BMC Chemistry

RESEARCH Open Access

Green stability-indicating RP-HPTLC approach for determining suvorexant in commercial tablet dosage forms

Prawez Alam[1], Faiyaz Shakeel[2], Mohammed H. Alqarni[1], Ahmed I. Foudah[1], Tariq M. Aljarba[1], Fatma M. Abdel Bar[1], Mohd Imran[3] and Mohammad Ali[4*]

Abstract

A novel sedative/hypnotic drug called suvorexant (SUV) is advised for treating insomnia. From a forensic standpoint, it is important medicine because of its sedative/hypnotic and depressing effects. There are no green "high-performance thin-layer chromatographic (HPTLC)" techniques for measuring SUV in the literature. Therefore, this study aims to develop and validate a reverse-phase HPTLC approach that indicates green stability for SUV measurement in commercially available tablet dosage forms. SUV was detected at 255 nm in wavelength. The suggested SUV analysis approach's greenness was assessed using the "analytical eco-scale (AES), ChlorTox, and analytical GREEnness (AGREE)" tools. The current SUV analysis method showed linearity in the 10–1200 ng/band range. Furthermore, the SUV analytical method was robust, accurate (% recoveries = 98.18–99.30), sensitive (LOD = 3.32 ng/band and LOQ = 9.98 ng/band), precise (% CV = 0.78–0.94), and environmentally friendly. The "AES, total ChlorTox, and AGREE" scales were derived to be 93, 0.96 g, and 0.88, respectively, using the current SUV analytical method, demonstrating an exceptional greenness profile. SUV was shown to be suitably unstable under oxidative degradation conditions and suitably stable under acid, base, and heat degradation conditions. Furthermore, the SUV analytical method's stability-indicating component identified SUV in the presence of its breakdown products. It was observed that marketed SUV tablet brands A and B contained, respectively, 98.18 and 101.32% of SUV. The findings of the study indicated that SUV in marketed tablet dosage forms may be monitored on a regular basis with the use of the current green HPTLC methodology.

Clinical trial number

Not applicable.

Keywords Forensic medicine, Greenness assessment, RP-HPTLC, Suvorexant, Validation

*Correspondence:
Mohammad Ali
alimohammad973@gmail.com
[1]Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia
[2]Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University, Rafha 91911, Saudi Arabia
[4]Department of Pharmacology, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, BG Nagara, Mandya 571448, India

Introduction

The family of medications known as central nervous system (CNS) drugs includes sedative/hypnotic drugs [1]. From a forensic standpoint, these drugs are important because of their widespread use, propensity for abuse, incapacitating effects, and capacity to mix with other CNS depressants to produce compounding effects [2]. A relatively new family of sedative/hypnotic medications is represented by the pharmaceutical product suvorexant (SUV), which is tablet-based [3, 4]. Figure 1 shows the SUV's molecular structure. Insomnia is treated with it [5, 6]. SUV is allegedly a very potent dual orexin receptor (OX1R and OX2R) antagonist that suppresses the OX neurons in the arousal system that promote alertness, accelerating the onset of sleep [3, 6]. Like other sedative/hypnotic medicines, SUV has the potential to be abused, which is why the US Drug Enforcement Administration placed it under Schedule IV of the Federal Controlled Substances Act shortly after it was approved [7]. The forensic toxicology community is likely to come with SUV more frequently now that it has been successfully detected in the postmortem specimens of three different autopsy cases [8]. The SUV assessment is essential for its commercial pharmaceutical products, both in terms of quality and quantity.

Numerous analytical approaches for analyzing and identifying SUV in a variety of biological samples and drug dosage forms have been described. A "high-performance liquid chromatography (HPLC)" approach has been documented for assessing SUV in tablet dosage forms [9]. SUV and lemborexant (LMB) concentrations in laboratory-prepared synthetic mixtures have recently been reported to be determined simultaneously using a greener HPLC method [10]. It has been reported that SUV can be detected in rabbit plasma samples using an HPLC bioanalytical approach [11]. There have also been reports of a bioanalytical approach using "LC-mass spectrometry (LC-MS)/MS (LC-MS/MS)" to detect SUV in blood samples [12]. Along with other sedatives and hypnotics, SUV has also been measured in whole blood samples using an LC-MS/MS bioanalytical technique [13]. Additionally, "LC-quadrupole/time of flight-MS (LC-Q/TOF-MS)" is a bioanalytical technique utilized to detect SUV in blood samples [14]. SUV in human plasma samples was also measured by an LC-MS/MS approach [15]. Several "ultra-performance LC-MS/MS (UPLC-MS/MS)"-based bioassays were also used to evaluate SUV in plasma samples [16–18]. Urine sample SUV has been reported to be measured using "gas chromatography-MS (GC–MS), LC-Q/TOF-MS, and UPLC-MS/MS"-based bioassays [19–21]. We recently developed a bioassay for identifying SUV in human urine samples using "high-performance thin-layer chromatography (HPTLC)" [22]. Our research team has also reported using a greener HPTLC technique to identify a comparable class of medication, LMB, in its pharmaceutical tablets [23].

The majority of analysis techniques that have been documented have been used to the determination of SUV in biological materials, including blood, serum, urine, and plasma. Only two analytical methods are available to determine SUV in pharmaceutical dosage forms [9, 10]. Additionally, there is a dearth of information in the literature about the greener HPTLC methods for determining SUV. The adoption of ecologically appropriate solvent alternatives to decrease the hazardous effects of toxic or hazardous solvents on the ecosystem is one of the 12 requirements of "green analytical chemistry (GAC)" [24]. A review of the literature indicated that during the past few decades, there has been a significant increase in the usage of greener solvents [25–30]. Numerous approaches for evaluating the greenness of analytical methodologies are available in the literature [31–39]. The current study used three different tools—"the Analytical Eco-Scale (AES) [34], ChlorTox [38], and the Analytical GREEnness (AGREE)" [39]—to assess the greenness of the proposed methodology. The present approach sought to establish and verify a reverse-phase HPTLC technique for the rapid, sensitive, environmentally benign, and stability-indicating identification of SUV in commercially available pharmaceutical tablets. The proposed SUV analysis method was validated using "The International Council for Harmonization (ICH)" Q2-R2 protocols [40].

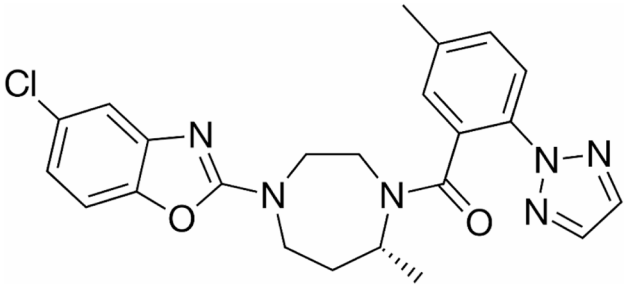

Fig. 1 Suvorexant (SUV)'s molecular structure

Materials and methods

Materials

The reference SUV (purity: 99.2% by HPLC) was acquired from "Beijing Mesochem Technology (Beijing, China)". The LC grade ethanol was acquired from "E-Merck (Darmstadt, Germany)". The "Milli-Q device (Lyon, France)" was used to obtain the purified water. We bought commercial SUV tablet brands A and B, each containing 10 mg of SUV, from a pharmacy in Mumbai, India. The materials that were remaining were of AR quality.

