Hindawi Publishing Corporation
Journal of Chemistry
Volume 2016, Article ID 5097364, 8 pages
http://dx.doi.org/10.1155/2016/5097364

Research Article

Highly Sensitive Micellar Enhanced Spectrofluorimetric Method for Determination of Mirtazapine in Tablets and Human Urine: Application to In Vitro Drug Release and Content Uniformity Test

Hany W. Darwish,[1,2] Ahmed H. Bakheit,[1,3] and Raed M. Alharbi[1,4]

[1]*Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*
[2]*Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo 11562, Egypt*
[3]*Department of Chemistry, Faculty of Science and Technology, El-Neelain University, P.O. Box 12702, 12702 Khartoum, Sudan*
[4]*Department of Aviation Medicine, King Abdulaziz Medical City, Ministry of National Guard, P.O. Box 22490, Riyadh 11426, Saudi Arabia*

Correspondence should be addressed to Hany W. Darwish; hdarwish75@yahoo.com

Received 5 April 2016; Revised 29 June 2016; Accepted 10 July 2016

Academic Editor: Ana M. García-Campaña

A highly sensitive and simple micelle enhanced spectrofluorimetric method was developed for assaying mirtazapine (MRZ) in REMERON® tablets and spiked human urine directly without the need of derivatizing agent. The basis of the current procedure is the examination of the relative fluorescence intensity (RFI) of MRZ in sodium lauryl sulphate (SLS) micellar medium. The RFI of MRZ in water was enhanced markedly on addition of SLS. The RFI was measured at 403 nm after excitation at 320 nm. The fluorescence-concentration relationship was linear over the range 1–500 ng/mL, with lower detection limit of 0.399 ng/mL. The proposed method was successfully applied to the determination of MRZ in dosage form and spiked human urine. Recovery percentages of MRZ utilizing the current method were 99.05±1.83, 98.37±1.96, and 100.41±2.61% for pure powder, pharmaceutical dosage form, and spiked human urine, respectively. The application of the proposed method was extended to test content uniformity and the in vitro drug release of REMERON tablets, according to USP guidelines.

1. Introduction

Mirtazapine (MRZ; [1,2,3,4,10,14b-hexahydro-2-methylpyrazino-[2,1a] pyrido [2,3-c] benzazepine]) (Figure 1) belongs to chemical class of piperazinoazepines. MRZ is an antidepressant of the second generation and a member of noradrenergic and specific serotonergic class. MRZ acts by increasing serotonin and norepinephrine discharge, maybe due to its antagonistic action on α_2-adrenergic heteroreceptors and autoreceptors. High efficiency of MRZ as an antidepressant drug is due to its unique mechanism which differs from the other drugs belonging to the same class [1]. Currently, MRZ is utilized for treatment of number of disorders including obsessive-compulsive disorder [2], posttraumatic stress disorder [3], generalized anxiety disorder [4], and postchemotherapy nausea and vomiting [5] (due to its antiemetic properties). Regarding MRZ dose, it initiates with 15–30 mg in the first day of administration and then increases according to the patients' needs [6]. Upon extensive survey in the literature, it was found that several techniques were adopted for assaying MRZ in REMERON tablets including spectrophotometry [7], spectrofluorimetry [8], high performance liquid chromatography (HPLC) [8], and capillary zone electrophoresis (CZE) [8]. MRZ was determined in biological fluids utilizing spectrofluorimetry [9], HPLC [10–12], gas chromatography (GC) [13], capillary electrophoresis (CE) [14], and high performance thin layer chromatography (HPTLC) [15]. For analysis of MRZ in urine, there are

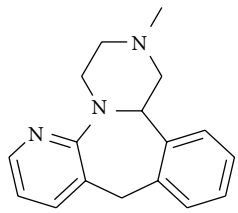


Figure 1: Chemical structure of mirtazapine (MRZ).

only few techniques reported for that aim. These techniques include GC coupled with tandem mass spectrometry [16, 17], liquid chromatography coupled with mass spectrometry [18], and CE [19]. These techniques suffered from several disadvantages such as being laborious, time consuming, and in need for costly apparatuses that lessened their availability in developing communities. Therefore, the aim of the current study is to develop simple and sensitive spectrofluorimetric method for assaying MRZ in dosage form and urine samples. Moreover, the adopted method was applied for testing REMERON tablets content uniformity and in vitro drug release from REMERON tablets following USP guidelines.

