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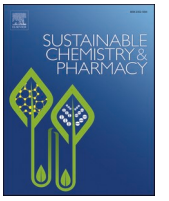

Facile one-pot green chemistry approach with whiteness assessment for unveiling micellar-mediated fluorescence amplification of midodrine: A comprehensive study of analytical significance

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ABSTRACT

The importance of green chemistry has never been greater than it is today. With growing concerns about environmental sustainability, resource depletion, and the impact of chemical processes on human health and the ecosystem, the principles of green chemistry offer a crucial framework for addressing these challenges. This approach encompasses the entire analytical process, from sample preparation to instrumental analysis and waste management aiming to contribute to a more sustainable and eco-friendly analytical practice. The present work introduces a new spectrofluorimetric method characterized by high sensitivity for the quantification of midodrine hydrochloride, based on the amplification of midodrine's intrinsic fluorescence (at λ_{ex} 291 nm and λ_{em} 324 nm) through the addition of sodium dodecyl sulfate (SDS) above its critical micelle concentration, and Torell buffer (pH 6.0). The suggested approach was effectively validated according to ICH guidelines, and a statistical evaluation was conducted. The current method demonstrated high sensitivity, with linear range from 0.025 to 2.0 μg mL^{-1}. The lower quantification and detection limit were 30 and 9.8 ng mL^{-1}, in the respective order. It was efficiently employed to assess the drug concentration in its marketable tablet formulations, achieving exceptional recovery and without any disruptive impact from excipients. Additionally, this technique was employed to assess the uniformity of tablet contents in accordance with USP guidelines. Moreover, the whiteness profile using Red Green Blue algorithm and greenness profile using AGREE software were evaluated proving that the proposed technique demonstrates an outstanding level of environmental friendliness.

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1. Introduction

Midodrine (MDR) hydrochloride is 2-amino-N-[2-(2,5-dimethoxyphenyl)-2-hydroxyethyl]-acetamide monohydrochloride. Through a process of biotransformation, the drug undergoes metabolic alterations, leading to the production of desglymidodrine, which functions as the active metabolite. The resulting desglymidodrine exerts effects that imitate those of the sympathetic nervous system, resulting in elevated vascular tone and consequent hypertension. MDR is also utilized to manage hypotension associated with the dialysis process, orthostatic hypotension, and dysautonomia. (Axelrod et al., 1995; Jans et al., 2015; Karwa and Woodis, 2009; Low, 1997; Prakash et al., 2004).

There have been a few reports of analytical strategies for the quantification of MDR in commercial pharmaceutical formulations. The majority of these employed expensive and environmentally problematic liquid chromatography methods (Abdelgaleel et al., 2023; Ali et al., 2015; Barth et al., 2013; Byran et al., 2021; Jain et al., 2016; Jia et al., 2006; Lee et al., 2010; Narenderan et al., 2020; Patel et al., 2016; Yoshida et al., 2003). There are very few other documented techniques, such as spectrofluorimetric (Derayea et al., 2023; Hamad et al., 2024; Khashaba et al., 2022; Omar et al., 2019a, 2019b, 2019c), spectrophotometric (Elazazy, 2015; Nair et al., 2015a, b), and electrochemical techniques (Al-Qahtani et al., 2021; Elzanfaly et al., 2013; Salem, 2020). These procedures were time-consuming, expensive, or have low sensitivity. Therefore, it was crucial to establish a sensitive, sustainable and cost-effective method to analyze MDR in bulk powders and commercially available medications.

After careful evaluation, the spectrofluorimetric approach was deemed the optimal technique for the present inquiry due to its versatility and applicability, which includes high sensitivity, inherent simplicity, and effective selectivity. The method's objective is to enhance MDR 's natural fluorescence in order to develop a more accurate spectrofluorimetric method for its determination using an anionic surfactant as SDS over its critical micelle concentration.

The novel spectrofluorimetric technique is considered a significant advancement in the analysis of MDR against the previously published spectrophotometric techniques, which are subject to low sensitivity and employing complex chemical derivatization or toxic solvents. These drawbacks are circumvented in the novel technique. Sodium dodecyl sulfate (SDS) used in the proposed process enhances the inherent fluorescence of MDR without the need for heating or complex reagents and is extremely appropriate for day-to-day laboratory use in quality control. The analytical process also avoids the use of expensive and hazardous solvents demanded by liquid chromatography, hence improving its greenness profile. The newly developed technique has the lowest LOD compared to other techniques and also is quick, simple, affordable, and economical. The new technique not only makes the analytical process easier but also results in a greener practice of pharmaceutical analysis. Therefore, it is an ideal green approach for quantitation of MDR in its pharmaceutical tablet dosage forms and checking content uniformity.

