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Article

Comparing the Greenness and Validation Metrics of Traditional and Eco-Friendly Stability-Indicating HPTLC Methods for Ertugliflozin Determination

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ABSTRACT: The literature does not provide any "high-performance thin-layer chromatographic (HPTLC)" techniques for the determination of a novel antidiabetic medicine, ertugliflozin (ERZ). Additionally, there are not many environmentally friendly analytical methods for ERZ measurement in the literature. A rapid, sensitive, and eco-friendly reversed-phase-HPTLC (RP-HPTLC) method was designed and validated in an attempt to analyze ERZ in marketed pharmaceutical tablets more precisely, accurately, and sustainably over the traditional normal-phase HPTLC (NP-HPTLC) method. The stationary phases used in the NP- and RP-HPTLC procedures were silica gel 60 NP-18F254S and 60 RP-18F254S plates, respectively. For NP-HPTLC, a chloroform/methanol (85:15 v/v) mobile phase was used. However, ethanol−water (80:20 v/v) was the preferred method for RP-HPTLC. Four distinct methodologies, including the National Environmental Method Index (NEMI), Analytical Eco-Scale (AES), ChlorTox, and Analytical GREEnness (AGREE) approaches, were used to evaluate the greenness of both procedures. For both approaches, ERZ detection was carried out at 199 nm. Using the NP- and RP-HPTLC techniques, the ERZ measurement was linear in the 50−600 and 25−1200 ng/band ranges. The RP-HPTLC method was found to be more robust, accurate, precise, linear, sensitive, and eco-friendly compared to the NP-HPTLC approach. The results of four greenness tools demonstrated that the RP strategy was greener than the NP strategy and all other reported HPLC techniques. The fact that both techniques can assess ERZ when its degradation products are present implies that they both have characteristics that point to stability-indicating features. 87.41 and 99.28%, respectively, were the assay results for ERZ in commercial tablets when utilizing the NP and RP procedures. Based on several validation and greenness metrics, it was determined that the RP-HPTLC approach was better than the NP-HPTLC method. As a result, it is possible to determine ERZ in pharmaceutical products using the RP-HPTLC approach.

1. INTRODUCTION

Since sodium-glucose cotransporter-2 (SGLT2) inhibitors are largely expressed in the proximal renal tubules, 90% of the glucose that is originally filtered by the kidneys is reabsorbed.[1,2] As a result, SGLT2 inhibitors have become a cutting-edge treatment option for type 2 diabetes.[2,3] Ertugliflozin (ERZ) is one SGLT2 inhibitor that is approved for the management of type 2 diabetes mellitus.[4,5] Figure S1 illustrates the chemical structure. It has been approved as monotherapy and in fixed-dose combination with metformin hydrochloride and sitagliptin.[6,7] For its commercial products, qualitative and quantitative assessment of ERZ is essential. Hence, the high-performance thin-layer chromatography (HPTLC) technique was applied for the qualitative and quantitative analysis of ERZ.

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Several analytical techniques for ERZ analysis in biological materials and pharmaceutical products were found through a review of the literature. Pharmaceutical formulations and pure forms of ERZ can be determined using the "high-performance liquid chromatographic (HPLC)" method.[8] A number of HPLC methods have also been used to determine ERZ in combination with either sitagliptin or metformin hydrochloride.[9−17] To ascertain ERZ in rat plasma samples, an HPLC technique incorporating fluorescence detection has also been created.[18] ERZ has also been determined in combination with sitagliptin using LC mass spectrometry (MS)/MS (LC-MS/MS) method in fixed-dose combination products.[19] Using LC-MS/MS techniques, ERZ has also been quantified in rat plasma samples in conjunction with sitagliptin or metformin.[20,21] The determination of ERZ in pure forms and pharmaceutical preparations, either in conjunction with sitagliptin or alone, has also been performed utilizing various ultraperformance LC-MS/MS (UPLC-MS/MS) techniques.[22−24] Using a UPLC-MS/MS approach, ERZ and sitagliptin have also been measured simultaneously in rat plasma samples.[25] A green spectrofluorometric method was also used to determine ERZ in dosage forms and human urine samples.[26] To the best of our knowledge, no reports of ERZ measurement in commercial products have been made using traditional or eco-friendly HPTLC techniques. HPTLC analytical procedures present numerous benefits such as low solvent usage/waste, nondestructive mode of detection, ease of use, minimal pretreatment, efficiency, simultaneous detection of multiple samples, nontoxic, and greenness features over conventional liquid chromatography methods (HPLC, LC-MS, and UPLC methods) for the analysis of pharmaceutical compounds.[27,28] Currently, HPTLC approaches have been utilized for the green analysis of various pharmaceutical compounds and medications.[27−30] One of the 12 principles of "green analytical chemistry (GAC)" is the use of ecologically acceptable solvent replacements to lessen the harmful effects of toxic or hazardous eluents on the ecosystem.[31] A literature search revealed that the use of greener solvents has grown dramatically during the last few decades.[32−35] Numerous qualitative and quantitative techniques for assessing the greenness profiles of analytical processes have been described in the literature. The aforementioned tools are the National Environmental Method Index (NEMI),[36] the Green Analytical Procedure Index (GAPI),[37] the Analytical Eco-Scale (AES),[38] Red, Green, and Blue (RGB),[39] the Environmental Assessment Tool (EAT),[40] the Analytical Method Volume Intensity (AMVI),[41] the Analytical Method GREEnness Score (AMGS),[42] ChlorTox,[43] and the Analytical GREEnness (AGREE).[44] The present investigation employed four discrete tools, specifically NEMI,[36] AES,[38] ChlorTox,[43] and AGREE,[44] to assess the greener profile of the current approaches. In comparison to the conventional stability-indicating normal-phase HPTLC (NP-HPTLC) procedure for measuring ERZ in commercial tablets, the current strategy aimed to develop and validate a stability-indicating reversed-phase HPTLC (RP-HPTLC) procedure that would be more precise, accurate, sensitive, robust, and environmentally friendly. Following "The International Council for Harmonization (ICH)" Q2-R2 procedures, both procedures for ERZ analysis were validated.[45]

2. RESULTS AND DISCUSSION

2.1. Method Development and Optimization. For the method development and optimization procedures, preliminary investigations were performed to optimize the best solvent systems for the NP- and RP-HPTLC methods. For NP procedures, different binary solvent combinations were used, such as chloroform ($CHCl_3$)/methanol (MeOH), MeOH/ethyl acetate, hexane/acetone, and ethyl acetate/cyclohexane. The best results were obtained using the $CHCl_3$/MeOH combination. Hence, different proportions of $CHCl_3$/MeOH were investigated in NP procedures. For RP procedures, different binary solvent combinations were used, such as acetone/water (H_2O), ethanol (EtOH)/H_2O, EtOH/ethyl acetate, and EtOH/acetone. The best results were obtained using an EtOH/H_2O combination. Hence, different proportions of EtOH/H_2O were investigated for the RP procedures. Table 1 provides a summary of the system suitability parameters for each approach. The "retardation-factor (R_f), tailing-factor (A_s), and theoretical plates number per meter (N/m)" for ERZ analysis were found to be trustworthy for both approaches.

Table 1. Ertugliflozin (ERZ) Assessment by Mobile Phase and Chromatographic Parameter Optimization for NP-HPTLC and RP-HPTLC Techniques (Mean ± SD, n = 3)

mobile phase	A_s	N/m	R_f
	NP-HPTLC		
$CHCl_3$/MeOH (45:55 v/v)	1.33 ± 0.06	1789 ± 2.32	0.42 ± 0.05
$CHCl_3$/MeOH (55:45 v/v)	1.28 ± 0.05	1992 ± 2.45	0.38 ± 0.04
$CHCl_3$/MeOH (65:35 v/v)	1.23 ± 0.04	2412 ± 3.41	0.35 ± 0.03
$CHCl_3$/MeOH (75:25 v/v)	1.18 ± 0.03	3281 ± 3.83	0.32 ± 0.02
$CHCl_3$/MeOH (85:15 v/v)	1.06 ± 0.02	4472 ± 4.22	0.29 ± 0.01
$CHCl_3$/MeOH (95:5 v/v)	1.15 ± 0.03	3842 ± 4.03	0.34 ± 0.02
	RP-HPTLC		
EtOH/H_2O (40:60 v/v)	1.34 ± 0.05	1452 ± 1.61	0.78 ± 0.03
EtOH/H_2O (50:50 v/v)	1.27 ± 0.04	1943 ± 1.78	0.75 ± 0.03
EtOH/H_2O (60:40 v/v)	1.22 ± 0.03	2861 ± 3.16	0.73 ± 0.03
EtOH/H_2O (70:30 v/v)	1.19 ± 0.03	3544 ± 3.74	0.71 ± 0.02
EtOH/H_2O (80:20 v/v)	1.08 ± 0.03	4652 ± 4.02	0.68 ± 0.01
EtOH/H_2O (90:10 v/v)	1.17 ± 0.04	3772 ± 3.93	0.70 ± 0.02

Chamber saturation conditions were used in the development of the TLC plates for both techniques. As the traditional mobile phases for the ERZ analysis by the NP procedure, a variety of $CHCl_3$/MeOH combinations between 45 and 95% $CHCl_3$ were examined.[46] Table 1 lists the combinations of traditional mobile phases and other chromatographic responses. The traditional mobile phase $CHCl_3$/MeOH (85:15 v/v) produced a well-eluted and sharp chromatographic signal for ERZ at R_f = 0.29 ± 0.01 (Figure 1A), according to the results. Furthermore, it was discovered that the A_s values of 1.06 ± 0.02 for ERZ are suitable for ERZ evaluation. As a result, $CHCl_3$/MeOH (85:15 v/v) was chosen as the optimal mobile phase for the NP-HPTLC method's ERZ assessment.