Chromatography and instrumental settings

SUV in commercial tablets was measured utilizing the "HPTLC system (Muttenz, Switzerland)". The solutions were applied using an "Automatic TLC Sampler 4 (ATS4) Sample Applicator (CAMAG, Geneva, Switzerland)" in the shape of 6 mm bands. "Microliter Syringe (Hamilton, Bonaduz, Switzerland)" was filled with the sample applicator. The application rate for SUV analysis was 150 nL/s. Silica gel with a particle size of 5 µm was pre-coated on 60 RP-18F254S glass-coated (10 × 20 cm) plates, which served as the stationary phase for SUV separation. The plates were developed in an "automated developing chamber 2 (ADC2) (CAMAG, Muttenz, Switzerland)" at a distance of 8 cm using a linear ascending mode. The best developing system for SUV analysis was a 75:25 v/v ethanol/water mixture. The developing system's fumes were forced into the development chamber and held there for thirty minutes at 22 °C. SUV was found utilizing a UV detector in densitometry mode at a wavelength of 255 nm. Two parameters were set: the scan speed (20 mm/s) and the slit size (4 × 0.45 mm^2). We utilized either three or six replications for every measurement. It was "WinCAT's (version 1.4.3.6336, CAMAG, Muttenz, Switzerland)" software that was utilized.

SUV calibration curve

In order to produce a stock solution containing 100 µg/mL of SUV, which is the working standard, 10 mg of SUV that had been precisely weighed was dissolved in 100 mL volumes of ethanol/water (75:25 v/v). To further produce SUV concentrations in the range of 10–1200 ng/band, the developing system was diluted with varying volumes of the SUV stock solution using the current approach. For the current procedure, TLC plates were filled with approximately 10 µL of each SUV concentration. The proposed methodology was utilized to measure the peak area for every SUV concentration. The SUV calibration curve was produced by plotting the observed peak area against the SUV concentrations using six replications ($n = 6$).

Sample Preparation for the analysis of SUV in marketed tablets

A total of twenty-five tablets, each having 10 mg of SUV, were randomly taken in order to ascertain the quantity of SUV present in marketed tablet brands A and B. Next, the two tablet brands' average weights were calculated. To obtain the fine powder, the tablets were first crushed and then triturated. The fine powder was divided using 10 mL of the developing system, and each brand's 10 mg of SUV was contained in it. The resultant mixes were filtered using a 0.45 µm membrane filter after 15 min of sonication [41]. The resulting mixture was diluted utilizing the appropriate development system to get the sample at the SUV concentration of 200 ng/band. Using the current approach, 10 µL samples were injected to determine the SUV in commercial tablet brands A and B.

Validation assessment

In compliance with ICH-Q2-R2 recommendations, the current SUV measuring method was validated for several validation criteria [40]. SUV linearity was determined by plotting the measured peak area against the SUV concentrations. To evaluate the linearity of the current SUV analysis method in the 10–1200 ng/band range, six independent replicates ($n = 6$) were used.

The parameters for the system adequacy for the current technique were calculated using the following parameters: "retardation factor (R_f), peak asymmetry factor (As), and number of theoretical plates per meter (N/m)". For the current procedure, the "R_f, As, and N/m" were calculated using their reported formulae [42, 43].

Using spiking technology and a typical addition strategy, the accuracy of the current method was computed and expressed as a percentage of recoveries [40]. The pre-analyzed SUV solution (300 ng/band) was spiked with extra 50, 100, and 150% SUV solution using the current methodology. SUV levels for low-quality control (LQC) at 450 ng/band, middle-quality control (MQC) at 600 ng/band, and high-quality control (HQC) at 750 ng/band were the outcome of this. In order to evaluate the accuracy of the existing methodology, three different SUV QC samples were investigated. Six replications ($n = 6$) were carried out in order to ascertain the percent recovery at each QC level.

The current method's SUV intra- and inter-batch precision was assessed. The intra-batch variation for SUV was calculated using six replicates ($n = 6$) of freshly made SUV solutions at LQC, MQC, and HQC on the same day using the current approach. SUV inter-batch variation was assessed for the current method utilizing six replications ($n = 6$) of freshly produced SUV samples at the same QC levels distributed over the course of three days.

Certain deliberate modifications to the content of the pertinent developing system can be made to measure the SUV's robustness for the current methodology. Six replicate ($n = 6$) were utilized to measure the errors in spot area, a quantitative metric, and R_f, a separation parameter [40]. The developing system of ethanol/water (75:25 v/v) in the current approach was modified to ethanol/water (77:23 v/v) and ethanol/water (73:27 v/v).

The sensitivity of the current SUV analysis method was calculated using a standard deviation approach, and it was stated as "limit of detection (LOD) and limit of quantification (LOQ)". After a blank sample—one without SUV—was injected six times for the purposes of this investigation, the sample's standard deviation was calculated. Six replications ($n = 6$) for the current methodology

were used to determine the SUV "LOD and LOQ" using the published equations [40].

The specificity/peak purity of the current procedure were evaluated by contrasting the R_f data and UV-absorption spectrum of SUV in marketed tablets (brand A and B) to that of reference SUV [40].

Forced-degradation investigations

The current approach's forced-degradation tests were carried out in environments with oxidative, thermal stress, alkaline, and acidic conditions [41, 44]. The SUV was exposed to thermal stress conditions for 24 h using a hot air oven set at 55 °C, 1 M HCl (acid), 1 M NaOH (base), and 30% v/v H_2O_2 (oxidative) at a concentration of 600 ng/band. The samples were diluted by the developing system. The thorough protocols outlined in our most recent publication [41] were followed for these investigations. SUV chromatograms were obtained for the current procedure under the previously mentioned stress settings, and degradation products were checked.

Application of current methodology in the analysis of SUV in marketed tablets

The current method was applied in three replicates ($n = 3$) to get the peak responses for SUV using marketed tablet solutions on TLC plates. With the current methodology, the SUV calibration plot was used to assess the SUV content of the pharmaceutical tablets.