2. Experimental

2.1. Apparatus

In order to conduct the spectrofluorimetric measurements, a JASCO FP-8350 spectrofluorimeter was used. A PMT was used in the device to run a 150 W Xenon arc light at 400 V of power. For the emission and excitation monochromators, the slit width was 5 nm while the scan rate was 1000 nm min^{-1}.

2.2. Chemicals and reagents

MDR hydrochloride (99.5 % pure) was provided by the Nile Company for Pharmaceuticals and Chemical Industries. Tween-80 (prepared as 2 % v/v), SDS (as 2.0 % *w/v*), beta cyclodextrin (β-CD as 2 % w/v), methyl cellulose (MC as 2 % w/v), hydroxypropyl methyl cellulose (HPMC, as 2 % w/v), carboxy methyl cellulose (CMC as 2 % w/v) and poly ethylene glycol 400 (PEG 400 as 2 % v/v) were purchased from Sigma-Aldrich (St. Louis, MO, USA). NaOH, HCl, boric acid, citric acid, and phosphoric acid were purchased from El Nasr Company for Intermediate Chemicals (Cairo, Egypt). Torell–Stenhagen buffer (pH 2.0–12.0) was prepared by mixing 1 M solutions of phosphoric acid, citric acid monohydrate, and sodium hydroxide. The pH was adjusted using 0.1 M hydrochloric acid (Pesez and Bartos, 1974).

2.3. Standard drug solution

To prepare MDR hydrochloride stock solution, an exact weight of 10.0 mg of the pure drug was carefully measured and then placed in a 100 mL calibrated flask. Following this, distilled water was used to dissolve the medication, and the same solvent was then used to bring the solution up to the final volume. Additionally, aliquots of the stock solution were diluted to 100 mL to prepare working solutions that achieve the required concentrations.

2.4. Procedure for standard calibration curve

A series of calibrated flasks of 10 mL was used to construct the plot for the standard calibration. A final concentration of the drug ranging from 0.025 to 2 μg mL^{-1} was achieved by transferring varying volumes of the working solution into these flasks. Following this, 0.5 mL of Torell-Stenhagen buffer (pH 6.0) and 1 mL of a 2 % SDS solutions were added to each flask. The volume was then adjusted to the mark with distilled water. Fluorescence measurements were performed at an excitation wavelength of 291 nm and an

emission wavelength of 324 nm. A blank experiment was conducted concurrently. By correlating the drug concentration with the corresponding relative fluorescence intensity, the standard calibration plot was established.

2.5. Procedure for tablet dosage form

The initial step involved transferring an exact weight of finely ground powder, obtained from the grinding of twenty tablets of midodrine formulation, into a 100-mL calibrated flask. The powder was dissolved in approximately 50 mL of distilled water. The resulting solution was then subjected to sonication for duration of 20 min and subsequently filtered using double filter paper. Upon dilution with the same solvent to the mark, the solution was ready for the analysis. A portion of the obtained filtrate was further diluted to reach a concentration that lies in the recommended linear range of the method. This solution was analyzed by the previously mentioned general procedure. An equation based on linear regression was used to estimate drug concentration in the sample solutions.

2.6. Procedure for content uniformity testing

The USP guidelines (Rockville, 2007) were followed to evaluate the content uniformity of MDR dosage tablet units. Ten individual tablets of MDR commercial tablets were analyzed as described before.

3. Results and discussion

It is well established that the fluorescence activity of various fluorophores is substantially amplified when a surfactant is added above its critical micellar concentration (Eman and El-Yazbi, 2016; Mohammed et al., 2020). The micellar enhancement may be arising from the double effects by changing some solution physical properties as well as physicochemical properties of the analytes. Upon moving the solute molecules from the aqueous solution into the micellar core, a couple of characteristics (e.g., reactivity, solubility, or spectroscopic behaviors) experience significant alterations, one of which is the rise in the fluorescence intensity. The core of the micelle provides a spatial environment for the enclosed solute that differs greatly from the bulk aqueous solution restricting the free rotational movement of the solute molecule that competes with the emission process and this could reduce the possibility of a radiation-less process. In addition, the core imparts a relatively viscous microenvironment that may also enhance the emission through inhibition of the quenching effect of dissolved molecular oxygen. This may stabilize or protect the singlet excited state, and thus prevents the quenching of the fluorescence by non-radiative deactivation processes. Micellar enhancement approach has been applied to elevate the sensitivity of the spectrofluorimetric assay of some pharmaceutical compounds (Eman and El-Yazbi, 2016; Mohammed et al., 2020).

MDR exhibits a weak native fluorescence in aqueous medium at 324 nm when excited at 291 nm. A previous study has shown that, the fluorescence of MDR was improved by using ethanol as a diluting solvent. In the present study, to improve the environmental safety and greenness of the analytical procedure, a completely aqueous solution will be utilized with fluorescence enhancement achieved by adjusting the pH and using a micellar system (Fig. 1).