As the eco-friendly mobile phases for the ERZ analysis by the RP procedure, a variety of EtOH/H_2O combinations between 40 and 90% EtOH were studied. Table 1 summarizes the combinations of the RP-HPTLC method's several chromatographic parameters and eco-friendly mobile phases. The results showed that an intact and well-resolved ERZ chromatographic signal at R_f = 0.68 ± 0.01 was obtained with

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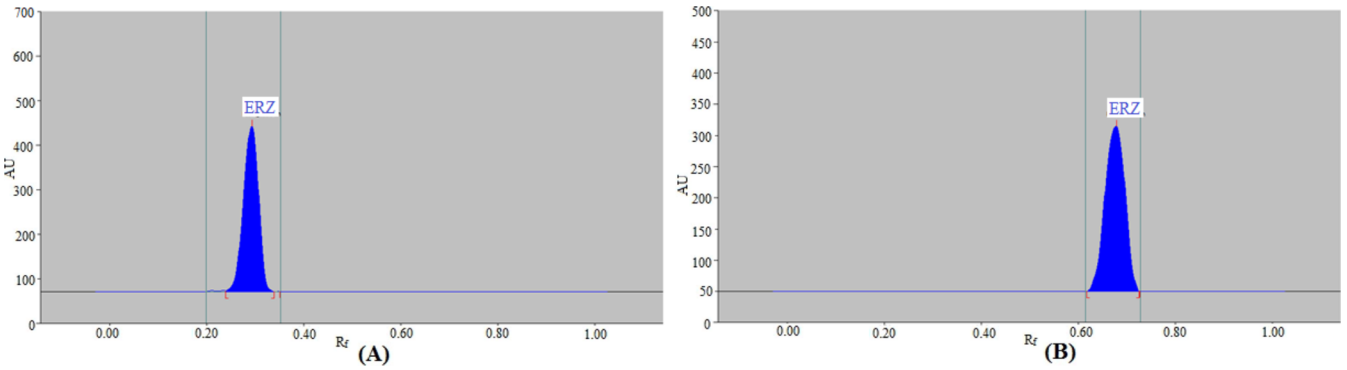

Figure 1. Representative spectrodensitograms of standard ERZ obtained by (A) NP- and (B) RP-HPTLC methods.

the EtOH/H_2O (80:20 v/v) mixture (Figure 1B). Furthermore, a projection of 1.08 ± 0.03 for ERZ's A_s value was made, which was suitable for ERZ evaluation. Because of this, EtOH/H_2O (80:20 v/v) was selected as the optimal eco-friendly mobile phase for the ERZ evaluation using the RP-HPTLC method. When the ERZ spectral bands were examined under spectrodensitometry mode, the maximum TLC response was recorded at 199 nm. It was performed through the peak area integration corresponding to the spectrodensitogram plot at 199 nm. As a result, the entire analysis of ERZ was performed at 199 nm.

2.2. Validation Studies. The protocols ICH-Q2-R2 were used to record the different ERZ validation parameters.[45] The results of evaluation of the linearity of ERZ calibration plots using both methods are presented in Table 2. Between 50 and 600 ng/band, the ERZ calibration curve for the NP method was linear. The ERZ calibration plot for the RP procedure was linear in the range of 25−1200 ng/band. For the NP-HPTLC method, the correlation coefficient (R) and determination coefficient (R^2) for ERZ were, respectively, 0.9965 and 0.9932. ERZ's R^2 and R for the RP-HPTLC method were 0.9987 and 0.9988, respectively. These findings showed a significant correlation between the ERZ concentrations and the measured spot areas. These results all showed that the two ERZ measurement methods were linear. However, the RP-HPTLC approach was more linear than the NP-HPTLC method.

Table 2. Results of the Linearity Assessment of ERZ for the NP-HPTLC and RP-HPTLC Procedures (Mean ± SD; n = 6)

parameters	NP-HPTLC	RP-HPTLC
linear range (ng/band)	50−600	25−1200
regression equation	y = 12.805x + 1326.8	y = 12.956x + 641.86
R^2	0.9932	0.9987
R	0.9965	0.9988
standard error of slope	0.92	0.34
standard error of intercept	11.36	1.50
95% confidence interval of slope	8.81−16.79	11.46−14.44
95% confidence interval of intercept	1277.88−1375.71	635.37−648.34
LOD ± SD (ng/band)	7.17 ± 0.16	0.94 ± 0.01
LOQ ± SD (ng/band)	21.52 ± 0.48	2.82 ± 0.03

Both ERZ measurement techniques were evaluated for accuracy in terms of % recovery. Table 3 presents the accuracy measurement results for both approaches. The recoveries of ERZ at three different quality control (QC) samples for the NP-HPTLC technique were determined to be 94.15−104.52%. Using the RP-HPTLC technique, the recoveries of ERZ in three distinct QC samples were assessed and found to be 98.77−100.96%. These outcomes demonstrated the validity of both methods for the ERZ measurement. However, the RP-HPTLC method performed more accurately than the NP-HPTLC method when it came to quantifying the ERZ.

Table 3. Results of Accuracy Assessment of ERZ for the NP-HPTLC and RP-HPTLC Procedures (Mean ± SD; n = 6)

conc. (ng/band)	conc. found (ng/band) ± SD	recovery (%)	CV (%)
	NP-HPTLC		
150	141.23 ± 3.87	94.15	2.74
200	188.74 ± 4.87	94.37	2.58
250	261.31 ± 5.12	104.52	1.95
	RP-HPTLC		
300	296.32 ± 3.11	98.77	1.04
400	397.65 ± 3.84	99.41	0.96
500	504.81 ± 4.41	100.96	0.87

The data were reported as a % of the coefficient of variance (%CV), and the intra- and interassay precisions of both methods were evaluated in order to measure ERZ. Table 4 displays the intra- and interassay precisions for both ERZ measuring techniques. The intra-assay CVs of ERZ for NP procedure varied from 2.63 to 3.12%. The NP procedure's ERZ interassay CVs varied from 2.70 to 3.33%. The intra-assay CVs of ERZ for the RP procedure varied from 0.87 to 0.97%. The range of the ERZ CVs for interassay in the RP procedure was 0.88−0.99%. These measurements demonstrated the precision of both ERZ measurement techniques. However, the RP-HPTLC approach was more precise than the NP-HPTLC method for measuring the ERZ.

In order to measure the robustness of both ERZ measurement techniques, intentionally planned modifications were made to the mobile phase components. Table 5 displays the results of the robustness measurements for both techniques. The CVs for ERZ using the NP-HPTLC technique were 3.14−3.30%. The ERZ R_f values for the NP-HPTLC technique were found to be 0.28−0.30. The ERZ CVs for the RP-HPTLC technique varied from 0.89 to 0.93%. The RP procedure's ERZ R_f values were found to range from 0.67 to 0.69. These measurements demonstrated the robustness of both approaches for the ERZ measurement. In terms of ERZ measurements, however, the RP-HPTLC approach fared better than the NP-HPTLC method.

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Table 4. Measurement of ERZ Precision for NP-HPTLC and RP-HPTLC Procedures (Mean ± SD; n = 6)

	intraday precision			interday precision		
conc. (ng/band)	conc. (ng/band)	SE	CV (%)	conc. (ng/band)	SE	CV (%)
			NP-HPTLC			
150	156.35 ± 4.89	1.99	3.12	159.63 ± 5.32	2.17	3.33
200	211.21 ± 5.91	2.41	2.79	214.52 ± 6.38	2.60	2.97
250	239.14 ± 6.31	2.57	2.63	262.35 ± 7.10	2.90	2.70
			RP-HPTLC			
300	304.15 ± 2.97	1.21	0.97	295.61 ± 2.95	1.20	0.99
400	406.31 ± 3.61	1.47	0.88	395.35 ± 3.58	1.46	0.90
500	492.89 ± 4.32	1.76	0.87	510.23 ± 4.53	1.84	0.88

Table 5. Results of Robustness Evaluation of ERZ for NP-HPTLC and RP-HPTLC Procedures (Mean ± SD; n = 6)

	mobile phase combination ($CHCl_3$-MeOH)			results		
conc. (ng/band)	original	used		conc. (ng/band)	CV (%)	R_f
			NP-HPTLC			
200	85:15	87:13	+2.0	186.41 ± 5.87	3.14	0.28
		85:15	0.0	192.56 ± 6.13	3.18	0.29
		83:17	−2.0	208.51 ± 6.89	3.30	0.30
			RP-HPTLC			
			mobile phase composition (EtOH-H_2O)			
400	80:20	82:18	+2.0	389.41 ± 3.47	0.89	0.67
		80:20	0.0	392.61 ± 3.58	0.91	0.68
		78:22	−2.0	407.64 ± 3.81	0.93	0.69

Both ERZ measurement techniques' sensitivity was assessed in terms of "limit of detection (LOD) and limit of quantification (LOQ)". Table 2 lists the obtained values of "LOD and LOQ" for ERZ for both approaches. The "LOD and LOQ" for ERZ for the NP procedure were derived to be 7.17 ± 0.16 and 21.52 ± 0.48 ng/band, respectively. The "LOD and LOQ" for ERZ for the RP procedure were calculated to be 0.93 ± 0.01 and 2.82 ± 0.03 ng/band, respectively. These results demonstrated that both approaches were highly sensitive to ERZ measurement. However, compared to the NP procedure, the RP procedure was more sensitive in measuring ERZ.

