzerland). As an environmentally friendly solvent system, EtOH−H_2O−NH_3 (50:45:5 v/v/v) was employed. The green solvent system's vapors were completely saturated in the development chamber for 30 min at 22 °C. TNX was quantified at a wavelength of 375 nm. The slit diameter was fixed at 4 × 0.45 mm^2, and the scan speed was set to 20 mm/s. For each analysis, three or six replications were used. Results were decoded by the CAMAG WinCAT program (version 1.4.3.6336, Muttenz, Switzerland).

4.3. TNX Calibration Plot. A TNX stock solution with a concentration of 100 μg/mL was created by combining 10 mg of TNX with 100 mL of an eco-friendly solvent system. To obtain TNX concentrations ranging from 25 to 1400 ng/band, this stock solution was serially diluted. Each TNX solution was placed in 200 μL portions on TLC plates, and the necessary response was taken. Utilizing a graph of the measured peak area vs TNX concentrations, the TNX calibration curve was created. Six replicates (n = 6) of each of these solutions and experiments were completed.

4.4. Sample Processing for TNX Analysis in Procured Tablets and Capsules. Twenty-five commercial tablets (formulation A) and capsules (formulation B), each containing 20 mg of TNX, had an average mass that was calculated. Twenty-five capsules' worth of material were removed from their shells. In a glass pestle and mortar, 25 tablets and capsules were ground into a fine powder. The average mass of the obtained powder from formulations A and B was combined with 10 mL of an eco-friendly solvent system. Then, 1 mL of solution of formulations A and B was diluted using 50 mL of the eco-friendly solvent system, separately. The produced solutions of formulations A and B were sonicated at 25 °C for 30 min to eliminate any insoluble materials and then filtered. The acquired samples of formulations A and B were examined for TNX contents using the suggested procedure.

4.5. Validation Studies. The proposed TNX analytical method was verified using ICH-Q2-R1 protocols for numerous parameters as explained below.[47]

4.5.1. Parameters of System Suitability. To determine the parameters of system suitability for the proposed method of TNX analysis, the estimation of R_f, As, and N/m was performed. The reported formulas for R_f, As, and N/m were used to generate the data.[40]

4.5.2. Linearity. By plotting the measured peak area vs TNX concentrations, the TNX linearity was ascertained. The linear range of the proposed assay for TNX analysis in the 25−1400 ng/band magnitude range was assessed with six replicates (n = 6).

4.5.3. Accuracy. The proposed approach for TNX quantification's intra-assay and interassay accuracy was evaluated using the spiking methodology, and the results were represented as % recoveries.[47] To establish low-QC (LQC) solutions of TNX of 300 ng/band, moderate-QC (MQC) solutions of 400 ng/band, and high-QC (HQC) solutions of 500 ng/band, extra 50, 100, and 150% TNX solutions were spiked into the previously measured TNX solution (200 ng/band). To forecast intra-assay accuracy, the aforementioned TNX QC samples underwent reanalysis on the

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same day. To evaluate interassay accuracy, the identical QC solutions were reassessed over a three-day period. The % recovery was obtained for both accuracies and at each QC level. Six replicates (n = 6) were used to measure both accuracies.

4.5.4. Precision. The current approach for TNX analysis was evaluated for its intra- and interassay precision. It was possible to assess freshly generated TNX solutions at previously defined QC levels on the same day. This allowed the TNX intra-assay precision to be established. The TNX interassay precision was assessed by measuring freshly made TNX solution over a period of 3 days at previously established QC levels. To evaluate both precisions, six replicates (n = 6) were used. The precisions were expressed as % RSD.

4.5.5. Sensitivity. The present TNX method's sensitivity was measured in terms of LOD and LOQ by standard deviation method.[47] For the current procedure, the blank solution (without TNX) was assessed using six replicates (n = 6) and the standard deviation was computed. The standard deviation and slope of the TNX calibration plot were then used to get the data for TNX's LOD and LOQ using the formulas that were provided.[47,50]

4.5.6. Specificity. The R_f data and UV-absorption spectrum of TNX in commercial formulations A and B was contrasted with those of standard TNX for the assessment of specificity of the suggested approach for TNX analysis.

4.6. Forced-Degradation Studies. Studies on forced degradation were conducted utilizing four different stress settings, including acid (HCl), base (NaOH), oxidative (H_2O_2), and thermal degradation conditions.[47,51] The MQC solution of TNX (400 ng/band) was freshly made using the proposed solvent system for all degradation studies. Acid and base hydrolysis was performed by mixing 1 mL of the MQC sample with either 4 mL of 1 M HCl or 1 M NaOH. Acid and base hydrolysis solutions were successfully diluted by the proposed solvent system. These samples were subjected to the proposed method for the evaluation of TNX breakdown following 48 h of refluxing at 60 °C.[40]

Fresh TNX MQC samples (400 ng/band) were produced by utilizing the proposed solvent system for oxidative degradation conditions. The next step was to oxidize 1 mL of this solution with 4 mL of 30% H_2O_2. We successfully diluted this mixture using the proposed solvent system. The degradation of TNX in this mixture was assessed using the proposed approach after refluxing for 48 h at 60 °C.[40]

An aliquot of the MQC (400 ng/band) sample was placed into a hot air oven and heated to 60 °C for 48 h after being properly diluted with the suggested solvent mixture. This resulted in thermal hydrolysis of the MQC (400 ng/band) solution. The proposed method was then used to evaluate TNX degradation.[40]

4.7. Application of the Current Methodology in the Assay of TNX in Commercial Formulations. Commercial samples of tablets and capsules were spotted on TLC plates for the current method. The same experimental setup was utilized to determine standard TNX, and the peak area was observed in triplicates (n = 3). Using the TNX calibration curve, the current methodology was used to determine the % content of TNX in commercial tablets and capsules.

4.8. Greenness Determination. By the AGREE methodology, the greener feature for the current method of TNX analysis was determined.[46] The AGREE: The Analytical Greenness Calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020) estimated the AGREE score for the current technique.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project no. IFKSUOR3-066.

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