The ability of various surface-active agents to boost MDR emissions was investigated. In comparison to the aqueous solution, adding SDS as a surface-active agent at micellar concentration improved the emission of MDR (by a factor of 1.5). As a result, SDS was selected as a key component for developing a new spectroscopic approach for MDR analysis.

3.1. Study of experimental parameters

All parameters that could influence the experiment as pH, surfactant type and concentration, buffer type and volume, and diluents were separately studied to reach the optimal experimental conditions.

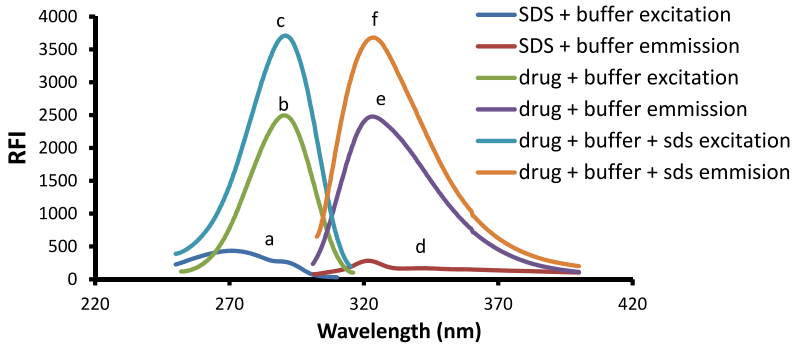

Fig. 1. MDR fluorescence spectra (1.0 μg ml^{-1}). Blank, drug-in-buffer, and drug-buffer-SDS system excitation spectra are shown by a, b, and c, respectively, while their emission spectra are represented by d, e, and f.

3.1.1. Various forms of organized media influence

To improve the fluorescence of MDR solution, different types of surfactants in addition to some macromolecules were examined. The study included SDS as an anionic surfactant, PEG 400 and Tween-80, as nonionic surfactants, and other compounds including MC, CMC, HPMC and β-CD (Fig. 2). While β-CD, MC, CMC and HPMC showed minimal enhancement effect, nonionic surfactants, (tween 80 and PEG 400) negatively impacted drug emission. The anionic surfactant, SDS, on the other hand, considerably enhanced the natural MDR fluorescence by a factor of 1.5. In acidic medium, MDR has a positive charge because of its mild basicity, pKa value of 7.8. Thus, the protonated primary amine group of MDR may interact with the negatively charged sulfonyl group of SDS to form an ion pair complex. As a consequence, the drug's excited singlet state was sustained or shielded, preventing fluorescence drop through a non-radiative process (Tran and Van Fleet, 1988). Furthermore, micelle-induced enhancement could be due to the restriction of the free rotation of the drug molecule owing to its complexation with SDS. This free rotation can reduce fluorescence intensity (Darwish and Bakheit, 2015). In addition, micelle binding may improve MDR's fluorescence activity through reducing the collision and electrostatic attraction among MDR molecules (Alarfaj and El-Tohamy, 2013). The enhancement of MDR fluorescence could be additionally due to the improvement of its microenvironment arising from the drug's encapsulation inside micelles.

3.1.2. Influence of volume of SDS

An incremental increase in surfactant volume was used to examine the impact of surfactant volume on MDR's fluorescence by adding specific amounts of a 2 % reagent solution in a systematic manner. This resulted in a corresponding improvement in the emission intensity, indicating a direct relationship between surfactant volume and fluorescence enhancement peaking at 0.8 mL beyond which no more improvement was noticed. However, at higher volumes than 1.8, the intensity of emission starts to decline gradually (Fig. 3). As a result, the chosen optimal SDS volume was 1.0 mL.

3.1.3. Influence of pH, buffer volume and type

Using Torell-Stenhagen buffer solutions with different pH (2.0–12.0), the impact of pH on fluorescence of MDR was investigated in the presence of SDS. At pH 5.0–8.0, the greatest RFI of MDR was obtained (Fig. 4). Meanwhile, the measured emission intensity was decreased with increasing pH, thus 6.0 was the chosen pH. Additionally, different buffer systems at pH 6.0 were tried; including; borate, phosphate, Mcllvaine and Torell - Stenhagen buffer solutions. The use of Torell - Stenhagen buffer gave the highest fluorescence with most stable and reproducible reading, thus it was selected as the buffer system.

The impact of buffer volume was investigated by using different volumes (0.1–2.0 mL) of the Torell – Stenhagen solution (pH 6.0). The maximum fluorescence value was observed using volumes starting from 0.4 mL of the buffer solution. The intensity of emission was not influenced by larger volumes but at 1.5 mL, a little decrease was observed (Fig. 5). Based on these findings, 0.5 mL of the Torell - Stenhagen buffer solution was deemed optimal.