By contrasting the R_f values, superimposed UV spectrum, and 3D spectrum of ERZ in marketed pharmaceutical tablets with those of pure ERZ, we were able to assess the specificity and peak purity of the recommended method for ERZ assessment. The superimposed UV spectra of marketed pharmaceutical tablets' ERZ and pure ERZ are shown in Figure 2. Figure S2 shows the 3D spectrum of marketed pharmaceutical tablets and pure ERZ. By contrasting the spectrum at the peak start (S), peak apex (M), and peak end (E) positions of the spot, the peak purities of standard ERZ and ERZ in marketed pharmaceutical tablets were evaluated.[47,48] Standard ERZ and marketed pharmaceutical tablets' computed values of $r(S,M)$ and $r(M,E)$ were found to be more than 0.99, demonstrating the homogeneity of the peaks.[49,50] The highest chromatographic response was observed for ERZ in standard and commercial tablets at a wavelength of 199 nm. The usage of the same UV spectrum, 3D spectrum, R_f data, and wavelengths found in standard and marketed pharmaceutical tablets demonstrated the specificity of the current methods for ERZ assessment.

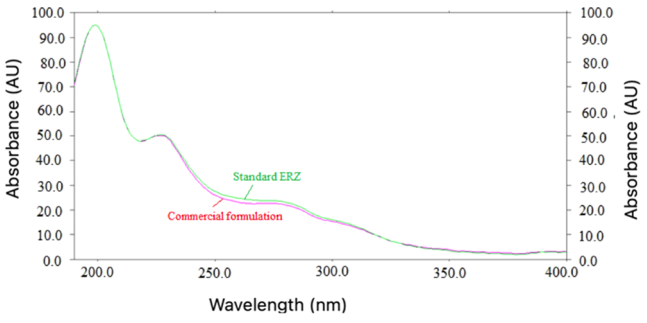

Figure 2. UV spectrum of standard ERZ and marketed products.

2.3. Forced-Degradation Evaluation. Under varied stress circumstances, the forced degradation of the NP and RP procedures was examined. The purpose of these investigations was to evaluate the stability-indicating properties of the current approaches. Figure 3 and Table 6 present the results obtained from the NP-HPTLC approach. At different stress levels, the ERZ peaks were clearly separated (Figure 3). After deterioration, the quantity of ERZ was measured. The quantity of degradation was computed based on the residual amount of ERZ. Under conditions of acid breakdown (Figure 3A), 51.95% of ERZ was degraded and 48.05% remained intact. Consequently, under acid breakdown conditions, ERZ was extremely unstable. Under acid degradation conditions, the ERZ R_f value remained constant (R_f = 0.29). The molecules detected under acid degradation (peaks 2, 3, and 4 in Figure 3A) showed separation at R_f = 0.51, 0.64, and 0.71, in that order. ERZ was maintained at 100.0% (Table 6) during the base degradation (Figure 3B), oxidative degradation (Figure 3C), and thermal degradation (Figure 3D) settings, and no signs of ERZ degradation were found. The tailing was observed in the drug peak under base degradation condition. We tried to resolve this issue by changing the degradation conditions by reducing and increasing the NaOH concentrations. However, no degradation was observed under those conditions. As a result, we considered 100% drug recovery under base degradation conditions. The tailing of the drug peak under such circumstances might be due to the presence of some unknown impurities in NaOH. Thermal chromatogram baseline was also shifted slightly, but the drug was recovered at 100%. In both cases, the drug concentration was not below LOD. ERZ was therefore resistant to heat, oxidative, and base stress situations. During base degradation settings, the ERZ R_f value was slightly displaced (R_f = 0.28). Nonetheless, the ERZ R_f value remained constant (R_f = 0.29) in both the oxidative and thermal degradation scenarios.

Table 7 and Figure 4 show the results of the RP-HPTLC approach. At various stress levels, the ERZ peak exhibited good

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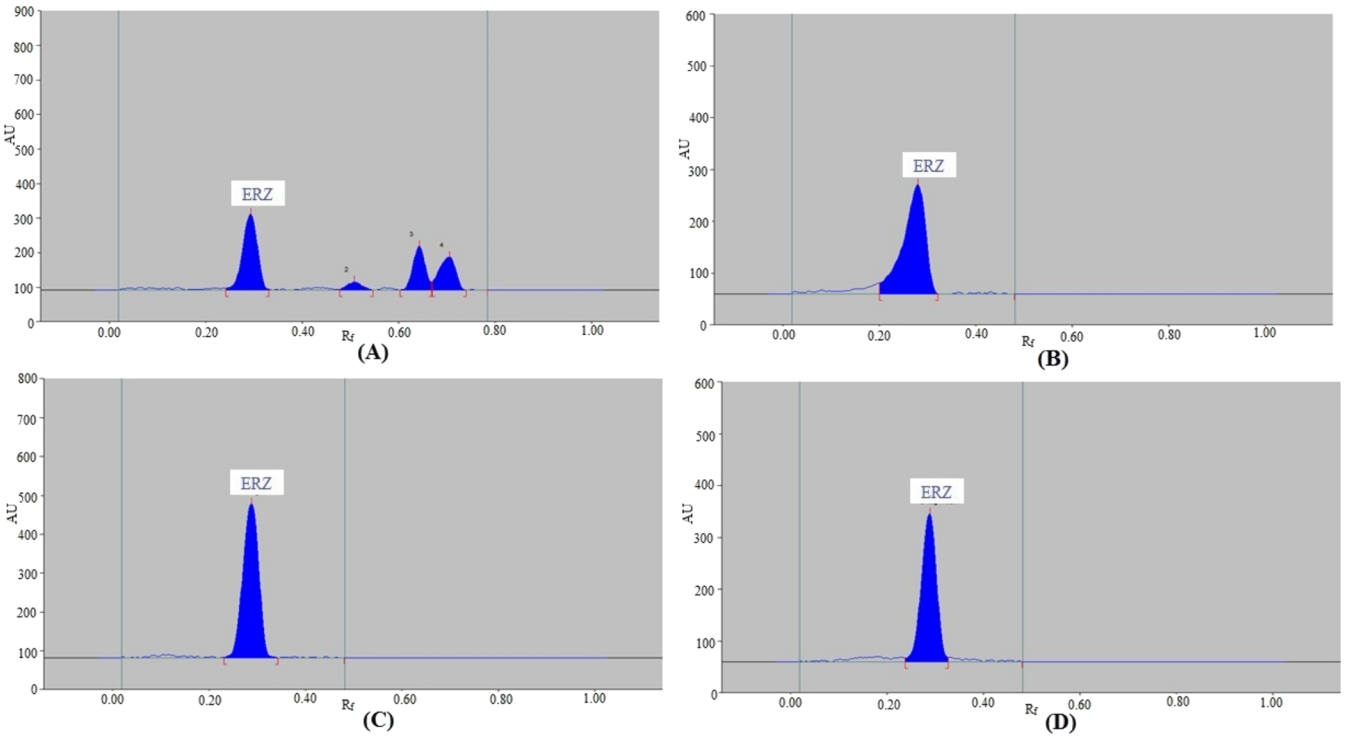

Figure 3. Chromatograms of ERZ recorded under (A) acid, (B) base, (C) oxidative, and (D) thermal degradations of ERZ by the NP-HPTLC method.

Table 6. Outcomes of Forced-Degradation Experiments of ERZ for the NP-HPTLC Assay under Varied Stress Conditions (Mean ± SD; n = 3)

degradation setting	degradation products (R_f)	ERZ R_f	ERZ remained (ng/band)	ERZ recovered (%)
1 M HCl	3 (0.51, 0.64, 0.71)	0.29	240.25	48.05 ± 1.86
1 M NaOH	0	0.28	500.00	100.00 ± 0.00
30% H_2O_2	0	0.29	500.00	100.00 ± 0.00
thermal	0	0.29	500.00	100.00 ± 0.00

Table 7. Results of Forced-Degradation Evaluation of ERZ for the RP-HPTLC Method under Varied Stress Conditions (Mean ± SD; n = 3)

degradation setting	degradation products (R_f)	ERZ R_f	ERZ remained (ng/band)	ERZ recovered (%)
1 M HCl	2 (0.49, 0.57)	0.68	369.75	73.95 ± 2.38
1 M NaOH	0	0.69	500.00	100.00 ± 0.00
30% H_2O_2	0	0.69	500.00	100.100 ± 0.00
thermal	0	0.69	500.00	100.00 ± 0.00

separation as well (Figure 4). 26.05% of the ERZ had degraded after the application of acid stress, leaving 73.95% intact (Table 7 and Figure 4A). The ERZ R_f value (R_f = 0.68) did not change in the acid degradation settings. At R_f = 0.49 and 0.57, respectively, the acid degradation compounds (peaks 1 and 2 in Figure 4A) were separated. Consequently, in conditions of acid breakdown, ERZ was extremely unstable. ERZ was maintained at 100.0% (Table 7) during the base degradation (Figure 4B), oxidative degradation (Figure 4C), and thermal degradation (Figure 4D) settings, and no signs of ERZ degradation were found. ERZ was therefore resistant to heat, oxidative, and base stress situations. During base, oxidative, and thermal degradation settings, the ERZ R_f value changed minimally (R_f = 0.69 in all of these cases). For the RP-HPTLC method, some shoulders were observed especially under base, oxidative, and thermal degradation conditions. We tried to resolve shoulder issues by changing the degradation conditions. However, no degradation was observed under those conditions. Both techniques were used to record the highest decomposition of ERZ during acid degradation settings. These findings showed that in the presence of its breakdown products, ERZ might be detected by both NP- and RP-HPTLC techniques. The stability-indicating characteristics of both processes were indicated by these results. Both approaches were stability-indicating for ERZ detection.