Greenness assessment

Three different approaches were used to analyze the greenness profile of the current approach: AES [34], ChlorTox [38], and AGREE [39]. AES is a semi-quantitative method that takes instrumentation, waste, and each step of the analysis process into account. For the solvents and reagents that use little to no reagent, low energy, and no waste, a perfect analysis with 100 points is expected. Penalty points are awarded and deducted from the final score of 100 if any of these conditions are not met [34]. Equation (1) [38] is employed, in accordance with the ChlorTox scale technique, to determine the ChlorTox scale.

$$\text{ChlorTox} = \frac{\text{CH}_{\text{sub}}}{\text{CH}_{\text{CHCl3}}} \times \text{m}_{\text{sub}} \quad (1)$$

Where CH_{sub} denotes the substance of interest's chemical risks, CH_{CHCl3} denotes the standard $CHCl_3$'s chemical risk, and m_{sub} denotes the mass of the substance of interest needed for a single measurement. With the use of the safety data sheet from "Sigma Aldrich (St. Louis, MO, USA)", the values of CH_{sub} and CH_{CHCl3} for the weighted hazards number (WHN) model could be calculated [38]. The AGREE score for the current SUV analysis methodology was obtained using the AGREE-metric technique [39]. The AGREE scale for the present method was computed using the "AGREE: The Analytical Greenness Calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020)". The values ranged from 0.0 to 1.0 and were established by 12 different GAC principles.

Results and discussion

Development of the green HPTLC method

An illustration of a typical TLC image is shown in Fig. 2. The chamber saturation conditions were used to establish the TLC plates for the current procedure. A range of ethanol/water combinations between 35 and 95% ethanol were studied as the development systems for the SUV analysis by the current approach. The ethanol/water (75:25 v/v) mixture produced an uninterrupted and well-resolved SUV chromatographic peak at R_f = 0.47 ± 0.01, according to the data (Fig. 3A). Furthermore, the SUV peak did not show any fronting, which may have been caused by the high sample solubility. Additionally, an As value forecast of 1.07 ± 0.03 was established, which was reliable for SUV evaluation. For the SUV assessment utilizing the current methodology, the ethanol/water (75:25 v/v) combination was determined to be the best

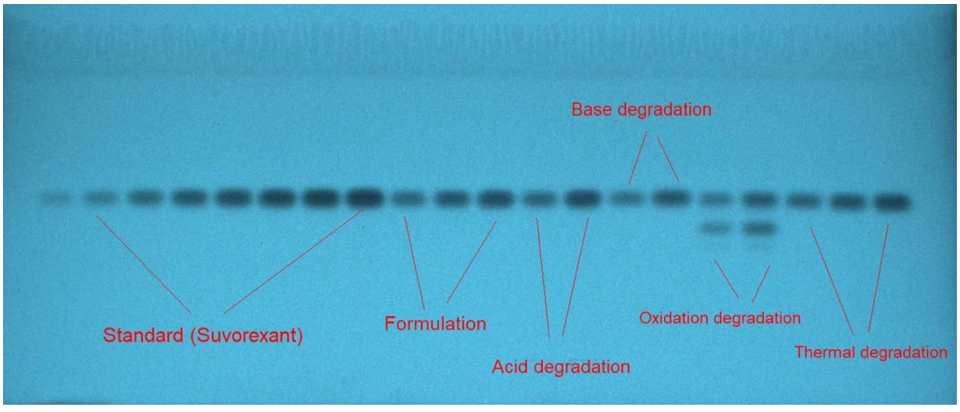

Fig. 2 A typical TLC image for reference SUV, marketed formulations, and forced-degradation samples derived using the present method

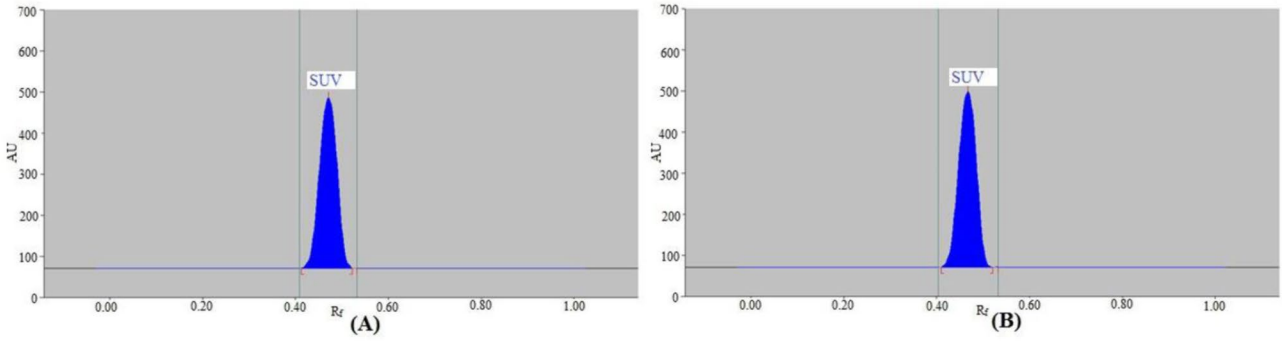

Fig. 3 Representative chromatograms of (**A**) reference SUV and (**B**) commercial tablet dosage form

Table 1 Statistical data for the linearity assessment of SUV for the present method (mean ± SD; *n* = 6)

Parameters	Values
Linear range (ng/band)	10-1200
Regression equation	y = 12.229x + 1153.7
R^2	0.9969
R	0.9984
Standard error of slope	0.31
Standard error of intercept	2.29
95% confidence interval of slope	10.94–13.64
95% confidence interval of intercept	1143.82-1163.57
LOD ± SD (ng/band)	3.32 ± 0.08
LOQ ± SD (ng/band)	9.98 ± 0.24

Table 2 System suitability parameters of SUV assessment for the present method (mean ± SD, *n* = 3)

Parameters	Value
R_f	0.47 ± 0.01
As	1.07 ± 0.03
N/m	4688 ± 4.65

environmentally friendly developing system. The SUV spectral bands in the 200–400 nm range were evaluated using spectrodensitometry mode, and 255 nm was found to have the greatest TLC response. As a result, 255 nm was used for the entire SUV analysis.

Validation studies

The various SUV validation parameters were computed following the ICH-Q2-R2 protocols [40]. The statistical results for the SUV calibration plots linear regression analysis carried out utilizing the proposed methodology are indicated in Table 1. For the proposed methodology, the SUV calibration curve was linear between 10 and 1200 ng/band. Using the proposed methodology, the SUV's "correlation coefficient (R) and coefficient of determination (R^2)" were 0.9984 and 0.9969, respectively. Furthermore, the slope and intercepts' standard error values were abnormally low when in comparison to corresponding average values for the proposed methodology. These results demonstrated a strong relationship between the evaluated peak area and the SUV concentrations. These findings demonstrated the linear nature of the present SUV assessment process.

Table 3 Accuracy results of SUV for the present analysis approach (mean ± SD; *n* = 6)

Conc. (ng/band)	Conc. found (ng/band) ± SD	Recovery (%)	CV (%)
450	446.88 ± 4.93	99.30	1.10
600	591.13 ± 5.88	98.52	0.99
750	736.41 ± 7.18	98.18	0.97

The findings of the system suitability parameters for the current approach are displayed in Table 2. The SUV study yielded measurements of 0.47 ± 0.01, 1.07 ± 0.03, and 4688 ± 4.65 for "R_f, As, and N/m" using the current approach. The current method's recommended system suitability parameters were accurate and appropriate for SUV analysis.