3.1.4. Influence of diluting solvent

Previous studies indicated that the use of alcohols and other organic solvents can reduce the fluorescence intensity in micellar enhancement approaches (Abu-Hassan et al., 2020; Hamad et al., 2021; Mohammed et al., 2020). The decrease in the fluorescence activity induced by these organic solvents was linked to their destructive nature on the micelles. Alcohols such as methanol and ethanol are miscible with water which can diminish the micelle aggregation by changing the solution's characteristics. In addition, at high concentrations, alcohol could destroy the micelle aggregates. A previous report mentioned that MDR fluorescence was not enhanced by the addition of SDS (Omar et al., 2019a). We think that the detrimental effect of SDS in that study was the destructive effect of ethanol on the micellar system used as the diluting solvent. However, in the present work, owing to using water, a distinct fluorescence improvement was observed by SDS addition as detailed under the effect of SDS concentration. Typically, water is the diluting solvent of choice in micellar enhancement protocols; It has the dual benefits of being an excellent green solvent and being very cheap.

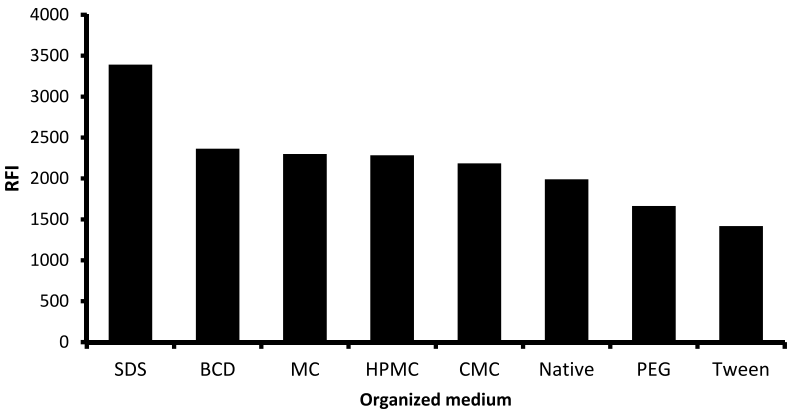

Fig. 2. Impact of surfactant type on the RFI of MDR (1.0 μg ml^{-1}) in Torell buffer.

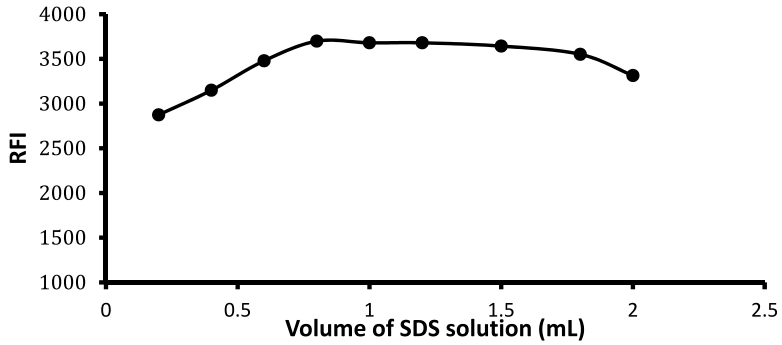

Fig. 3. Impact of the volume of SDS (2.0 % w/v) solution on the RFI of MDR (1.0 μg ml^{-1}).

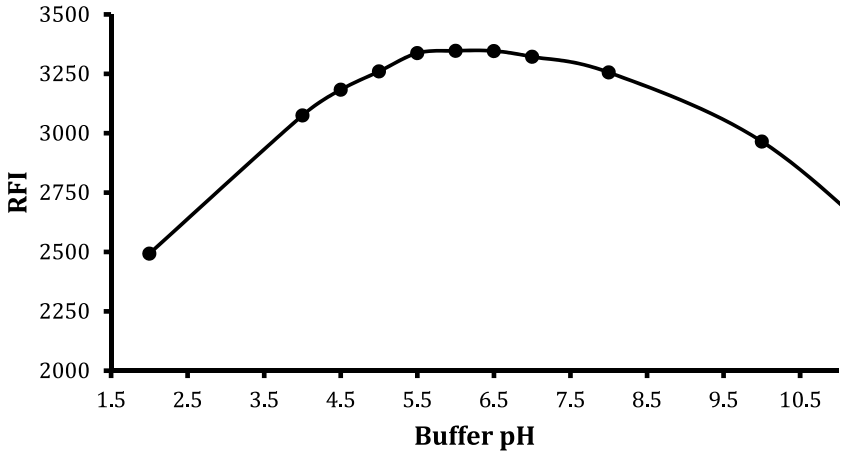

Fig. 4. Impact of pH of the buffer solution on the RFI of MDR (1.0 μg ml^{-1}) in the presence of SDS micelles.