2. Experimental

2.1. Apparatus

(i) All fluorescence measurements were performed on Jasco FP-8200 Fluorescence Spectrometer (Jasco Corporation, Japan) equipped with 150 W xenon lamp and 1 cm quartz cells with bandwidth of 5 nm for excitation and emission monochromators. All data acquisition was handled utilizing SpectraManager® software.

(ii) pH was measured and adjusted using Hanna pH-Meter (Romania).

(iii) In vitro drug release was tested using automatic dissolution tester (8-cup system) (Abbota Corporation, New Jersey, United States).

2.2. Reagents and Materials. MRZ was kindly gifted by Organon, OSS, and utilized without additional purification. All the chemicals used were of Analytical Reagents grade, and the solvents were of HPLC grade.

The dosage form analyzed was REMERON tablets (Lot number 783147) claimed to contain 30 mg MRZ and other inactive ingredients including hydroxypropyl cellulose, hydroxypropyl methyl cellulose, maize starch, colloidal silicone dioxide, magnesium stearate, lactose, polyethyleneglycol 8000, titanium dioxide (E171), and yellow and red iron oxides (E172 and E172, resp.).

(i) SLS (sodium lauryl sulphate; 95%) was procured from Winlab (Pontefract, London, UK) and prepared as 1% w/v in water.

(ii) Cremophor RH 40 and Cremophor EL were obtained from BASF (Ludwigshafen, Germany) and prepared as 1% v/v aqueous solutions.

(iii) CMC (carboxymethylcellulose) and β-CD (β-cyclodextrin) were acquired from Merck (Darmstadt, Germany) and utilized as 1% w/v in water.

(iv) Tween surfactants (20, 80, and 85) were acquired from Techno Pharmchem Haryana Company (New Delhi, India) and prepared as 1% v/v in water.

(v) Acetonitrile (ACN) was purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany). Short chain alcohols, such as ethanol and methanol, were bought from VWR Prolabo (Fontenay Sous Bois, France)

(vi) 0.1 M phosphate buffer and 0.1 M borate buffer, covering the pH ranges 2–12, were freshly prepared.

(vii) Ultrapure water was acquired through a Millipore Milli-Q® UF Plus water purifier (MA, USA).

2.3. Standard Solutions. MRZ stock solution (1 mg/mL) was prepared by dissolving 25 mg of MRZ reference standard material into 25 mL methanol in a 25 mL calibrated volumetric flask and completing the volume properly. This stock solution was then diluted with acetonitrile (ACN) to prepare different working standard solutions of 0.5, 2, and 5 μg/mL. These standard MRZ solutions were stable for 14 days when refrigerated.

2.4. Construction of the Calibration Graph. Calibration samples were prepared by accurately transferring aliquots of MRZ standard solutions to five mL calibrated flasks followed by addition of 0.3 mL of 1% w/v SLS and 0.2 mL of H_2SO_4 (0.1 N) and completing volume by ultrapure distilled water to yield final concentrations of 1–500 ng/mL. Mixing of the flasks' contents took place and then RFI was measured at 403 nm after excitation at 320 nm. Calibration curve was generated by plotting RFI of MRZ calibration samples versus MRZ concentrations in ng/mL. Finally, the regression equation was computed and utilized to calculate MRZ concentrations in different samples depending on their RFIs.

2.5. Determination of Tablet Samples. Number of REMERON tablets (30 mg tablets; Lot number 784571) were weighed and powdered followed by transferring accurate amount of powder correspondent to one tablet (30 mg of MRZ) to a 100 mL volumetric flask and dissolved in thirty milliliters of methanol. The volumetric flask was sonicated for 30 min and then diluted to the mark with the same solvent. This solution (0.3 mgmL^{-1}) was filtered with chromafil® Xtra 0.2 μm filter paper and diluted with ACN to get appropriate concentrations of MRZ for subsequent analysis. Finally, the procedures designated under "Construction of Calibration Graph" were followed.