2.4. Application of NP- and RP-HPTLC Methods in ERZ Analysis in Marketed Pharmaceutical Tablets. Both techniques were applied in order to determine the ERZ in marketed pharmaceutical tablets. By utilization of the NP procedure to compare the single TLC spot at R_f = 0.29 ± 0.01 for ERZ with standard ERZ, the chromatogram of ERZ from marketed pharmaceutical tablets was assessed. By use of the NP procedure, the chromatographic peak of ERZ in pharmaceutical tablets was the same as that of pure ERZ (Figure 5A). By employing the RP procedure to compare the single TLC spot at R_f = 0.68 ± 0.01 for ERZ with that of standard ERZ, the chromatogram of pharmaceutical tablets was evaluated. By using the RP procedure, the chromatographic peak of ERZ in pharmaceutical tablets was similar to that of pure ERZ (Figure 5B). Additionally, neither of the two approaches revealed any additional signals related to the tablet contents in the pharmaceutical tablets, suggesting that there was no interaction between ERZ and the tablet ingredients. The amount of ERZ in pharmaceutical tablets was ascertained using the ERZ calibration plot for both methods. Using the NP procedure, the amount of ERZ in pharmaceutical tablets was found to be 87.41 ± 1.24%. Using the RP procedure, the

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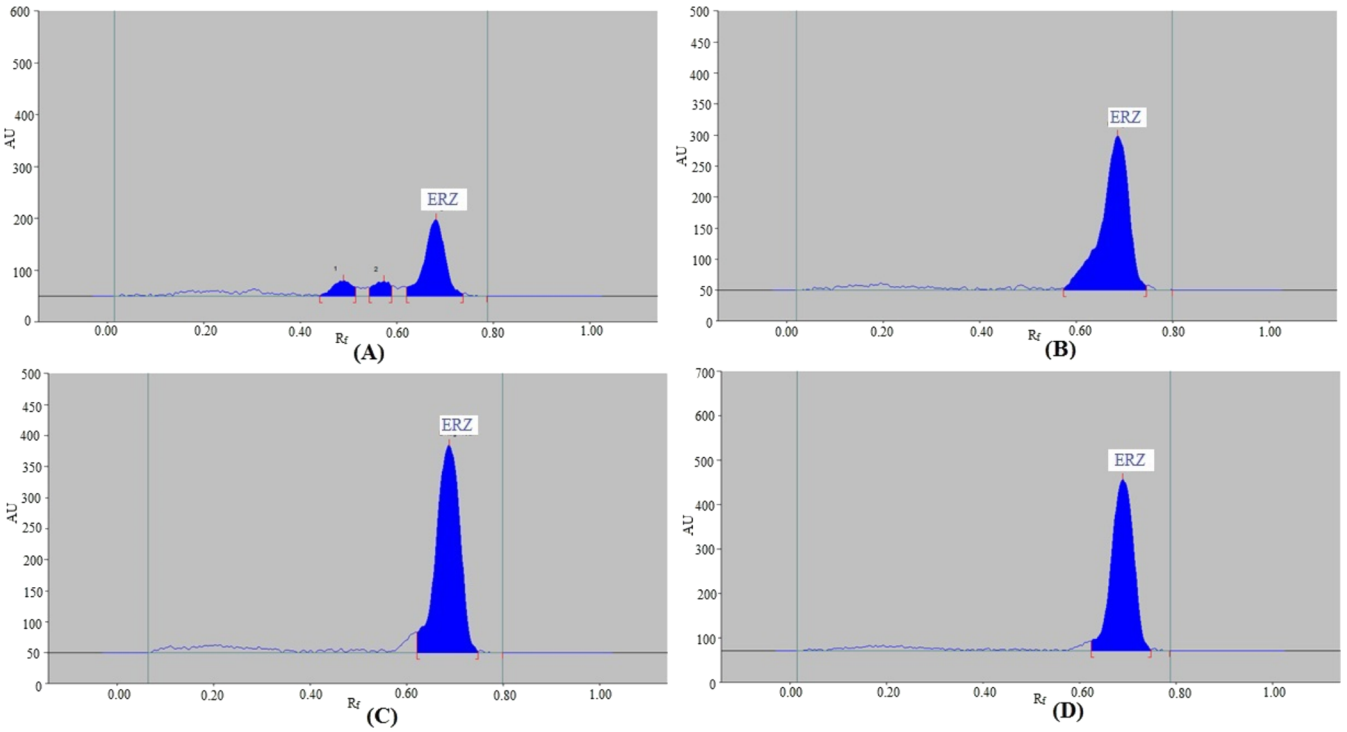

Figure 4. Chromatograms of ERZ recorded under (A) acid, (B) base, (C) oxidative, and (D) thermal degradations of ERZ by the RP-HPTLC method.

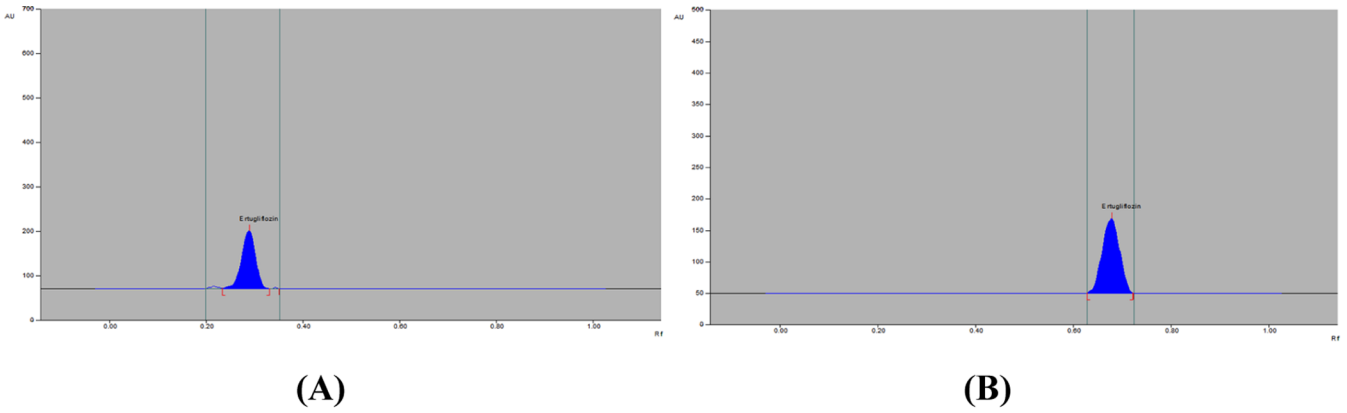

Figure 5. Representative spectrodensitograms of ERZ in marketed tablets obtained by (A) NP- and (B) RP-HPTLC methods.

amount of ERZ in pharmaceutical tablets was found to be 99.28 ± 1.34%. The % ERZ contents in two different brands of commercial tablets, T_1 and T_2, were determined to be 100.99 and 99.98%, respectively in the literature using the HPLC method.[8] The results of the proposed NP- and RP-HPTLC methods of ERZ analysis in commercial tablets were compared with the reported HPLC method using the Student's *t* test and the variance ratio *F*-test. The obtained *t* and *F* values between the present NP procedure and the reported HPLC method were found to exceed their theoretical values, suggesting that there were significant variations in the accuracy and precision of the compared methods. However, the obtained t and *F* values between the present RP procedure and the reported HPLC method did not exceed their theoretical values, suggesting that there were no significant variations in the accuracy and precision of the compared methods.[8] As a result, the present NP procedures were inferior to the reported HPLC method and the present RP procedures were similar to the reported HPLC method.[8] These findings demonstrated that the RP procedure worked better than the NP procedure for the measurement of ERZ. As a result, RP procedures can be efficiently applied in determining ERZ in ERZ-containing pharmaceutical formulations compared to NP procedures.