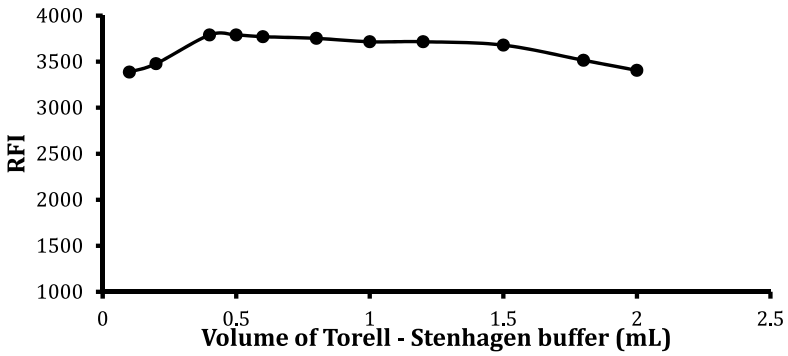

Fig. 5. Impact of the buffer solution volume on the RFI of MDR (1.0 μg ml^{-1}) in the presence of SDS micelles.

3.2. Validation of the suggested approach

The suggested approach was validated following the ICH guiding rules (Guideline and others, 2005). The validation process encompassed various parameters including range, linearity, detection limit (LOD), limit of quantitation (LOQ), robustness, accuracy, and precision.

3.2.1. Range and linearity

Both the linear range and linearity of the method were assessed by analyzing various concentrations of standard MDR solutions. The analytical procedure was conducted, and the data were subjected to statistical evaluation by least square linear curve's fitting. The

calibration graph was built up through correlating the values of fluorescence emission with the drug concentrations. It was observed that the measured emission values were linear with MDR concentrations in a range of 0.025–2.0 μg mL^{-1}. The high linearity of the suggested strategy was indicated from the excellent values of the correlation (r) and determination (r^2) coefficients (0.9999). Statistical data for analyzing the drug using the current methodology are shown in Table 1.

3.2.2. Limits of detection (LOD) and quantitation (LOQ)

To investigate the sensitivity of the current protocol, both detection and quantitation limits were estimated based on the recommendations of ICH (Guideline and others, 2005). The data from the calibration equation including slope (s), and the standard deviation of the intercept (σ) were utilized for the limit's computation. The employed formulae were: LOD = 3.3σ/S and LOQ = 10 σ/S. The estimation revealed values of 0.03 and 0.0098 μg mL^{-1}, for LOQ and LOD in respective order demonstrating the high sensitivity of the current method.

3.2.3. Accuracy

The existing method's accuracy was evaluated by finding the % recovery of three distinct MDR concentrations (0.05, 1.0, and 2.0 μg mL^{-1}). Each experiment was conducted in triplicate, and the results, presented in Table 2 as the mean and standard deviation of percentage recovery. The calculated %recovery values were consistently close to 100 % (ranging from 99.50 % to 102.49 %), indicating a high level of acceptance of the current approach.

3.2.4. Precision

The proposed methods' precision was investigated at two levels; intra-day and inter-day precisions. The assessment of the intra-day level was carried out by applying the general analytical protocol to three different concentrations of MDR solution (0.05, 1.0, 2.0 μg mL^{-1}) in three replicate analyses on the same day. While, the inter-day level was evaluated similarly but over 3 consecutive days. In both levels, the values of % recovery and relative standard deviation (RSD) were computed. The data presented in Table 3 indicate the highly repeatable and reproducible nature of the suggested micellar approach being had small RSD values ranging from 0.28 to 2.1 %).

3.2.5. Robustness

In the evaluation of this parameter, little change was performed in the susceptible parameters of the experiment, and its effect on the method's performance was monitored. The examined parameters included pH, volume of SDS, and buffer solutions. Results listed in Table 4 show that the incorporated variations in the experimental factors didn't have any significant effect on the reliability of MDR quantification at the studied concentration level. The obtained percent recovery was high (100.16–101.68 %), and standard deviation values were within the permissible range (0.15–0.76 %). These results prove the adequate degree of robustness of the current micellar method.

3.3. Applications

3.3.1. Analysis of tablets formulations

Midodrine® tablet was assayed as a representative example for commercial formulation of MDR by adopting both the suggested and reported methods (Omar et al., 2019a). At a confidence level of 95 %, a statistical comparison of the data obtained from the two methods was performed using Student's t-test and F-test. As presented in Table 5, the computed values for both parameters are less than the theoretical values, giving evidence of the acceptable levels of precision and accuracy for the current approach. The present method is safer than the previously reported fluorescence-based method (Omar et al., 2019a) as water is the solvent of dilution in the present method, while the previous method utilized methanol for dilution. Water is the number one 'green' solvent, being cheap and readily available. Moreover, the present method is more sensitive with a detection limit of 0.0098 μg mL^{-1}, while that of the previous method was 0.030 μg mL^{-1}. The obtained high percent recovery for the tablet analysis provides an indication of the absence of any interfering effect that may arise from the existence of pharmaceutical additives in the tablet's formulations. Thus, the present method could be an appropriate alternative for the assay of MDR dosage forms in quality assurance laboratories.