2.6. Determination of Human Urine Samples. One mL of free drug human urine was spiked with 20 μL of various MRZ standard solutions and mixed for 30 min. A volume of one milliliter of NaOH 100 mM/glycine buffer (pH~11) was added and then mixing took place for 10 s. Liquid-liquid extraction was accomplished using five milliliters of diethyl ether and

the solution was placed in the vortex mixer for 30 seconds followed by centrifugation for 15 minutes at 10,000 rpm (for complete phases separation). Then three milliliters of the upper organic layer was transferred into glass vials and dried utilizing gentle stream of nitrogen. Finally, the residue was reconstituted in methanol and appropriate dilutions were performed with ACN to yield final MRZ concentrations of 100 ng/mL, 200 ng/mL, 300 ng/mL, and 400 ng/mL. The procedures termed under "Construction of Calibration Graph" were performed. Treatment of a blank urine sample took place in a similar way. FI was measured at 403 nm after excitation at 320 nm. Linearity was checked by computing regression equation and MRZ concentrations were calculated.

2.7. Procedure for Content Uniformity Testing for MRZ. Analysis of number of REMERON tablets was carried out by applying the procedure adopted under "Determination of Tablet Samples" for assaying MRZ. USP guidelines [20] (Chapter 905: Uniformity of Dosage Units) were followed for studying the uniformity of REMERON tablets.

2.8. Procedure for In Vitro Drug Release Test (Dissolution Test) for MRZ. For testing in vitro release of REMERON tablets, dissolution procedure of USP [20] was followed utilizing USP apparatus II. A volume of 900 mL of 0.1 N HCl solution, kept at ambient temperature, was utilized for dissolution of the cited tablets and stirred at a speed of 50 rpm for 25 min. A volume of five mL of REMERON tablet sample was taken and filtered using 0.45 μm syringe filter. The volume was kept constant by substitution of the withdrawn volume by dissolution medium prepared freshly at the selected time intervals. Afterwards, the same procedures mentioned under "Construction of the Calibration Graph" were followed for assaying of MRZ in these filtered samples.

3. Results and Discussion

Spectrofluorimetry, as an analytical technique, was characterized by number of advantages including sensitivity and simplicity which offered multiapplication for this technique. Sensitivity allowed the determination of MRZ in urine while simplicity was beneficial for analysis of its dosage form in addition to content uniformity and dissolution rate testing. In this study, sensitivity of spectrofluorimetry was enhanced by addition of surface active agents which resulted in micelle formation. Micellar enhanced spectrofluorimetry was adopted and applied in assaying several active ingredients in many reports [21–27] due to the ability of the micelle to enhance RFI of these drugs in addition to the exclusion of organic solvents and thus increase the greenness of the adopted procedure. RFI of the cited drug (MRZ) was affected by number of factors including pH, type, and concentration of surfactant, diluting solvent, and others. Optimization of these factors was done by adjusting each factor individually while the other factors kept constant or what we call "one factor at a time optimization strategy."

3.1. Fluorescence Spectra and Characteristics of MRZ. Molecular emission behavior of a certain compound is represented by two spectra. The first one represents the process of electronic excitation and the second one represents the process of emission. Thus molecular emission spectra are characterized by two wavelengths: excitation and emission wavelengths ($\lambda_{ex}/\lambda_{em}$). Figures 2(a) and 2(b) showed that MRZ have $\lambda_{ex}/\lambda_{em}$ of 320/403 nm. This labeled the native fluorescence property of MRZ. Upon addition of SLS to MRZ standard solution, the RFI was improved remarkably as shown in peak number (2) in Figure 2(a).

3.2. Optimization of the Experimental Conditions

3.2.1. Effect of Organized Media. Different types of organized media were tested in this study. Consequently, 0.5 mL of 1% w/v solution of each surfactant was added to MRZ sample solution. A concentration of 1% w/v for different surfactants was chosen as it is higher than the reported critical micelle concertation (cmc) of these surfactants. Maximum RFI was attained utilizing SLS (Figure 3). For optimization of the concentration of organized media, various volumes of 1% w/v SLS were added to MRZ solution. It was clear from Figure 4 that optimum RFI for MRZ was reached by utilizing a final concentration of 1.5×10^{-3} Molar SLS (equivalent to 0.2 mL 1% SLS) and any extra addition of SLS had no influence on the MRZ response. Therefore, 1.75×10^{-3} Molar SLS (equivalent to 0.3 mL 1% w/v) was added in all subsequent experiments.