The % recovery was used to evaluate the accuracy of the existing methodology. Table 3 displays the accuracy measurement results for the current methodology. Using the current methodology, the SUV recoveries at three different QC levels were analyzed; the findings indicated a range of 98.18–99.30%. These results proved that the current SUV analysis approach is accurate.

The intra- and inter-batch variance of the suggested methodology was acquired in order to compute the SUV. As a proportion of the coefficient of variation (%CV), the data are displayed. Table 4 presents the current method's intra- and inter-assay precisions. Under the proposed methodology, the intra-batch CVs of SUV ranged from 0.78 to 0.87%. The SUV inter-assay CVs using this approach ranged from 0.85 to 0.94% respectively. The precision of the proposed method was demonstrated by these measurements.

The developing systems were subjected to intentional, prearranged changes to evaluate the robustness of the existing methodology. Table 5 indicates the outcomes of the current technique's robustness examination. The SUV CVs with the current methodology varied from 1.09 to 1.16%. The SUV R_f values obtained with the current

Table 4 Precision results of SUV for the proposed analysis method (mean ± SD; *n* = 6)

Conc. (ng/band)	Intra-day precision			Inter-day precision		
	Conc. (ng/band) ± SD	SE	CV (%)	Conc. (ng/band) ± SD	SE	CV (%)
450	458.61 ± 4.02	1.64	0.87	445.71 ± 4.23	1.72	0.94
600	612.13 ± 5.10	2.08	0.83	590.12 ± 5.30	2.16	0.89
750	765.14 ± 6.02	2.45	0.78	735.44 ± 6.26	2.55	0.85

Table 5 Results of SUV robustness for the current analysis method (mean ± SD; *n* = 6)

Conc. (ng/band)	Developing system (Ethanol/water)			Results		
	Original	Used	Level	Conc. (ng/band) ± SD	CV (%)	R_f
		77:23	+ 2.0	588.72 ± 6.43	1.09	0.46
600	75:25	75:25	0.0	596.74 ± 6.71	1.12	0.47
		73:27	-2.0	611.22 ± 7.11	1.16	0.48

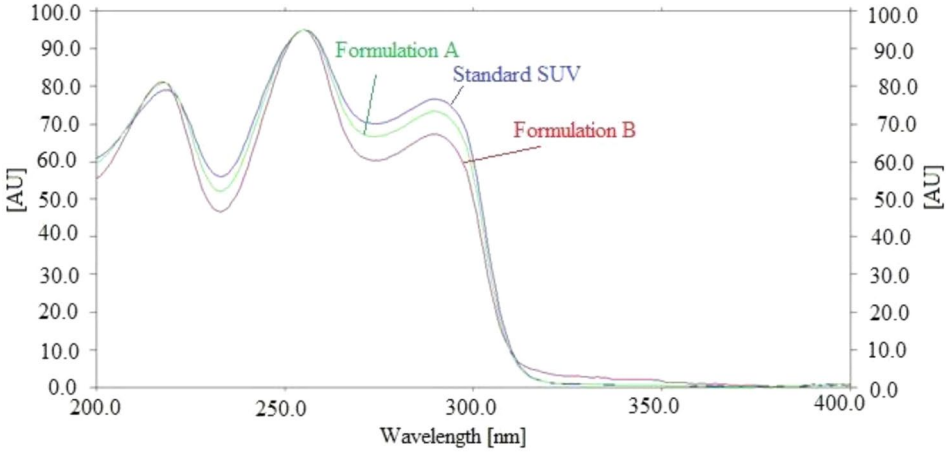

Fig. 4 UV-absorption spectrum of reference SUV and commercial tablet dosage forms

approach were found to vary between 0.46 and 0.48. These measurements demonstrated the existing method's robustness.

Sensitivity for the suggested technique was determined with "LOD and LOQ." Table 1 lists the projected "LOD and LOQ" values for SUV based on the present methodology. The SUV's "LOD and LOQ" were found to be 3.32 ± 0.08 and 9.98 ± 0.24 ng/band, respectively, using the current methodology. These results demonstrated the degree of sensitivity of the existing SUV evaluation methodology.

By contrasting the R_f data and superimposed UV, and 3D spectrum of SUV in marketed tablet brands A and B with that of reference SUV, we evaluated the specificity and peak purity of the proposed SUV assessment technique. Figure 4 displays the superimposed UV spectrum of the reference SUV and the A and B SUV from commercial tablet brands. Figure 5 displays the 3D spectrum of reference SUV and the A and B SUV from commercial tablet brands. The peak purities of conventional SUV and SUV in commercial tablet dosage forms were assessed by comparing the spectra at the peak start (S), peak apex (M), and peak end (E) positions of the spot [45, 46]. The estimated values of r (S, M) and r (M, E) for standard SUV and commercial tablets were found to be greater than 0.99, indicating the homogeneity of the peaks [47, 48]. SUV was found to exhibit the highest chromatographic response at 255 nm in both standard and commercial tablets. The specificity of the currently employed SUV analysis approach was shown utilizing the similar UV spectra, 3D spectra, R_f values, and wavelengths recorded in both reference and marketed tablets. These results also suggest that a simple spectroscopic approach can measure SUV without interference from any matrix ingredients. Simple spectroscopic methods, however, are less sensitive than separation techniques like HPTLC and HPLC. Consequently, the SUV analysis in this work employed HPTLC as the separation technique.

Forced-degradation evaluation

The forced degradation of the proposed SUV analysis approach was examined under four different stress situations. Figure 6 displays the chromatograms from the forced-degradation experiments. Table 6 summarizes the results of forced-degradation studies. The results showed that SUV exhibited remarkable stability during