Table 1
Statistical results for analyzing MDR with the suggested micellar spectrofluorimetric approach.

Parameter	Value
Range of linearity (μg mL^{-1})	0.025–2.0
Slope	3368.8
Intercept	−10.962
SD of intercept (S_a)	9.992
SD of slope (S_b)	10.531
LOD (ng mL^{-1})	9.8
LOQ (ng mL^{-1})	30
Determination coefficient (r)	0.9999
Correlation coefficient (r^2)	0.9999

Table 2
Assessment of accuracy of the proposed micellar method for the analysis of MR at three concentration levels.

Taken conc. (μg mL^{-1})	Found conc. (μg mL^{-1})	% Recovery ± SD[a]	Er% [a]	RSD[a]
0.05	0.0512	102.49 ± 0.91	2.49	0.88
1.0	1.012	101.28 ± 0.63	1.28	0.62
2.0	1.99	99.50 ± 0.99	−0.50	1.0

[a] Value is the mean of three replicate measurements. SD, standard deviation; Er%, Error %; and RSD, relative standard deviation.

Table 3
Evaluation of the intra-day and inter-day precisions for the estimation of MDR using the micellar spectrofluorimetric method.

Precision level	Conc. (μg mL^{-1})	% Recovery ± SD[a]	Er%	RSD%
Interday	0.05	102.88 ± 1.49	2.88	1.45
	1.0	100.9 ± 0.13	0.90	0.13
	2.0	99.26 ± 0.73	−0.74	0.74
Intraday	0.05	102.68 ± 2.1	2.86	2.1
	1.0	100.96 ± 0.23	0.96	0.23
	2.0	99.87 ± 0.28	−0.13	0.28

[a] Average of three determinations. SD, standard deviation; Er%, Error %; RSD, relative standard deviation.

Table 4
Robustness evaluation of the suggested micellar method for the assay of MDR (1.0 μg mL^{-1}).

Parameter	Parameter value	% recovery • ± SD	RSD%
pH	5.8	100.16 ± 0.19	0.19
	6.0[a]	101.68 ± 0.37	0.36
	6.2	100.87 ± 0.36	0.36
Buffer volume (mL)	0.4	100.33 ± 0.24	0.23
	0.5[a]	101.38 ± 0.76	0.75
	0.6	101.58 ± 0.25	0.25
SDS volume (mL)	0.8	101.27 ± 0.19	0.19
	1.0[a]	101.6 ± 0.15	0.15
	1.2	101.1 ± 0.34	0.34

*Average of three determinations.
[a] Optimum (normal) values.

Table 5
Estimation of the MDR in its tablet form applying the suggested micellar approach and the reported method (Omar et al., 2019a).

Parameter	Suggested approach	Reported strategy
% Recovery	99.51	99.21
Standard deviation	0.19	0.3
Number of determinations	5	5
F-value [a]	2.611	
t- value [a]	1.448	

[a] Tabulated values at 95 % confidence limit are $t = 2.306$ and $F = 6.338$.

3.3.2. Application to content uniformity test

It is advisable to perform content uniformity testing in cases where the drug substance ratio in the dosage units is less than 25 % or if the drug dosage is below 25 mg (Rockville, 2007). The content of MDR in Midodrine® tablets is 2.5 mg per tablet, thus, testing the content uniformity of dosage units for MDR tablets can be performed. Testing the content uniformity is a lengthy process if a conventional procedure is utilized. However, the current micellar-based protocol is very simple and fast allowing rapid analysis of samples with satisfactory accuracy and minimal effort. Thus, the suggested micellar approach is excellently suited for this purpose. After the determination of the drug contents in ten individual tablets by the suggested assay procedure, the acceptance value (AV) was estimated by adopting the formula, $AV = |M - \overline{\mathscr{X}}| + KS$. M and K are the reference value and acceptability constant, respectively, while $\overline{\mathscr{X}}$ and S are the average of the individual content and its standard deviation, respectively. A dosage form may fulfill the requirement for content uniformity if AV of 10 tablets is $\leq$ L1 (the maximum allowed acceptance value). Calculation revealed that the AV value for the contents of ten Midodrine® tablets obtained by the proposed method was less than L1 as shown in Table 6, which means that the content uniformity of the investigated tablets is acceptable.

Table 6
Results for testing uniformity of tablets contents using the proposed micellar spectrofluorimetric method.