3.2.2. Effect of pH. Different buffers and acids (covering pH range 1–12) were examined to optimize the influence of pH on RFI of MRZ and MRZ-SLS and SLS, as shown in Figure 5. It was clear from that figure that RFI of MRZ alone was optimum at highly acidic solutions and decreased remarkably as pH increased. This may be attributed to alteration of MRZ ionization. Regarding SLS surfactant, its RFI was almost nil in all pH range which considered an advantage for utilizing this anionic surfactant for MRZ determination in this work. Ultimately, maximum RFI for MRZ-SLS was attained at acidic solutions (specifically 0.1 N H_2SO_4) and RFI decreased noticeably on increasing pH values. These findings assure that MRZ at acidic pH attained positive charge and hence it is logic to interact well with the negatively charged sulphonyl group of SLS. This thought was supported by Chemicalize [28] calculations. The interaction of MRZ-SLS was proposed in Scheme 1.

3.2.3. Effect of Diluting Solvent. Various solvents were examined as diluting solvents in the current study. Among the tested solvents, the highest response was attained using water as displayed in Figure 6. This offered an advantage for the proposed procedure due to the absence of the organic solvent. This behavior may be attributed to the increment of medium polarity by water which in turn increased the interaction between water molecules and MRZ ones (Figure 6).

3.2.4. Effect of Time. The effect of time on the stability of the RFI of MRZ was also studied. It was found that RFI of MRZ in

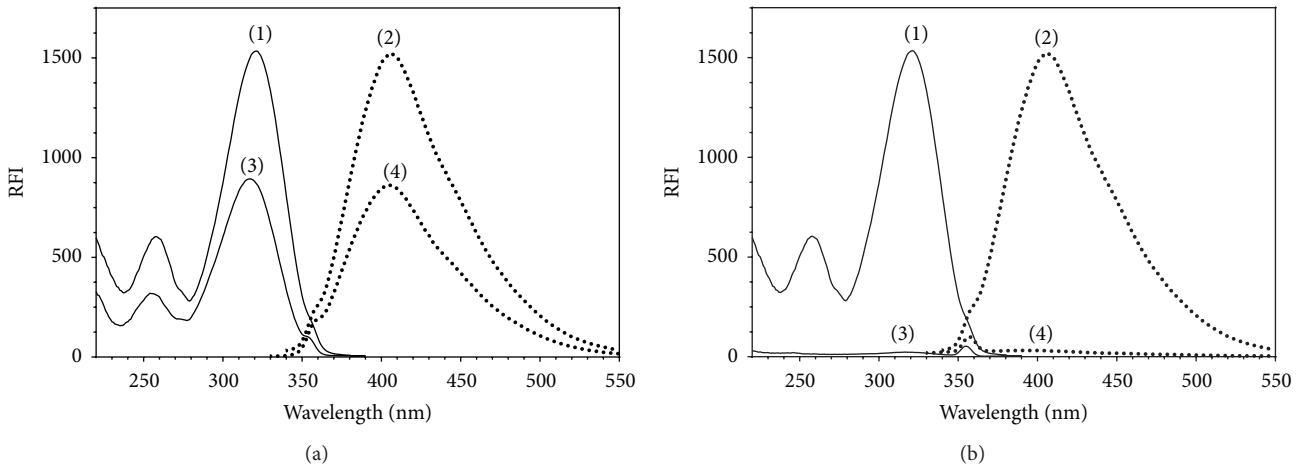


Figure 2: Excitation (1) and emission (2) spectra of MRZ (100 ng/mL) in SLS (1%, w/v); (a) excitation (3) and emission (4) spectra of MRZ (100 ng/mL) in acidic aqueous solution; (b) excitation (3) and emission (4) spectra of SLS (1%, w/v) in water.

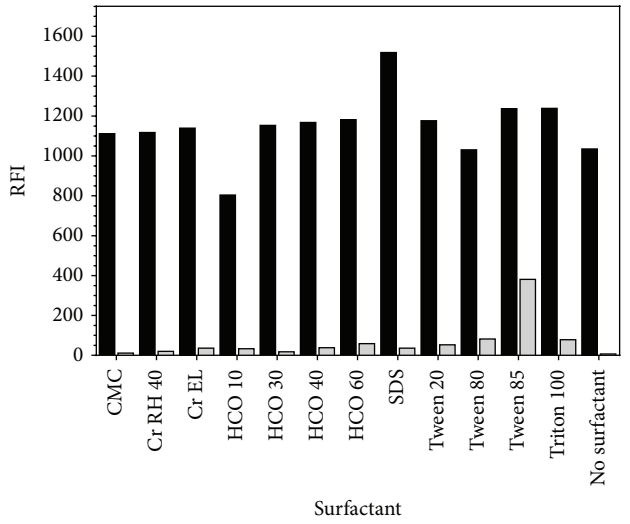


Figure 3: Effect of the type of organized media (0.5 mL 1% w/v solution of each) on RFI of MRZ (100 ng/mL) (black column MRZ-SLS, white column MRZ alone).