2.5. Greenness Evaluation. A number of techniques, such as NEMI,[36] GAPI,[37] AES,[38] RGB,[39] EAT,[40] AMVI,[41] AMGS,[42] ChlorTox,[43] and AGREE,[44] can be used to assess the greenness of developed pharmaceutical analysis methods. In the current work, the greenness of both strategies was evaluated using four different approaches: NEMI,[36] AES,[38] ChlorTox,[43] and AGREE.[44] The typical pictograms for the NEMI of both approaches are displayed in Figure S3. Just two of the circles for the NP-HPTLC approach were green (Figure S3A). However, the RP-HPTLC method yields a four-quadrant green circle (Figure S3B), reflecting the method's greenness, because all of the chemicals used are neither toxic, persistent, bioaccumulative, and toxic (PBT), or corrosive and

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Table 8. Proposed Methodology's Greenness Evaluation Using the Analytical Eco-Scale (AES) and Penalty Points and a Comparison with Published HPLC Methods

	penalty points							
reagents/instruments/waste	HPLC [9]	HPLC [10]	HPLC [13]	HPLC [16]	HPLC [8]	HPLC [17]	present NP-HPTLC	present RP-HPTLC
EtOH								4
H_2O			0		0			0
$CHCl_3$							12	
MeOH		18		18	18	18	18	
acetonitrile	12		12	12				
acetate buffer				0				
phosphate buffer					0			
orthophosphoric acid	12		12			8		
KH_2PO_4 (0.5 mM)		0						
instruments	0	0	0	0	0	0	0	0
waste	5	5	5	5	5	5	3	3
total penalty points	29	23	29	35	23	31	33	7
AES scale	71	77	71	65	77	69	67	93

Table 9. Results of the ChlorTox Scales for the Current Approach Compared to Reported HPLC Methods in Terms of the Relative Dangers Concerning Chloroform (CH_{sub}/CH_{CHCl_3}) Calculated Using the WHN Model

analytical stage	solvent/reagent	relative hazard (CH_{sub}/CH_{CHCl_3})	m_{sub} (mg)	ChlorTox (g)	total ChlorTox (g)	ref
sample preparation	$CHCl_3$	1.00	1700	1.70	3.74	present NP-HPTLC
	MeOH	0.56	300	0.17		
HPTLC analysis	$CHCl_3$	1.00	1700	1.70		
	MeOH	0.56	300	0.17		
sample preparation	EtOH	0.26	1600	0.41	0.82	present RP-HPTLC
HPTLC analysis	EtOH	0.26	1600	0.41		
sample preparation	acetonitrile	0.39	314	0.12	3.12	9
	orthophosphoric acid	0.56	713	0.40		
HPLC analysis	acetonitrile	0.39	1572	0.61		
	orthophosphoric acid	0.56	3564	1.99		
sample preparation	MeOH	0.56	356	0.20	1.40	10
HPLC analysis	MeOH	0.56	2138	1.20		
sample preparation	acetonitrile	0.39	275	0.11	3.77	13
	orthophosphoric acid	0.56	772	0.43		
HPLC analysis	acetonitrile	0.39	1651	0.64		
	orthophosphoric acid	0.56	4633	2.59		
sample preparation	MeOH	0.56	792	0.44	1.10	16
HPLC analysis	acetonitrile	0.39	1698	0.66		
sample preparation	MeOH	0.56	277	0.16	1.40	8
HPLC analysis	MeOH	0.56	2218	1.24		
sample preparation	MeOH	0.56	791	0.44	5.10	17
	orthophosphoric acid	0.56	119	0.07		
HPLC analysis	MeOH	0.56	7121	3.99		
	orthophosphoric acid	0.56	1069	0.60		

produce little waste. The RP procedure fared better than the NP procedure based on the NEMI results.

Table 8 displays the outcomes of the AES scales, with penalty points for both strategies. The AES scale for the NP procedure was calculated to be 67. On the other hand, the AES scale of the RP procedure was found to be 93. The RP procedure fared better than the NP procedure, according to the AES results. Additionally, we calculated the AES scales of several HPLC methods found in the literature and compared them to the NP and RP procedures currently used for ERZ assessment (Table 8). AES scales ranging from 65 to 77 were calculated using several HPLC methods found in the literature.[8−10,13,16,17] Based on AES scales, it was discovered that all previously published HPLC methods for ERZ determination were significantly inferior to the current RP-HPTLC approach.[8−10,13,16,17] According to AES scales,[8−10,13,16,17] the majority of documented HPLC methods for ERZ determination were judged to be better than the current NP-HPTLC approach.

Table 9 displays the total ChlorTox and individual solvent ChlorTox scale results for both procedures in relation to published HPLC methods. The current NP-HPTLC method's estimated total ChlorTox scale was 3.74 g, indicating that it was hazardous and unsafe.[47] On the other hand, the current RP-HPTLC method's computed total ChlorTox scale was 0.82 g, indicating that it was environmentally benign and safe.[43] Additionally, we calculated the ChlorTox scales for several HPLC methods found in the literature and compared them to NP and RP procedures currently used for ERZ assessment (Table 9). It was calculated that the ChlorTox scales for

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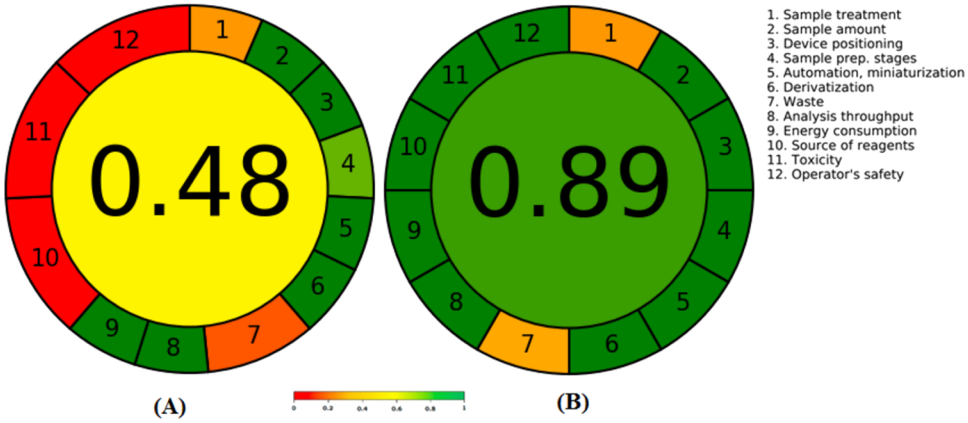

Figure 6. Representative images for AGREE scales for (A) NP-HPTLC and (B) RP-HPTLC methods derived by the AGREE calculator.

several literature HPLC methods ranged from 1.40 to 5.10 g.[8−10,13,16,17] Based on ChlorTox scales, it was discovered that all previously published HPLC methods for ERZ detection were significantly less effective than the present RP-HPTLC method.[8−10,13,16,17] On the basis of ChlorTox scales, the majority of documented HPLC methods for ERZ detection were discovered to be better than the current NP-HPTLC approach.[8−10,13,16,17]

The most popular quantitative approach for assessing greenness is the AGREE methodology, which considers all 12 GAC criteria.[44] The overall AGREE scale for both approaches is shown in Figure 6. The total AGREE scale was predicted by the current NP-HPTLC approach to be 0.48 (Figure 6A). Nonetheless, the total AGREE scale of 0.89 (Figure 6B) was estimated by the current RP-HPTLC technique. The AGREE results once more showed that, in terms of the AGREE scale, the present RP-HPTLC approach performed better than the NP-HPTLC method. Overall greenness approaches compared with all documented HPLC methods show that the current RP procedure for ERZ analysis in pharmaceutical tablets has an excellent greener profile.

3. CONCLUSIONS

Stability-indicating HPTLC methods for ERZ measurement are lacking in the literature. As an alternative to the conventional stability-indicating NP-HPTLC approach, the goal of this study was to develop and evaluate a sensitive and environmentally friendly stability-indicating RP-HPTLC strategy for ERZ assessment in pharmaceutical tablets. For measuring the ERZ, the RP method is more linear, accurate, precise, robust, sensitive, and environmentally friendly than the NP method. The ERZ % recovery values were higher upon using the RP procedure than when using the NP procedure. Selectivity- and stability-indicating traits were found in both techniques. The RP procedure fared better on the greenness scale than the NP procedure, according to the findings of the NEMI, AES, ChlorTox, and AGREE evaluations. These findings demonstrated that the RP strategy performed better than the NP strategy for assessing ERZ in the pharmaceutical tablets. The potential strategies such as low solvent usage, optimizing solvent consumption, reducing waste, and replacing organic solvents with aqueous buffers could further enhance the greenness of the developed HPTLC methods. Subsequent studies can evaluate ERZ in plasma samples and assess its pharmacokinetics using proven HPTLC techniques.

4. MATERIALS AND METHODS

4.1. Materials. "Beijing Mesochem Technology (Beijing, China)" is the source of pure ERZ. "E-Merck (Darmstadt, Germany)" provided the HPLC grade solvents, including EtOH, MeOH, and $CHCl_3$. The Milli-Q equipment provided H_2O of HPLC quality. Commercial ERZ tablets, each containing 15 mg of ERZ, were purchased at a pharmacy in Riyadh, Saudi Arabia. The remaining components were of AR grade.