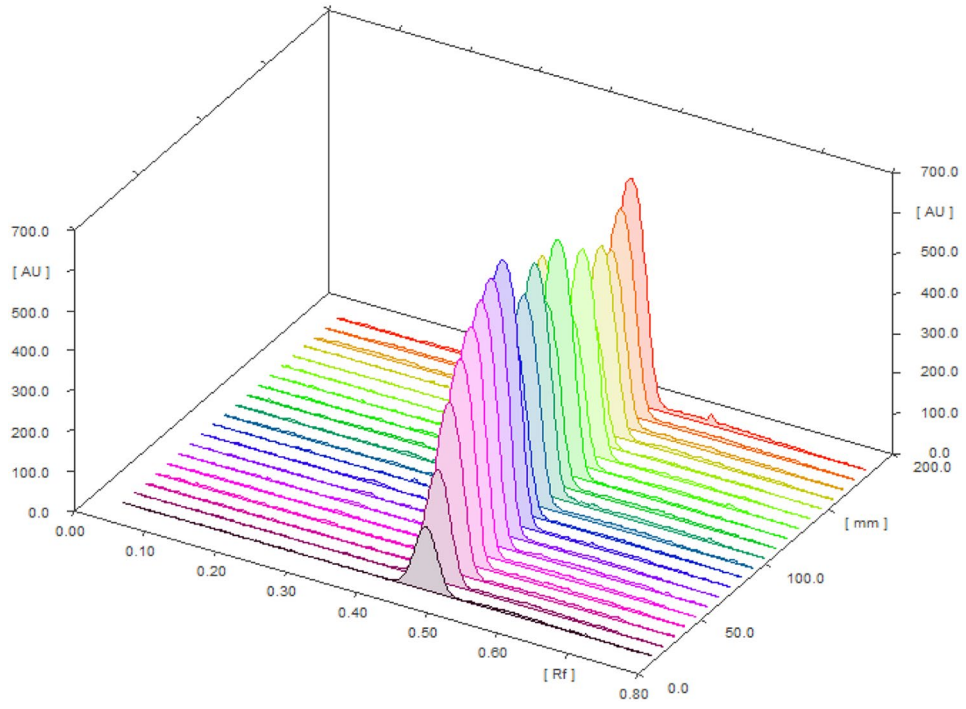

Fig. 5 3D spectrum of reference SUV and commercial tablet dosage form

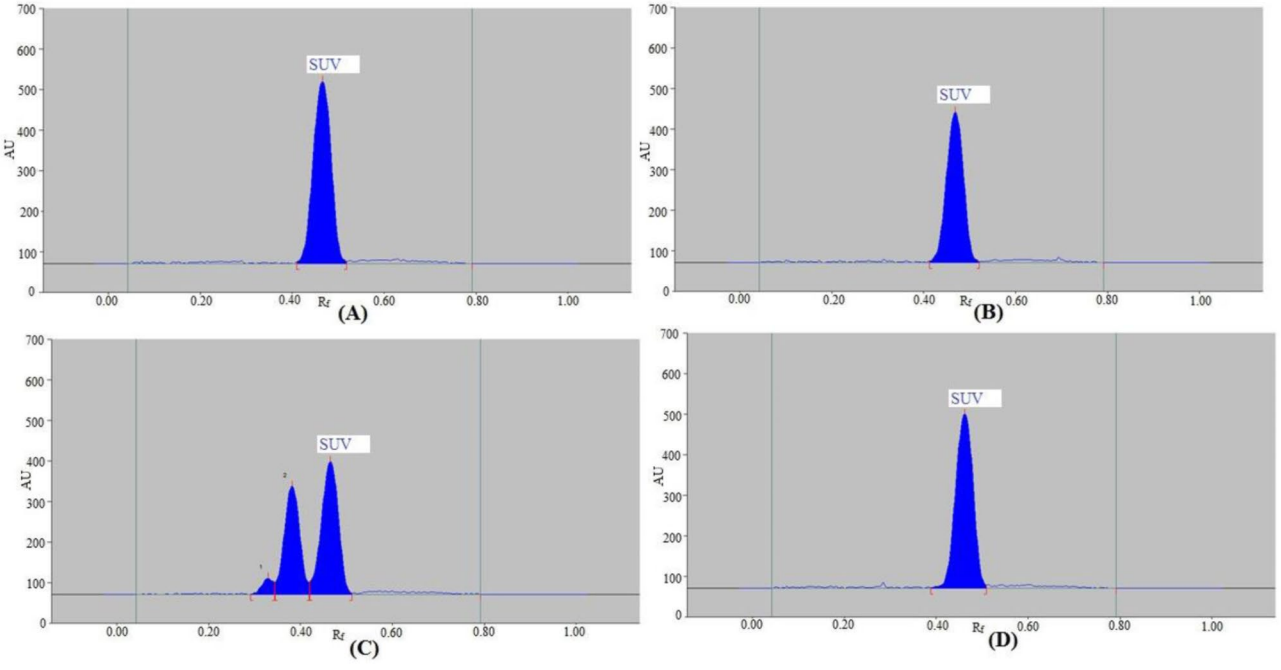

Fig. 6 SUV chromatograms obtained using the current approach under the following conditions: (**A**) acid, (**B**) base, (**C**) oxidative, and (**D**) heat degradations

acid, base, and thermal hydrolysis. Specifically, no degradation products were found under acid (Fig. 6A), base (Fig. 6B), or thermal (Fig. 6D) deterioration conditions. Under the acid, base, and heat hydrolysis stress settings, it was discovered that the SUV R_f values had changed slightly (R_f = 0.46 in all three cases). In comparison, it was shown that under oxidative hydrolysis stress conditions, SUV degraded at a rate of 45.22% while remaining at 54.78%. As a result, it was shown that SUV was highly unstable when oxidative hydrolysis was investigated. R_f values of 0.33 and 0.38, respectively, were used to separate the breakdown product signals represented by

Table 6 Findings from the forced-degradation assessment of SUV using the present analysis technique under various stress scenarios (mean ± SD; *n* = 3)

Degradation setting	Degradation products (R_f)	SUV R_f	SUV remained (ng/band)	SUV recovered (%)
1 M HCl	0	0.46	600.00	100.00 ± 0.00
1 M NaOH	0	0.46	600.00	100.00 ± 0.00
30% H_2O_2	2 (0.33, 0.38)	0.46	328.68	54.78 ± 1.71
Thermal	0	0.46	600.00	100.00 ± 0.00

chromatographic peaks 1 and 2 in Fig. 6C. The R_f value for SUV was similarly somewhat changed at 0.46 under oxidative hydrolysis. The settings for oxidative degradation produced the largest SUV breakdown, according to the recommended SUV analysis methodology. All of these data indicated that SUV could be identified even when their breakdown components were present, according to the methodology used in the study. These outcomes confirmed the current approach's selectivity and stability-indicating abilities.

Application of the present method in SUV determination in commercial tablets

The green HPTLC approach offers many benefits compared to traditional LC techniques, such as reduced solvent consumption, quicker sample analysis times, nondestructiveness, low preparation requirements, user-friendliness, ability to analyze multiple samples simultaneously, non-toxicity, and environmental friendliness [26, 41, 44, 48]. HPTLC techniques have numerous advantages, but they also have certain drawbacks, such as a lack of reproducibility and standardization, which might affect the overall outcomes [22, 47, 48]. To find the SUV in marketed tablets, the proposed SUV analysis method was utilized. The single TLC spot at R_f = 0.47 ± 0.01 for SUV and standard SUV were compared using the current method to evaluate the chromatogram of SUV from commercial tablet brands A and B. According to the current method, the chromatographic peak of SUV in commercial tablets (Fig. 3B) matched the peak of standard SUV. Additionally, there were no extra peaks of the ingredients in the marketed tablets, indicating that SUV and the ingredients in the tablets did not interact. The SUV calibration plot for the proposed technique was utilized to derive the quantity of SUV in marketed tablets. The amount of SUV in marketed tablet brands A and B was found to be 98.18 ± 1.36 and 101.32 ± 1.41%, respectively, using the current approach. According to Siddhartha et al. [9] and Iqbal et al. [10], the SUV content of commercial tablet dosage forms is 99.05 ± 0.16% and 101.31 ± 1.23%, respectively. The results of the current SUV analysis strategy in marketed tablets were compared with published HPLC methods using the Student's t-test and the variance ratio F-test. The findings are compiled in Table 7 [9, 10]. There were no discernible variations in the accuracy and precision of the tested methods, as shown by the obtained t and F values of the present HPTLC approach and the reported HPLC methods not surpassing their theoretical values [9, 10]. These findings demonstrated the comparability of the suggested HPTLC technology with the published HPLC processes [9, 10].