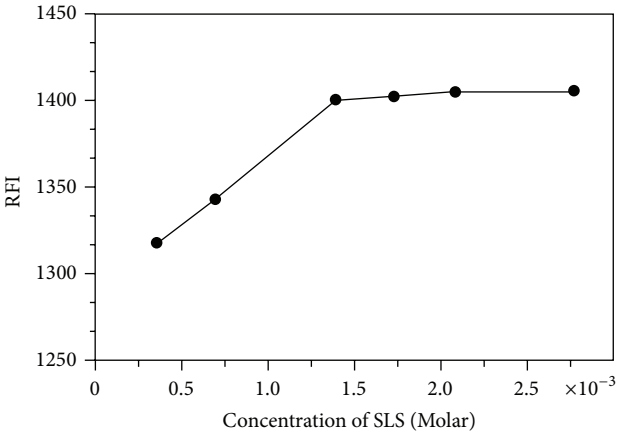


Figure 4: Effect of sodium lauryl sulfate (SLS) concentration (Molar) on fluorescence intensity of MRZ (100 ng/mL).

micellar medium was developed immediately and was stable for at least 30 minutes.

4. Validation of the Method

4.1. Linearity and Range. The calibration curve was linear through the concentration range of 1–500 ng/mL as displayed in Table 1.

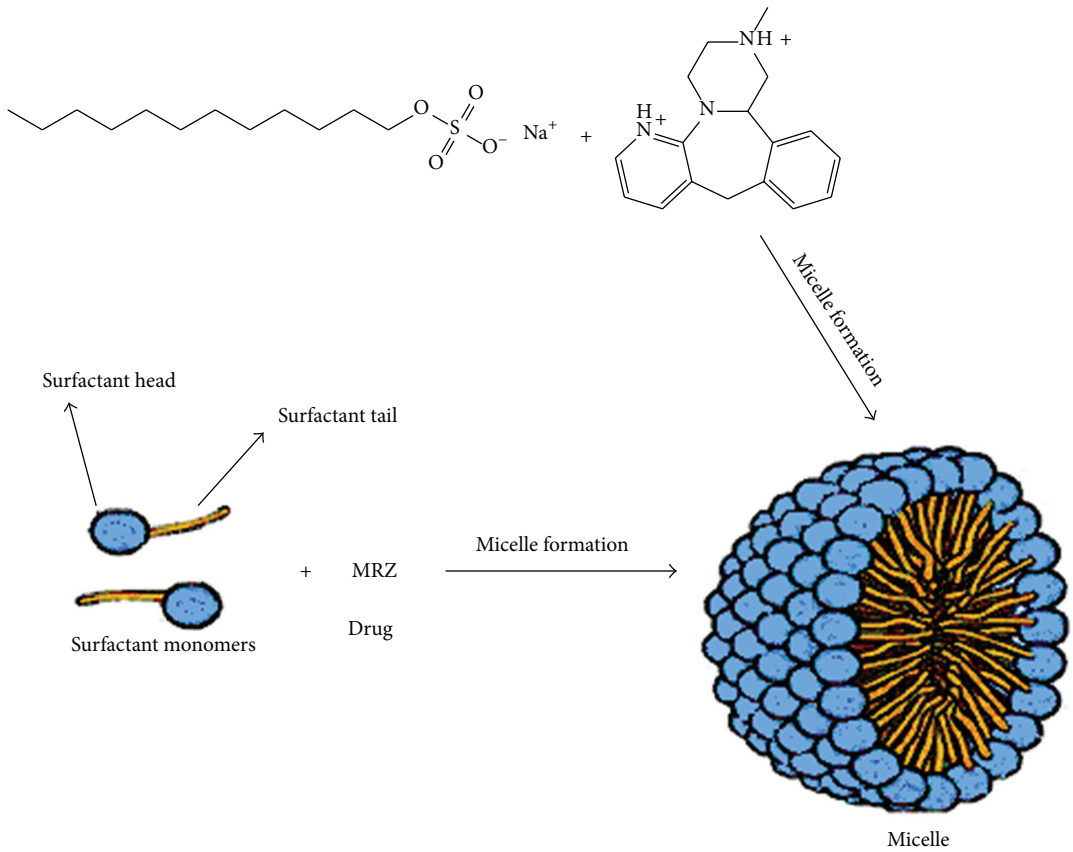
From Table 1, it can be seen that the linearity was proven from the high values of the determination coefficient (r) and low values of standard deviation of residuals ($S_{y/x}$), intercept (S_a), slope (S_b), % RSD, and % error [29].