4.2. Instrumentation and Analytical Settings. The "HPTLC CAMAG TLC system (CAMAG, Muttenz, Switzerland)" was used to determine the ERZ in commercial tablets. The solutions were spotted in the form of 6 mm bands with the help of a "CAMAG Automatic TLC Sampler 4 (ATS4) Sample Applicator (CAMAG, Geneva, Switzerland)". The sample applicator was filled with the "CAMAG microliter Syringe (Hamilton, Bonaduz, Switzerland)". 150 nL/s was used as the application rate for ERZ analysis. The glass-coated plates were developed using a linear ascending mode at a distance of 8 cm in a "CAMAG automated developing chamber 2 (ADC2) (CAMAG, Muttenz, Switzerland)". The development chamber was filled with the vapors of the respective mobile phase for 30 min at an ambient temperature of 22 °C. At a wavelength of 199 nm, ERZ was detected. The slit size and scan speed were set to 4 × 0.45 mm^2 and 20 mm/s, respectively. For every measurement, three or six replications were used. The program that was utilized was WinCAT's (version 1.4.3.6336, CAMAG, Muttenz, Switzerland).

Both assays employed the same equipment and analytical parameters. The mobile phases and stationary phase/TLC plates were the primary differences between the NP- and RP-HPTLC procedures. In the NP procedure, the optimal mobile phase was $CHCl_3$/MeOH (85:10 v/v), while in the RP procedure, the optimal mobile phase was EtOH/H_2O (80:20 v/v). The stationary phase was "glass-coated plates (plate size: 10 × 20 cm^2) pre-coated with silica gel (particle size: 5 μm) 60 NP-18F254S plates" in the NP procedure. The stationary phase was "glass-coated plates (plate size: 10 × 20 cm^2) pre-coated with silica gel (particle size: 5 μm) 60 RP-18F254S plates" in the RP procedure. The choices of silica gel 60 NP-18F254S and RP-18F254S plates will definitely influence the performance and selectivity of the NP- and RP-HPTLC methods. The particle sizes of both NP and RP plates were very low (5 μm) in addition to the thinner layer for both plates. The lower particle size provides a higher packing

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density and a smooth surface. In addition, the low particle size and thinner layer would result in enhanced detection sensitivity and analysis speed.

4.3. Calibration Curves for ERZ. A stock solution with a concentration of 100 μg/mL of ERZ was created by dissolving precisely weighed 10 mg of ERZ into the appropriate amounts of $CHCl_3$/MeOH (85:15 v/v) for NP-HPTLC and EtOH/H_2O (80:20 v/v) for RP-HPTLC. By diluting variable amounts of ERZ stock solution with $CHCl_3$/MeOH (85:15 v/v), ERZ concentrations in the 50−600 ng/band range were further created for the NP procedure. On the other hand, ERZ concentrations in the 25−1200 ng/band range were produced using the RP procedure, which entailed diluting the various amounts of ERZ stock solution using EtOH/H_2O (80:20 v/v). 10 μL of each ERZ concentration were spotted on NP and RP TLC plates, respectively, for the NP- and RP-HPTLC procedures. To determine each ERZ concentration's peak response, both techniques were applied. ERZ calibration curves were constructed by plotting the observed spot area against the ERZ concentrations using six replications (n = 6).

4.4. Sample Preparation for the Measurement of ERZ in Commercial Tablets. Twenty-five tablets containing 15 mg of ERZ each were randomly ingested in order to measure the amount of ERZ in commercial tablets. Next, the average weight was calculated. The fine powder was obtained by crushing and triturating the tablets. The fine powder, which contained 15 mg of total ERZ, was dispersed using 10 mL of the appropriate mobile phase. The resultant mixes were filtered and sonicated for 15 min.[35] The produced solutions were evaluated for ERZ in commercial tablets using both procedures.

4.5. Validation Assessment. Both ERZ measurement techniques were validated for multiple validation criteria in accordance with ICH-Q2-R2 guidelines.[45] Pharmaceutical analytical procedures are assessed using the ICH-Q2-R2 protocols. These protocols must be followed in order for analytical methods to be registered in the USA, Japan, and the EU. The analytical methods should be verified for linear range, sensitivity (LOD and LOQ), accuracy, precision, repeatability, specificity, robustness, and robustness, as per the ICH-Q2-R2 criteria.[45] ERZ linearity was assessed by plotting the observed peak response against the ERZ concentrations. The linearity for the NP technique of ERZ analysis in the 50−600 ng/band range was assessed using six replicates (n = 6). Six replicates (n = 6) of the RP technique were used to evaluate the ERZ linearity over the 25−1200 ng/band range.

R_f, A_s, and N/m were computed to determine the parameters for the system appropriateness for both ERZ measurement methods. Using their published formulas,[35] R_f, A_s, and N/m for both ERZ measurement procedures were determined.

The accuracy of the two ERZ measuring techniques was calculated utilizing spiking technology/standard addition strategy, expressed as % recoveries.[45] The preanalyzed ERZ solution (100 ng/band) was spiked with additional 50, 100, and 150% ERZ solution for the NP procedure in order to achieve low-QC (LQC) levels of 150 ng/band, middle-QC (MQC) levels of 200 ng/band, and high-QC (HQC) levels of 250 ng/band of ERZ. To attain LQC, MQC, and HQC levels of 300, 400, and 500 ng/band of ERZ by the RP procedure, an additional 50, 100, and 150% ERZ solution was mixed with the 200 ng/band preanalyzed ERZ solution. The selection of different concentrations for recovery studies was due to different linearity ranges for both methods. For NP procedures, the linearity range was 50−600 ng/band. However, the linearity range for RP procedures was 25−1200 ng/band. The % recovery was determined using the spiking methodology. The target concentration was selected as 100 ng/band from the middle of the linearity range for NP procedures. In the target concentration (100 ng/band), 50−150% extra concentration was spiked to obtain LQC, MQC, and HQC of 150, 200, and 250 ng/band, respectively. Similarly, the target concentration was selected as 200 ng/band from the middle of the linearity range for RP procedures. In the target concentration (200 ng/band), 50−150% extra concentration was spiked to LQC, MQC, and HQC of 300, 400, and 500 ng/band, respectively. An investigation was conducted on three distinct ERZ QC solutions in order to evaluate the accuracy of both approaches. For both approaches, six replications (n = 6) were used to calculate the % recovery at each QC level. The % recovery for both approaches was determined using eq 1:

$$\text{recovery (\%)} = \frac{\text{measured concentration}}{\text{theoretical concentration}} \times 100 \quad (1)$$

The precision of the NP- and RP-HPTLC techniques for ERZ was evaluated in terms of interassay (intermediate precision) and repeatability (intra-assay precision). Six replicates of freshly made ERZ solutions were tested for repeatability (intra-assay precision) for both procedures on the same day at LQC, MQC, and HQC (n = 6). Like recovery studies, three different concentrations based on LQC, MQC, and HQC for both methods were selected. Because the linearity ranges for both methods were different, the selected concentrations for precision studies were different. For each approach, ERZ interday precision was assessed using six replicates (n = 6) of freshly generated ERZ solutions at the same QC samples spread over 3 days.[45]

A number of deliberate modifications were made to the relevant mobile phase's content in order to evaluate the robustness of ERZ for both approaches. The standard $CHCl_3$/MeOH (85:15 v/v) mobile phase for ERZ was changed to $CHCl_3$/MeOH (87:13 v/v) and $CHCl_3$/MeOH (83:17 v/v) for the NP-HPTLC experiment. Six replications (n = 6) were used to record the variations in peak response and R_f. The eco-friendly mobile phase EtOH/H_2O (80:20 v/v) for the RP-HPTLC method was changed to EtOH/H_2O (82:218 v/v) and EtOH/H_2O (78:22 v/v), and six replications (n = 6) were used to record the uncertainties in spot area and R_f.[45]

Using a standard deviation approach, the sensitivity of both techniques for the ERZ was assessed in terms of "LOD and LOQ". For each of the two procedures, a blank sample (one without ERZ) was injected six times, and the sample's standard deviation was calculated. The published equations for both approaches were utilized to calculate ERZ "LOD and LOQ" through six replications (n = 6).[45]

To evaluate the specificity and peak purity of both ERZ techniques, the R_f values, UV-absorption spectra, and 3D spectra of ERZ in commercial tablets were compared with that of pure ERZ.

4.6. Forced-Degradation Studies. The forced-degradation studies were conducted for both techniques under acidic, alkaline, oxidative, and thermal stress conditions.[35,51] The ERZ in the concentration of 500 ng/band for both techniques was subjected to 24 h of thermal stress conditions in a hot air oven at 55 °C, 1 M HCl (acid), 1 M NaOH (alkaline), and 30% v/v H_2O_2 (oxidative). The solutions were diluted with corresponding mobile phases. For these investigations, the comprehensive

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protocols as described in our most previously published article[35] were adhered to. Under the previously stated stress conditions, ERZ chromatograms were acquired for both approaches and examined for degradation products.

4.7. Application of NP- and RP-HPTLC Methods in the Measurement of ERZ in Commercial Tablets. Commercial tablet solutions were placed on NP and RP TLC plates, respectively, to record the peak responses for ERZ in three replicates (n = 3) using NP and RP procedures. The commercial tablet's ERZ content was determined using the ERZ calibration plot for both approaches.