Table 7 Comparison of assay results of the current analysis method with reported HPLC methods in tablets using student t-test and the variance ratio F-test (mean ± SD; *n* = 3)

Marketed product	Recovery (%)		t[a]	F[a]
	Present HPTLC	Reported HPLC		
Brand A	98.18 ± 1.36	99.05 ± 0.16 [9]	0.167	0.443
Brand B	101.32 ± 1.41		0.016	0.871
Brand A	98.18 ± 1.36	101.31 ± 1.23 [10]	0.174	0.541
Brand B	101.32 ± 1.41		0.025	0.916

[a]Theoretical values of t and F are 4.303 and 4.256, respectively at 95% confidence limit

Table 8 The comparison of the recommended methodology's greenness to published HPLC and HPTLC techniques, as well as its assessment using the analytical eco-scale (AES) and penalty points

Reagents/instruments/waste	Penalty points			
	HPLC [9]	HPLC [10]	HPTLC [22]	Present RP-HPTLC
Ethanol	-	4	-	4
Water	0	-	-	0
Methanol	18	-	18	-
Chloroform	-	-	8	-
Orthophosphoric acid	6	-	-	-
KH_2PO_4 (10 mM)	-	0	-	-
Instruments	0	0	0	0
Waste	5	5	3	3
Total penalty points	29	9	29	7
AES scale	71	91	71	93

Greenness evaluation

The developed analytical processes' greenness can be evaluated using several greenness tools [31–39]. The present study determined the greenness of the current method using three different methods: "AES [34], ChlorTox [38], and AGREE [39]". Table 8 shows the outcomes of AES scales with penalty points for the current procedure. If the AES score was greater than 75, it was considered excellent; if it was less than 75 but greater than 50, it was considered adequate; and if it was less than

Table 9 Results of the WHN model-computed ChlorTox scales for the relative hazards associated with chloroform (CH_{sub}/CH_{CHCl3}) compared to previously published HPLC and HPTLC methods

Stage	Solvent/reagent	Relative hazard (CH_{sub}/CH_{CHCl3})	m_{sub} (mg)	ChlorTox (g)	Total ChlorTox (g)	Ref.
Sample preparation	Ethanol	0.26	1500	0.39	0.78	Present HPTLC
HPTLC analysis	Ethanol	0.26	1500	0.39		
Sample preparation	Methanol	0.56	792	0.44	2.75	[9]
HPLC analysis	Methanol	0.56	4118	2.31		
	Orthophosphoric acid	0.56	5.26	0.00		
Sample preparation	Ethanol	0.26	552	0.14	1.14	[10]
HPLC analysis	Ethanol	0.26	3866	1.00		
Sample preparation	Chloroform	1.00	1950	1.95	3.96	[22]
	Methanol	0.56	50	0.03		
HPTLC analysis	Chloroform	1.00	1950	1.95		
	Methanol	0.56	50	0.03		

50, it was considered unsatisfactory [34]. The current method's AES scale was determined to be 93. Furthermore, we compared the current SUV assessment method to the AES scales of two HPLC and one HPTLC method that were calculated and found in the literature (Table 8). Two reported HPLC methods were observed to have AES scales of 71 and 91, respectively [9, 10]. However, the AES scale for the reported HPTLC method was found to be 71 [22]. Based on AES scales, it was discovered that the current HPTLC approach for SUV assessment was much better than one of the documented HPLC methods and the HPTLC method found in the literature [9, 22]. One of the published HPLC approaches for SUV assessment was shown to be equivalent to the current HPTLC methodology based on AES scales [10].

Table 9 displays the total ChlorTox and solvent-specific ChlorTox scale results for the current methodology in relation to reported HPLC and HPTLC protocols. The ChlorTox scale of less than 1.00 g indicated that the method is environmentally safe. However, the ChlorTox scale of greater than 1.00 g indicated that the method is not environmentally safe [38]. The predicted total ChlorTox scale for the current approach was 0.78 g, which indicates that it is safe and benign for the environment [38]. Furthermore, we computed the ChlorTox scales for one reported HPTLC two HPLC approaches and compared them to the present HPTLC method of SUV analysis (Table 9). Two literature HPLC techniques were found to have ChlorTox scales of 2.75 g and 1.14 g, respectively [9, 10]. However, the ChlorTox scale for the HPTLC method that was described was 3.96 g [22]. Based on ChlorTox scales, it was demonstrated that the existing HPTLC approach is much more successful than one of the literature's HPLC methods and literature HPTLC method for SUV detection [9, 22]. One of the reported HPLC approaches for SUV assessment was found to be comparable to the current HPTLC method based on ChlorTox scales [10].

The AGREE method [39], which takes into account each of the 12 GAC criteria [24], is the most widely used quantitative method for evaluating greenness. Figure 7 displays the overall AGREE scale for the suggested approach in relation to methods for HPLC and HPTLC that have been published [10, 22]. If the AGREE score was greater than 0.75, it was considered excellent; if it was less than 0.75 but greater than 0.50, it was considered adequate; and if it was less than 0.50, it was considered unsatisfactory [39]. A total AGREE scale of 0.88 was predicted by the current method (Fig. 7A). For the stated HPLC and HPTLC procedures, the overall AGREE scale was computed to be 0.79 (Fig. 7B) and 0.52 (Fig. 7C), respectively [10, 22]. The current HPTLC and reported HPLC procedures have outstanding greenness properties and are therefore comparable with each other based on the AGREE scale. The current HPTLC method has been found to be much better than the previously published HPTLC methodology for SUV assessment, based on the AGREE scale [22]. When compared to one of the HPLC methods and HPTLC method found in the literature, the new method for SUV analysis in marketed tablets has an excellent greenness profile, as shown by all greenness techniques.