Tablet number	Recovery (%)	SD
1	100.78	0.29
2	101.68	0.75
3	98.95	0.72
4	100.42	0.39
5	99.87	0.52
6	100.83	0.50
7	99.90	0.36
8	99.98	0.43
9	100.16	0.43
10	101.16	0.44
Mean ($\bar{x}$)	100.37	0.78
Acceptance value	1.872	
Maximum allowed acceptance value	15	

3.4. Evaluation of the proposed spectrofluorimetric method against existing spectroscopic and chromatographic analytical approaches

When comparing the proposed spectrofluorimetric method with the reported spectrophotometric methods, the spectrofluorimetric method is preferred due to its high sensitivity. The reported spectrophotometric method utilizing hantzsch condensation reaction (Elazazy, 2015) for MDR determination suffered from relatively low sensitivity and tedious chemical derivatization procedure, which added complexity and reduced greenness. The other spectrophotometric methods based ion pair complexation with bromophenol blue or alizarin red S (Nair et al., 2015a,b); were not only have relatively low but also involved extraction with chloroform, which introduces toxic solvents and increased both the sample analysis time and environmental burden. A complete comparative study of the proposed method and reported spectrophotometric technique is provided in Supplementary Table S1.

The spectrofluorimetric methods also avoid the drawbacks associated with liquid chromatography, such as the use of hazardous and expensive solvents, sophisticated instruments and sometimes extremely expensive mass detector, all of which clearly compromise the method greenness and whiteness. A comparative study showing BAGI scores for the proposed method and existing HPLC methods for MDR determination was illustrated in Supplementary Table S2.

MDR has been previously analyzed using five spectrofluorimetric methods (Hamad et al., 2024; Khashaba et al., 2022; Omar et al., 2019a, 2019b, 2019c). Supplementary Table S1 presents a comparative analysis between the current approach and other published spectroscopic techniques which indicates the highest sensitivity of the current method, as reflected by its lowest LOD. Unlike ninhydrin and hantzsch condensation methods, the current approach does not require heating or complex reagents, making it more suitable for routine laboratory use, particularly in quality control. The procedures involving ninhydrin and *o*-phthalaldehyde are relatively time-consuming, whereas the proposed method is instantaneous and rapid. Furthermore, the reagent used in the proposed method (SDS) is cost-effective in contrast to the considerably expensive fluorescamine reagent. The current method also does not involve extraction process and thus eliminate the need of organic solvent such as methylene chloride which was used in case of dansyl chloride method or chloroform which was used in the ion-pair based method. Consequently, the proposed method is characterized by high sensitivity, speed, simplicity, environmental friendliness, and cost-effectiveness as reflected by its whiteness profile compared to other spectrofluorimetric techniques as shown in Supplementary Table S3.

4. Assessment of the greenness of the suggested approach

Researchers bear a great responsibility for protecting both humans as well as the surrounding environment from potential hazardous effects of the utilized reagents and organic solvents that find their way to the environment after their use in chemical and pharmaceutical applications. Hence, there is a strong recommendation for developing green analytical methods. Thus, access to assessment tools that can effectively evaluate the environmental sustainability of the analytical strategies is crucial (Gamal et al., 2021; Mikhail et al., 2021). The Analytical Greenness Calculator (AGREE), proposed by Pena-Pereira (Pena-Pereira et al., 2020), is a flexible, and over-frontal assessment tool that provides a clear and interpretable results. Its output is a clock-like pictogram, with the overall score and color representation in the center. A score close to 1 and a dark green color signifies a highly environmentally friendly procedure. Fig. 6 shows the AGREE assessment for the suggested spectrofluorimetric approach.

A comparative green assessment of the proposed method and some reported spectrofluorometric methods was conducted using AGREE calculator and presented in Supplementary Table S4. The proposed method is direct, simple, and rapid. It does not need extraction as in dansyl chloride method, or a time-consuming procedure as in *ortho*-phthalaldehyde/2-mercapto-ethanol method (15 min), and the dansyl chloride method (20 min). It is also not energy-consuming. The greenest methods identified were the current methodology as well as fluorescamine method, achieving AGREE score of 0.73, and 0.74, respectively. The proposed method has the advantage over fluorescamine of being more sensitive and using non expensive reagent without requiring derivatization process.

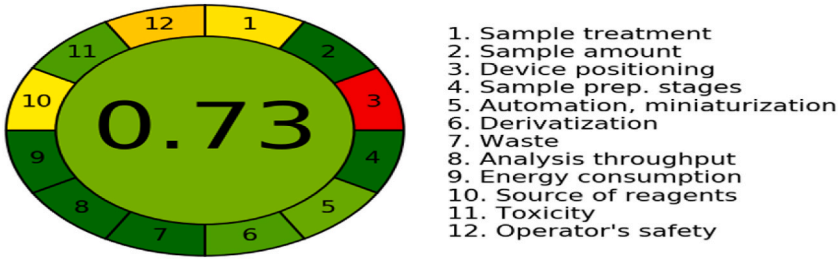

Fig. 6. Annotated results for the green assessment of the proposed spectrofluorometric method by AGREE calculator.