4.2. Sensitivity. Calculation of limit of quantitation (LOQ) and limit of detection (LOD) was carried out in accordance with the ICH Q2 (R1) recommendation [30]. Their values were abridged in Table 1. These values were computed by applying the next equation:

$$\text{LOD} = 3.3\frac{h}{l}, \qquad \text{LOQ} = 10\frac{h}{l}, \tag{1}$$

where h is the SD of the intercept of regression line of calibration curve while l is the slope of regression line of the calibration curve (Table 1).

4.3. Accuracy. Testing the accuracy of the suggested procedure took place by analyzing different samples of MRZ standard solution equivalent to different concentrations. Mean % recovery was 99.05 ± 1.83 as displayed in Table 2 which in turn reflects the high accuracy of the current spectrofluorimetric method. Additionally, the accuracy of the



Scheme 1: The suggested mechanism for CRZ-SLS micelle formation.

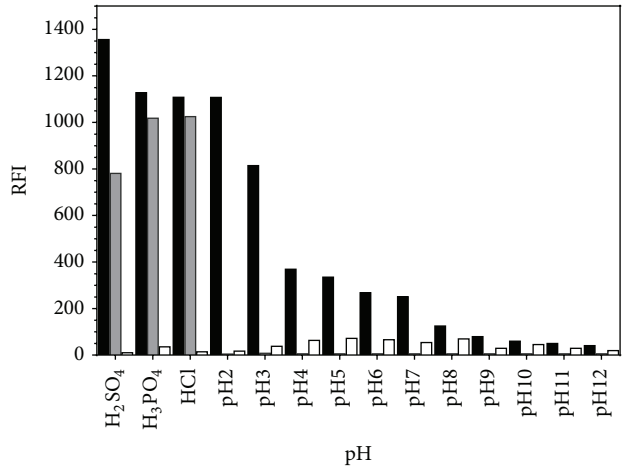


Figure 5: Effect of pH on the RFI of 100 ng/mL MRZ in 0.2 mL 1%, w/v sodium lauryl sulfate (SLS) in aqueous solution (black column MRZ-SLS, grey column MRZ alone, and white column SLS alone).

current procedure was assessed by investigating the results of analyzing REMERON tablets and spiked urine samples as seen in Table 2.

4.4. Repeatability and Reproducibility. Intra- and interassay precision were evaluated by analyzing various MRZ concentrations (20, 100, 200, and 400 ng/mL) in triplicate in one day and in three successive days, respectively. Mean % recoveries were almost equal to 100% and the relative standard deviation values (%RSD) were small. These values are evidence for the precision and accuracy of the suggested procedure (Table 3).

4.5. Robustness. Table 4 summarizes the method robustness by evaluating the liability of determinations to deliberate

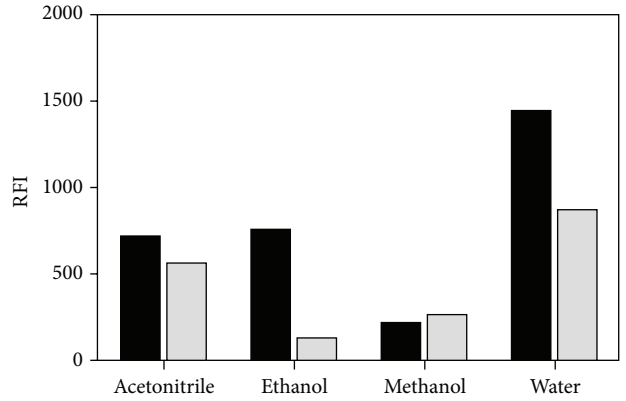


Figure 6: Effect of the diluting solvent on RFI of MRZ (100 ng/mL) (gray column MRZ alone and black column MRZ-SLS).

Table 1: Analytical performance data for the spectrofluorimetric determination of MRZ.