4.8. Greenness Assessment. Four different methodologies were used to analyze the greenness profile of both ERZ determination methods: NEMI,[36] AES,[38] ChlorTox,[43] and AGREE.[44] To obtain the initial evaluation based on PBT, hazardous, corrosive, and waste materials, NEMI is employed.[36] AES is a semiquantitative technique that considers instruments, waste, and each step of the analytical process. For the solvents/reagents that need minimal to no reagent use, little energy, and no waste, an ideal analysis with 100 points is predicted. Penalty points are awarded and deducted from the final score of 100 if any of these conditions are not met.[38] According to the ChlorTox scale approach, eq 2[43] is used to determine the ChlorTox scale.

$$\text{ChlorTox} = \frac{\text{CH}_{\text{sub}}}{\text{CH}_{\text{CHCl}_3}} \times m_{\text{sub}} \quad (2)$$

where m_{sub} is the mass of the substance of interest needed for a single analysis, $\text{CH}_{\text{CHCl}_3}$ is the chemical hazard of standard CHCl_3, and CH_{sub} is the chemical risk of the substance of interest. The safety data sheet from Sigma-Aldrich (St. Louis, MO) was used to aid in the computation of the values of CH_{sub} and $\text{CH}_{\text{CHCl}_3}$ using the weighted hazards number (WHN) model.[43] Using WHN method and safety data sheet from Sigma-Aldrich (St. Louis, MO), CH_{sub} values for the substance of interests such as CHCl_3, MeOH, and EtOH were derived using eq 3:

$$\text{CH}_{\text{sub}} = (1 \times N_{\text{cat1}}) + (0.75 \times N_{\text{cat2}}) + (0.5 \times N_{\text{cat3}}) + (0.25 \times N_{\text{cat4}}) \quad (3)$$

where the toxicity numbers for the 1, 2, 3, and 4 categories are denoted, respectively, by the letters N_{cat1}, N_{cat2}, N_{cat3}, and N_{cat4}.

For substance CHCl_3, $N_{\text{cat1}} = 1$, $N_{\text{cat2}} = 4$, $N_{\text{cat3}} = 3$, and $N_{\text{cat4}} = 1$ were taken from the safety data sheet of Sigma-Aldrich (St. Louis, MO).

Hence for CHCl_3, $\text{CH}_{\text{sub}} = (1 \times 1) + (0.75 \times 4) + (0.5 \times 3) + (0.25 \times 1) = 5.75$.

For the substance MeOH, $N_{\text{cat1}} = 1$, $N_{\text{cat2}} = 1$, $N_{\text{cat3}} = 3$, and $N_{\text{cat4}} = 0$ were taken from the safety data sheet of Sigma-Aldrich (St. Louis, MO).

Hence for MeOH, $\text{CH}_{\text{sub}} = (1 \times 1) + (0.75 \times 1) + (0.5 \times 3) + (0.25 \times 0) = 3.25$.

For the substance EtOH, $N_{\text{cat1}} = 0$, $N_{\text{cat2}} = 2$, $N_{\text{cat3}} = 0$, and $N_{\text{cat4}} = 0$ were taken from the safety data sheet of Sigma-Aldrich (St. Louis, MO).

Hence for EtOH, $\text{CH}_{\text{sub}} = (1 \times 0) + (0.75 \times 2) + (0.5 \times 0) + (0.25 \times 0) = 1.5$.

The CHCl_3 was used as a standard, and it was also the substance of interest under the NP procedure. The already calculated 5.75 value was used as the $\text{CH}_{\text{CHCl}_3}$ value for the standard CHCl_3. The values of m_{sub} required for a single analysis are included in Table 9. Finally, the ChlorTox values were obtained using eq 1.

The AGREE scale for both approaches to the ERZ analysis was derived using the AGREE-metric technique.[44] The AGREE: The Analytical Greenness Calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020) was used to calculate the AGREE scales for both approaches. The values ranged from 0.0 to 1.0 based on 12 different GAC principles.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c02399.

Molecular structure of ERZ (Figure S1); NEMI results for the present NP- and RP-HPTLC methods (Figure S2); and 3D spectra of standard ERZ and commercial tablets using NP- and RP-HPTLC methods (Figure S3) (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Plosker, G. L. Canagliflozin: a review of its use in patients with type 2 diabetes mellitus. *Drugs* **2014**, *74*, 807−824.
(2) Deeks, E. D.; Scheen, A. J. Canagliflozin: a review in type 2 diabetes. *Drugs* **2017**, *77*, 1577−1592.
(3) Alam, P.; Iqbal, M.; Foudah, A. I.; Alqarni, M. H.; Shakeel, F. Quantitative determination of canagliflozin in human plasma samples using a validated HPTLC method and its application to a pharmacokinetic study in rats. *Biomed. Chromatogr.* **2020**, *34*, No. 4929.
(4) Markham, A. Ertugliflozin: First global approval. *Drugs* **2018**, *78*, 513−519.
(5) Sharma, R.; Razdan, K.; Kuhad, A.; Kuhad, A. Ertugliflozin for the treatment of type 2 diabetes. *Drugs Today* **2019**, *55*, 167−175.
(6) Frias, J. P. Fixed-dose combination of ertugliflozin and metformin hydrochloride for the treatment of type 2 diabetes. *Expert Rev. Endocrinol. Metab.* **2019**, *14*, 75−83.
(7) Liu, J.; Tarasenko, L.; Terra, S. G.; Huyck, S.; Wu, L.; Pong, A.; et al. Efficacy of ertugliflozin in monotherapy or combination therapy in patients with type 2 diabetes: A pooled analysis of placebo-controlled studies. *Diabetes Vasc. Dis. Res.* **2019**, *16*, 415−423.
(8) Devika, G. S.; Anjana, A.; Isha, M. S.; Enija, E.; Jeevitha, T.; Nevish, P.; et al. Determination of ertugliflozin in pharmaceutical formulations by RP-HPLC method. *World J. Biol. Pharm. Health Sci.* **2023**, *15*, 35−42.
(9) Harshalatha, P.; Chandrasekhar, K. B.; Chandrasekhar, M. V. A novel RP-HPLC method for simultaneous determination of ertugliflozin and sitagliptin in bulk and tablet dosage form. *Int. J. Res. Pharm. Sci.* **2018**, *9*, 1042−1050.
(10) Babu, D. C.; Chetty, C. M.; Mastanamma, S. K. Novel stress indicating RP-HPLC method development and validation for the simultaneous estimation of ertugliflozin and sitagliptin in bulk and its formulation. *Orient. J. Chem.* **2018**, *34*, 2554−2561.
(11) Amtul, H. H.; Yunoos, M. A new validated stability indicating RP-HPLC method for the simultaneous estimation of sitagliptin and ertugliflozin in bulk and tablet dosage form. *Indo Am. J. Pharm. Sci.* **2018**, *5*, 8785−8794.
(12) Anjaneyulu, R. B.; Radhakrishnanand, P.; Irshad, A. M.; Ravi, K. P. A validated reverse-phase high-performance liquid chromatography-charged aerosol detector technique for the simultaneous estimation of sitagliptin and ertugliflozin in pure and pharmaceutical dosage forms. *Asian J. Pharm. Clin. Res.* **2019**, *12*, 236−240.
(13) Kumari, K. S.; Bandhakavi, S. Development and validation of stability-indicating RP-HPLC method for the simultaneous determination of ertugliflozin pidolate and metformin hydrochloride in bulk and tablets. *Future J. Pharm. Sci.* **2020**, *6*, No. 66.
(14) Suneetha, A.; Mounika, V.; Shaik, M. S. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of sitagliptin and ertugliflozin in bulk and tablet dosage forms. *Asian J. Pharm. Anal.* **2020**, *10*, 81−85.
(15) Sunkara, B.; Gampa, T. R.; Markanti, M.; Midthapallty, R. K. Stability indicating method development and validation for simultaneous estimation and quantification of ertugliflozin and metformin in bulk and tablet dosage from. *Future J. Pharm. Sci.* **2021**, *7*, No. 32.
(16) Gurrala, S.; Shivaraj, S.; Anumolu, P. D.; Haripriya, D.; Subrahmanyam, C. V. S. Chromatographic study of sitagliptin and ertugliflozin under quality-by-design paradigm. *Braz. J. Pharm. Sci.* **2023**, *59*, No. 21328.
(17) Mankar, S. D.; Sagar, P.; Bhawar, S.; Siddheshwar, S.; Dighe, S. Development and validation of RP-HPLC method for simultaneous estimation of ertugliflozin and sitagliptin in bulk drug and tablet dosage form. *Int. J. Exp. Res. Rev.* **2023**, *32*, 288−296.
(18) Han, D. G.; Yun, H.; Yoon, I. A. S. A novel high-performance liquid chromatographic method combined with fluorescence detection for determination of ertugliflozin in rat plasma: Assessment of pharmacokinetic drug interaction potential of ertugliflozin with mefenamic acid and ketoconazole. *J. Chromatogr. B* **2019**, *1122*−*1123*, 49−57.
(19) Khoja, S. S.; Patel, L. J. Development and validation of new analytical LC-MS/MS method for the estimation of antidiabetic drugs ertugliflozin and sitagliptin in combined pharmaceutical dosage form. *J. Pharm. Res. Int.* **2021**, *33*, 194−204.
(20) Rao, P. V.; Rao, A. L.; Prasad, S. V. U. M. Rapid quantitative estimation of metformin and ertugliflozin in rat plasma by liquid chromatography-tandem mass spectroscopy and its application to pharmacokinetic studies. *Egypt. Pharm. J.* **2021**, *20*, 1−7.
(21) Rao, P. V.; Rao, A. L.; Prasad, S. V. U. M. Development and validation of a method for simultaneous estimation of sitagliptin and ertugliflozin in rat plasma by LC−MS method. *Curr. Pharm. Anal.* **2021**, *17*, 1060−1074.
(22) Deepthi, R.; Gowri Sankar, D. A novel UPLC method for simultaneous estimation of ertugliflozin and sitagliptin in bulk and tablet dosage form. *Res. J. Pharm. Technol.* **2021**, *14*, 4859−4862.
(23) Kuber, B. R.; Addanki, S. Novel stability indicating RP-UPLC for simultaneous estimation of sitagliptin and ertugliflozin in bulk and pharmaceutical formulations. *Future J. Pharm. Sci.* **2021**, *7*, No. 86.
(24) Salakolusu, S.; Katari, N. K.; Sharma, G. V. R.; Kaliyaperumal, M.; Puppala, U.; Ranga, M.; Jonnalagadda, S. B. Identification, isolation, and structural characterization of novel forced degradation products of ertugliflozin using advanced analytical techniques. *Sci. Rep.* **2023**, *13*, No. 9472.
(25) Qiu, X.; Xie, S.; Ye, L.; Xu, R. UPLC-MS/MS method for quantification of ertugliflozin and sitagliptin in rat plasma. *Anal. Biochem.* **2019**, *567*, 112−116.
(26) Ahmed, R. M. Greenness assessment of spectrofluorometric method for quantification of ertugliflozin: Application to dosage from human urine. *Rec. Pharm. Biomed. Sci.* **2021**, *5*, 135−144.
(27) El-Yazbi, A. F.; Aboukhalil, F. M.; Khamis, E. F.; Youssef, R. M.; El-Sayed, M. A. Greenness assessment of a stability indicating simple inexpensive high performance thin layer chromatography-dual wavelength method for simultaneous determination of Mometasone furoate and salicylic acid in complex matrix using analytical eco scale. *J. Planar Chromatogr.* **2021**, *34*, 455−466.
(28) Alam, P.; Shakeel, F.; Iqbal, M.; Foudah, A. I.; Alqarni, M. H.; Aljarba, T. M.; Bar, F. A.; Alshehri, S. Quantification of pomalidomide using conventional and eco-friendly stability-indicating HPTLC assays: A contrast of validation parameters. *ACS Omega* **2023**, *8*, 30655−30664.
(29) Ragab, M. A. A.; Maher, H. M.; Tarek, S.; Mahgoub, H. An ecofriendly multi-analyte high performance thin layer chromatographic densitometric determination of amoxicillin, metronidazole, and famotidine in their ternary mixtures and simulated gastric juice: A promising protocol for eradicating *Helicobacter pylori*. *J. Sep. Sci.* **2023**, *46*, No. 2200951.
(30) Shakeel, F.; Alam, P.; Haq, N.; Alqarni, M. H. Eco-friendly high-performance thin-layer chromatography method for the determination of tenoxicam in commercial formulations. *ACS Omega* **2023**, *8*, 39936−39944.
(31) Gałuszka, A.; Migaszewski, Z.; Namiesnik, J. The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices. *TrAC, Trends Anal. Chem.* **2013**, *50*, 78−84.
(32) Abdelrahman, M. M.; Abdelwahab, N. S.; Hegazy, M. A.; Fares, M. Y.; El-Sayed, G. M. Determination of the abused intravenously administered madness drops (tropicamide) by liquid chromatography in rat plasma; an application to pharmacokinetic study and greenness profile assessment. *Microchem. J.* **2020**, *159*, No. 105582.
(33) Alam, P.; Ezzeldin, E.; Iqbal, M.; Anwer, M. K.; Mostafa, G. A. E.; Alqarni, M. H.; et al. Ecofriendly densitometric RP-HPTLC method for determination of rivaroxaban in nanoparticle formulations using green solvents. *RSC Adv.* **2020**, *10*, 2133−2140.