5. Assessment of the whiteness of the proposed spectrofluorometric method

Assessment using RGB12 algorithm quantitatively evaluates sustainability by determining the whiteness of the analytical approach based on the recently established Red-Green-Blue (RGB) model (Nowak et al., 2021). Analytical efficiency, as measured by validation criteria like accuracy, precision, LOD, sensitivity, and others, is represented by red. Green denotes adherence to green analytical chemistry principles concerning environmental safety, including reagent toxicity, its quantity and number, waste produced throughout the process, energy consumption, and overall environmental impact. Whereas, blue represents performance characteristics, such as minimal practice requirements, operational simplicity, cost and time efficiency. Results in Fig. 7 demonstrate that whiteness profile of the proposed method achieves a score of 90.4 %. A comparative study of the proposed methodology's whiteness profile against three reported spectrofluorometric methods (Derayea et al., 2023; Khashaba et al., 2022; Omar et al., 2019c) utilizing the RGB 12 model is shown in Supplementary Fig. S5.

6. Conclusion

Based on the augmentation of MDR's intrinsic fluorescence using SDS, a highly sensitive spectrofluorimetric approach was established. This method is deemed appropriate for the quantification of the investigated drug in both pure state and tablet formulations. In addition, the great simplicity of the employed procedure permitted its incorporation to assess the content uniformity of tablet formulations. The approach offers the benefits of being simple, rapid, highly sensitive, and environmentally safe. Moreover, the procedure was performed at ambient temperature without the need of heating or extraction steps. Thus, it can be applied efficiently for routine drug analysis in quality control laboratories. The greenness profile of the suggested strategy was evaluated using the AGREE metric tool. Additionally, whiteness assessment was conducted to accomplish a complete eco-friendliness profile and functionality of the designed methodology.

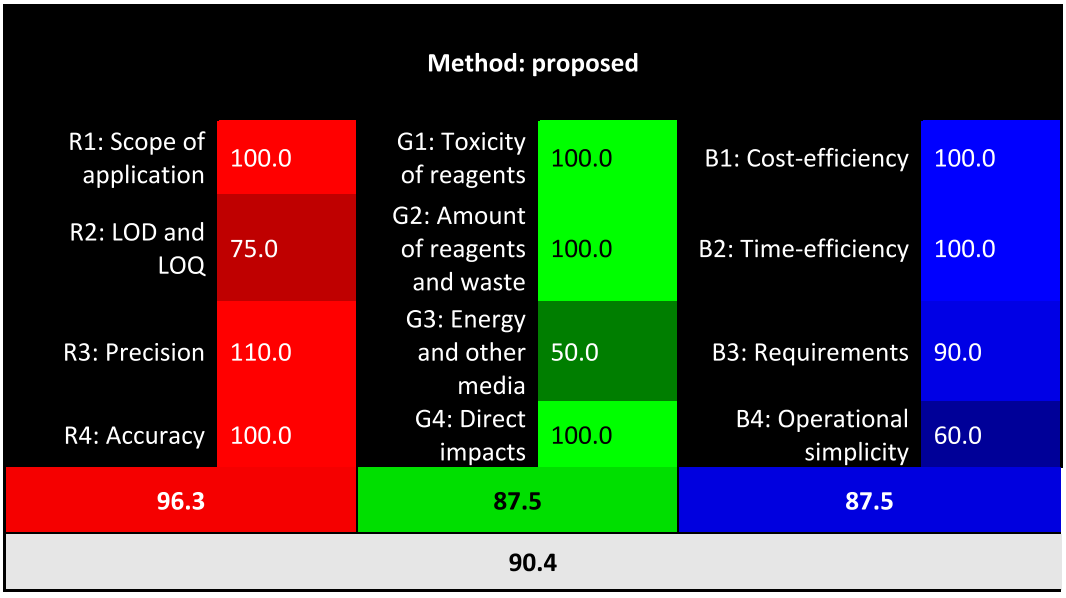

Fig. 7. Whiteness profile for the proposed method according to The RGB 12 algorithm.

CRediT authorship contribution statement

Pakinaz Y. Khashaba: Validation, Supervision, Software, Investigation. **Mahmoud Abdelgaleel:** Validation, Methodology, Data curation. **Dalia M. Nagi:** Writing – original draft, Conceptualization. **Mohamed Oraby:** Visualization, Data curation. **Sayed M. Derayea:** Writing – review & editing, Visualization, Supervision, Software, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scp.2025.102058.

Data availability

All data are included in manuscript and supplementary file

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