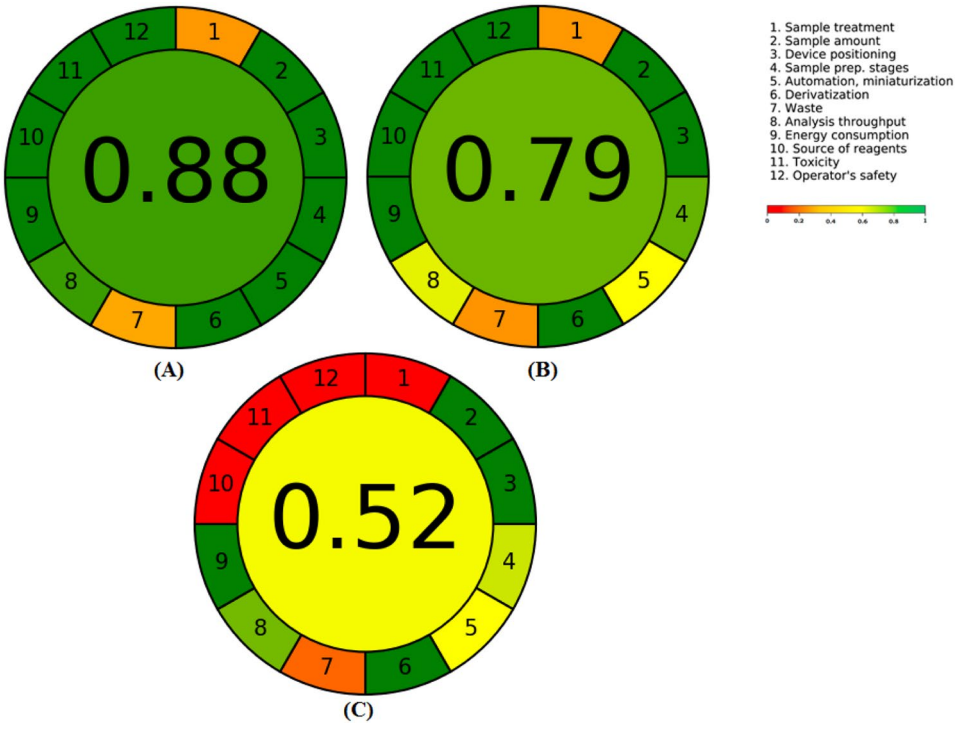

Fig. 7 Representative images for the AGREE scale produced by the AGREE calculator for the following three methods: (**A**) current HPTLC technique, (**B**) reported HPLC method (derived from reference [10]), and (**C**) calculated for reported HPTLC method [22]

Conclusions

Stability-indicating HPTLC approaches for SUV measurement are lacking in the literature. Developing and testing a green stability-indicating, sensitive reverse-phase HPTLC technique for SUV characterization in commercial tablets is the aim of this work. The present methodology for analyzing SUV is linear, robust, sensitive, accurate, precise, and sustainable. The SUV content of commercial tablets was successfully measured using the current methodology. The current approach was found to have stability-indicating qualities and selectivity. The present HPTLC strategy demonstrated a better greenness profile when compared to previously published HPLC and HPTLC methods for SUV analysis, as demonstrated by the AES, ChlorTox, and AGREE results. These findings demonstrated that the existing method can be regularly used to determine SUV in its commercial dosage forms. In the near future, SUV's pharmacokinetics may be examined in plasma samples using the existing HPTLC technology.

Acknowledgements

Authors are thankful to Researchers Supporting Project number (RSPD2025R1040), King Saud University, Riyadh, Saudi Arabia. The authors are also thankful to Prince Sattam bin Abdulaziz University for supporting this work via project number (PSAU/2025/R/1446).

Author contributions

Authors contribution PA: Conceptualization, Methodology, Investigation, Funding acquisition, Supervision, Project administration, Writing, review, and editing; FS: Software, Resources, Visualization, Funding acquisition, Data curation, Formal analysis, Validation, Writing original draft; MHA: Methodology, Investigation, Validation, Writing, review, and editing; AIF: Methodology, Investigation, Software, Writing, review, and editing; TMA: Methodology, Data curation, Formal analysis, Validation; FMAB: Data curation, Formal analysis, Software, Validation; MI: Software, Formal analysis, Validation, Writing, review, and editing; MA: Conceptualization, Supervision, Project administration, Validation, Writing, review, and editing.

Funding

This work was funded by the Researchers Supporting Project number (RSPD2025R1040), King Saud University, Riyadh, Saudi Arabia and Prince Sattam bin Abdulaziz University via project number (PSAU/2025/R/1446).

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 26 September 2024 / Accepted: 20 February 2025
Published online: 28 February 2025

References

1. Azevedo K, Johnson M, Wassermann M, Evans-Wall J. Drugs of abuse-opioids, sedatives, hypnotics. Crit Care Clin. 2021;37:501–16.