https://doi.org/10.1021/acsomega.4c02399
ACS Omega 2024, 9, 23001−23012

(34) Alam, P.; Salem-Bekhit, M. M.; Al-Joufi, F. A.; Alqarni, M. H.; Shakeel, F. Quantitative analysis of cabozantinib in pharmaceutical dosage forms using green RP-HPTLC and green NP-HPTLC methods: A comparative evaluation. *Sustainable Chem. Pharm.* **2021**, *21*, No. 100413.
(35) Foudah, A. I.; Shakeel, F.; Alqarni, M. H.; Alam, P. A rapid and sensitive stability-indicating green RP-HPTLC method for the quantitation of flibanserin compared to green NP-HPTLC method: Validation studies and greenness assessment. *Microchem. J.* **2021**, *164*, No. 105960.
(36) Keith, L. H.; Brass, H. J.; Sullivan, D. J.; Boiani, J. A.; Alben, K. T. An introduction to the national environmental methods index. *Environ. Sci. Technol.* **2005**, *39*, 173A−176A.
(37) Płotka-Wasylka, J. A new tool for the evaluation of the analytical procedure: Green analytical procedure index. *Talanta* **2018**, *181*, 204−209.
(38) Gałuszka, A.; Konieczka, P.; Migaszewski, Z. M.; Namiesnik, J. Analytical eco-scale for assessing the greenness of analytical procedures. *TrAC, Trends Anal. Chem.* **2012**, *37*, 61−72.
(39) Nowak, P. M.; Koscielnaik, P. What color is your method? Adaptation of the RGB additive color model to analytical method evaluation. *Anal. Chem.* **2019**, *91*, 10343−10352.
(40) Gaber, Y.; Tornvall, U.; Kumar, M. A.; Ali Amin, M.; Hatti-Kaul, R. HPLC-EAT (Environmental Assessment Tool): a tool for profiling safety, health and environmental impacts of liquid chromatography methods. *Green Chem.* **2011**, *13*, 2021−2025.
(41) Hartman, R.; Helmy, R.; Al-Sayah, M.; Welch, C. J. Analytical method volume intensity (AMVI): a green chemistry metric for HPLC methodology in the pharmaceutical industry. *Green Chem.* **2011**, *13*, 934−939.
(42) Hicks, M. B.; Farrell, W.; Aurigemma, C.; Lehmann, L.; Weisel, L.; Nadeau, K.; et al. Making the move towards modernized greener separations: introduction of the analytical method GREEnness score (AMGS) calculator. *Green Chem.* **2019**, *21*, 1816−1826.
(43) Nowak, P. M.; Wietecha-Posluszny, R.; Plotka-Wasylka, J.; Tobiszewski, M. How to evaluate methods used in chemical laboratories in terms of the total chemical risk? a ChlorTox Scale. *Green Anal. Chem.* **2023**, *5*, No. 100056.
(44) Pena-Pereira, F.; Wojnowski, W.; Tobiszewski, M. AGREE-Analytical GREEnness metric approach and software. *Anal. Chem.* **2020**, *92*, 10076−10082.
(45) International Conference on Harmonization (ICH). Q2 (R2): Guideline on Validation of Analytical Procedures − Text and Methodology, 2023.
(46) Alam, P.; Shakeel, F.; Mahdi, W. A.; Foudah, A. I.; Alqarni, M. H.; Aljarba, T. M.; Alshehri, S.; Ghoneim, M. M. Determination of gefitinib using routine and greener stability-indicating HPTLC methods: A comparative evaluation of validation parameters. *Processes* **2022**, *10*, No. 762.
(47) El-Kimary, E. I.; Youssef, R. M.; Allam, A. N. High-performance thin-layer chromatographic assay of metformin in urine using ion-pair solid-phase extraction: Application for bioavailability and bioequivalence study of new microbeads controlled release formulation. *J. Planar Chromatogr.* **2014**, *27*, 377−384.
(48) El-Kimary, E. I.; Ragab, M. A. A. A validated high-performance thin-layer chromatographic method for the assay of two binary mixtures containing omeprazole or its isomer esomeprazole in pharmaceutical dosage forms. *J. Planar Chromatogr.* **2015**, *28*, 74−82.
(49) El-Kimary, E. I.; Khamis, E. F.; Belal, S. F.; Moneim, M. M. A. Novel validated HPTLC method for the analysis of two binary mixtures containing tamsulosin hydrochloride with antimuscarinic agents. *J. Chromatogr. Sci.* **2018**, *56*, 81−91.
(50) Shakeel, F.; Alam, P.; Alqarni, M. H.; Iqbal, M.; Anwer, M. K.; Alshehri, S. A greener RP-HPTLC-densitometry method for the quantification of apremilast in nanoformulations and commercial tablets: Greenness assessment by analytical eco-scale, ChlorTox, and AGREE methods. *Arab. J. Chem.* **2024**, *17*, No. 105571.
(51) Alam, P.; Shakeel, F.; Alqarni, M. H.; Foudah, A. I.; Faiyazuddin, M.; Alshehri, S. Rapid, sensitive, and sustainable reversed-phase HPTLC method in comparison to the normal-phase HPTLC for the determination of pterostilbene in capsule dosage form. *Processes* **2021**, *9*, No. 1305.

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ACS Omega 2024, 9, 23001−23012