2. Silva-Bessa A, Forbes SL, Ferreira MT, Dinis-Oliveira RJ. Toxicological analysis of drugs in human mummified bodies and proposed guidelines. Curr Drug Res Rev. 2023;15:62–72.
3. Bennett T, Bray D, Neville MW. Suvorexant, a dual orexin receptor antagonist for the management of insomnia. Pharm Ther. 2014;39:264–6.
4. Yang LH. Suvorexant: first global approval. Drugs. 2014;74:1817–22.
5. Ahmed Z, Ahmad A, Khan SA, Husain A. Pharmacological, pharmaceutical and safety profile of suvorexant: a dual orexin receptors antagonist for treatment of insomnia. Int Educ Sci Res J. 2015;1:26–30.
6. Patel KV, Aspesi AV, Evoy KE. Suvorexant: a dual orexin receptor antagonist for the treatment of sleep onset and sleep maintenance insomnia. Ann Pharmacother. 2015;49:477–83.
7. U.S. Drug Enforcement Administration. Schedules of controlled substances: placement of suvorexant into schedule IV. Final rule. Fed Reg. 2014;79:51243–7.
8. Waters B, Hara K, Ikematsu N, Takayama M, Matsusue A, Kashiwagi M, Kubo SI. Tissue distribution of suvorexant in three forensic autopsy cases. J Anal Toxicol. 2018;42:276–83.
9. Siddhartha S, Ratna JV, Santosh T. Development and validation of HPLC method for the determination of suvorexant in pharmaceutical dosage forms. Int J Pharm Drug Anal. 2018;6:425–34.
10. Iqbal M, Haq N, Kalam MA, Imam F, Shakeel F. A simple, sensitive, and greener HPLC-DAD method for the simultaneous analysis of two novel orexin receptor antagonists. ACS Omega. 2024;9:23101–10.
11. Siddhartha S, Ratna JV, Tata SK. Development and validation of high performance liquid chromatographic method for the determination of suvorexant in rabbit plasma by HPLC-UV detection. J Emerg Technol Innov Res. 2019;6:194–203.
12. Skillman B, Kerrigan S. Identification of suvorexant in blood using LC-MS-MS: important considerations for matrix effects and quantitative interferences in targeted assays. J Anal Toxicol. 2020;44:245–55.
13. Garcia L, Tiscione NB, Yeatman DT, Richards-Waugh L. Novel and non-routine benzodiazepines and suvorexant by LC-MS-MS. J Anal Toxicol. 2021;45:462–74.
14. Skillman B, Kerrigan S. Quantification of suvorexant in blood using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. J Chromatogr B. 2018;1091:87–95.
15. Breidinger SA, Simpson RC, Mangin E, Woolf EJ. Determination of suvorexant in human plasma using 96-well liquid-liquid extraction and HPLC with tandem mass spectrometric detection. J Chromatogr B. 2015;1002:254–9.
16. Iqbal M, Ezzeldin E, Khalil NY, Al-Rashood STA, Al-Rashood KA. Simple and highly sensitive UPLC-ESI-MS/MS assay for rapid determination of suvorexant in plasma. J Anal Toxicol. 2017;41:114–20.
17. Xu RA, Chen KL, Jiao Y, Huo XL, Zhang Y. Quick method for the determination of suvorexant in plasma. Lat Am J Pharm. 2017;36:2185–9.
18. Iqbal M, Khalil NY, Ezzeldin E, Al-Rashood KA. Simultaneous detection and quantification of three novel prescription drugs of abuse (suvorexant, Lorcaserin and brivaracetam) in human plasma by UPLC-MS-MS. J Anal Toxicol. 2019;43:203–11.
19. Carson M, Kerrigan SJ. Quantification of suvorexant in urine using gas chromatography/mass spectrometry. J Chromatogr B. 2017;1040:289–94.
20. Sullinger S, Bryand K, Kerrigan S. Identification of suvorexant in urine using liquid chromatography-quadrupole/time-of-flight mass spectrometry (LC-Q/TOF-MS). J Anal Toxicol. 2017;41:224–9.
21. Iqbal M, Ezzeldin E, Khalil NY, Alam P, Al-Rashood KA. UPLC-MS/MS determination of suvorexant in urine by a simplified dispersive liquid-liquid microextraction followed by ultrasound assisted back extraction from solidified floating organic droplets. J Pharm Biomed Anal. 2019;164:1–8.
22. Alqarni MH, Iqbal M, Foudah AI, Aljarba TM, Bar FA, Alshehri S, Shakeel F, Alam P. Quantification of suvorexant in human urine using a validated HPTLC bioanalytical method. ACS Omega. 2023;8:39928–35.
23. Aljarba TM, Shakeel F, Alshehri S, Iqbal M, Foudah AI, Alqarni MH, Alhaiti A, Bar FMA, Alam P. Contrasting the validation parameters and greenness of normal-phase and reverse-phase stability-indicating HPTLC methods for lemborexant analysis. Arab J Chem. 2024;17:E105837.
24. Galuszka A, Migaszewski Z, Namiesnik J. The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices. Trends Anal Chem. 2013;50:78–84.
25. Abdelrahman MM, Abdelwahab NS, Hegazy MA, Fares MY, El-Sayed GM. 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:E105582.
26. Alam P, Ezzeldin E, Iqbal M, Anwer MK, Mostafa GAE, Alqarni MH, Foudah AI, Shakeel F. Ecofriendly densitometric RP-HPTLC method for determination of Rivaroxaban in nanoparticle formulations using green solvents. RSC Adv. 2020;10:2133–40.
27. Mohamed HM, Zaazaa HE, Abdelkawy M, Tantawy MA. Exploiting the power of UPLC in separation and simultaneous determination of Pholcodine, Guaiacol along with three specified Guaiacol impurities. BMC Chem. 2023;17:E35.
28. Tantawy MA, Wahba IA, Saad SS, Ramadan NK. Classical versus chemometrics tools for spectrophotometric determination of Fluocinolone acetonide, Ciprofloxacin HCl and Ciprofloxacin impurity-A in their ternary mixture. BMC Chem. 2023;17:E49.
29. Ahmed AR, Galal SM, Ragab MAA, Karany MA. Tri-chromic sustainable tools of derivative/discrete fourier transform HPLC convoluted peaks: application on LCZ696, an antihypertensive supramolecular complex, with its in process and degradation impurity analysis. Microchem J. 2024;200:E110307.
30. Suthar K, Desai S. A comparative greenness assessment of chromatographic methods for cilnidipine analysis and its combination. Green Anal Chem. 2024;9:E100112.
31. Keith LH, Brass HJ, Sullivan DJ, Boiani JA, Alben KT. An introduction to the National environmental methods index. Env Sci Technol. 2005;39:A173–6.
32. Gaber Y, Tornvall U, Kumar MA, 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;13:2021–5.
33. Hartman R, Helmy R, Al-Sayah M, Welch CJ. Analytical method volume intensity (AMVI): a green chemistry metric for HPLC methodology in the pharmaceutical industry. Green Chem. 2011;13:934–9.
34. Galuszka A, Konieczka P, Migaszewski ZM, Namiesnik J. Analytical eco-scale for assessing the greenness of analytical procedures. Trends Anal Chem. 2012;37:61–72.
35. Plotka-Wasylka J. A new tool for the evaluation of the analytical procedure: green analytical procedure index. Talanta. 2018;181:204–9.
36. Hicks MB, Farrell W, Aurigemma C, Lehmann L, Weisel L, Nadeau K, Lee H, Moraff C, Wong M, Huang Y, Ferguson P. Making the move towards modernized greener separations: introduction of the analytical method greenness score (AMGS) calculator. Green Chem. 2019;21:1816–26.
37. Nowak PM, Koscielnaik P. What color is your method? Adaptation of the RGB additive color model to analytical method evaluation. Anal Chem. 2019;91:10343–52.
38. Nowak PM, 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:E100056.
39. Pena-Pereira F, Wojnowski W, Tobiszewski W. AGREE-Analytical greenness metric approach and software. Anal Chem. 2020;92:10076–82.
40. International Conference on Harmonization (ICH), Q2 (R2). (2023) Guideline on validation of analytical procedures–text and methodology, Geneva, Switzerland.
41. Foudah AI, Shakeel F, Alqarni MH, 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:E105960.
42. Bele AA, Khale A. An overview of thin layer chromatography. Int J Pharm Sci Res. 2011;2:256–67.
43. Malinowska I, Studzinski M, Neizabitowska K, Gadzikowska M. Comparison of TLC and different micro TLC techniques in analysis of tropane alkaloids and their derivatives mixture from *Datura inoxia* mill. Extract. Chromatographia. 2013;76:1327–32.
44. Alam P, Shakeel F, Alqarni MH, Foudah AI, 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:E1305.
45. El-Kimary E, Ragab MAA. 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.
46. Alam P, Shakeel F, Alshehri S, Alhaiti A, Alqarni MH, Foudah AI, Aljarba TM. Analysis of the greenness to determine Risperidone in commercial products using a green stability-indicating HPTLC method. Arab J Chem. 2024;17:E105693.

47. El-Kimary EI, Khamis EF, Belal SF, Moneim MMA. Novel validated HPTLC method for the analysis of two binary mixtures containing Tamsulosin hydrochloride with antimuscarinic agents. J Chromatogr Sci. 2018;56:81–91.
48. Shakeel F, Alam P, Alqarni MH, Iqbal M, Anwer MK, 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:E105571.

Publisher's note