Parameter	MRZ
Wavelength [$\lambda_{ex}/\lambda_{em}$] (nm)	320/403
Linearity range (ng/mL)	1–500
Intercept (a)	16.79
Slope (b)	14.09
Correlation coefficient (r)	0.9998
SD of residuals ($S_{y/x}$)	48.51
SD of intercept (S_a)	8.517
SD of slope (S_b)	0.0368
% RSD[a]	1.123
% error[b]	0.195
LOD (ng/mL)[c]	0.399
LOQ (ng/mL)[d]	1.209

[a]Percentage relative standard deviation for three replicate samples.
[b]Percentage relative error for three replicate samples.
[c]Limit of detection.
[d]Limit of quantitation.

changes of the experimental conditions. It was found that small changes that possibly occur during the experimental runs will have no significant effect on the fluorescence intensity of the cited drug.

4.6. Specificity. For investigation of the specificity of the proposed procedure, interferences that may result from inactive ingredients of tablets were observed. It was clear from the results of analysis of REMERON tablets that these inactive ingredients did not exhibit any interference as can be seen in Table 2.

5. Applications

5.1. Application of Procedure to Analysis of MRZ in REMERON Tablets. REMERON tablets were assayed efficiently by the suggested spectrofluorimetric procedure. Mean percent recoveries of various concentrations depended on the mean of triplicate determinations. The % recovery ranged from 98.37 ± 1.96 as displayed in Table 2. These results indicated the high accuracy of the method.

5.2. Content Uniformity Testing for MRZ. The rapidity and simplicity of the current methods allowed us to test content uniformity of REMERON tablets following USP guidelines [20] (Chapter 905: Uniformity of Dosage Units). The acceptance value (AV) was computed and it was clear from data in Table 5 that it is less than the maximum allowed acceptance value (L1). These results established very good MRZ uniformity.

5.3. In Vitro Drug Release (Dissolution Test) for MRZ. Dissolution test was performed on REMERON tablet (30 mg). The concentration of MRZ released was then determined by substitution of the RFI for MRZ at the analytical wavelength (403 nm) in the regression linear equation and hence drug released % was computed. It was found that about 90% of the claimed amount of MRZ is released in less than 15 min as stated in the USP [20].

5.4. Application of Procedure to Analysis of MRZ in Urine. By virtue of the high sensitivity of the suggested procedure, MRZ could be assayed efficiently in spiked human urine samples. Less than 1% of MRZ dose (which is ranged from 15 to 45 mg daily) is excreted in the urine unchanged. Consequently, the unchanged drug level in urine (150–450 ng/mL) lies in the dynamic linear range of the current spectrofluorimetric method. Calibration curve constructed from results spiked urine analysis was linear and % recovery results of MRZ were displayed in Table 2.

6. Conclusion

Micellar enhanced spectrofluorimetric methodology was conducted for assaying MRZ in different matrices. The proposed method is characterized by its simplicity, sensitivity, and rapidity when compared to the reported chromatographic methodology. The current procedure was applied with a great success for assaying MRZ in REMERON tablets and spiked human urine. There were no interferences raised from the common inactive ingredients of REMERON tablets or the endogenous compounds in urine samples. The current study was extended for application of content uniformity testing. Additionally, it has been utilized for dissolution testing of MRZ tablets. Economically, the method offered cheap analytical procedure for assaying MRZ as it depended on measuring intrinsic fluorescence response of MRZ, rather than utilizing expensive derivatizing analytical reagents. Additionally, this technique belongs to green analytical methodology, as there is no utilization of organic solvent.

Table 2: Results of the determination of MRZ in pure form, REMERON tablets (Lot number 783147), and urine samples.

Parameter	Pure form			REMERON tablets			Urine samples		
	Amount taken (ng/mL)	Amount found (ng/mL)	% found	Amount taken (ng/mL)	Amount found (ng/mL)	% found	Amount added (ng/mL)	Amount found (ng/mL)	% found
	20	19.29	96.43	20	20.24	101.2	100	103.54	103.54
	100	100.2	100.2	100	96.66	96.66	200	198.38	99.19
	200	198.38	99.19	200	195.61	97.81	300	292.63	97.54
	400	401.57	100.39	300	293.42	97.81	400	405.46	101.36
Mean			99.05			98.37			100.41
±SD			1.83			1.96			2.61

Table 3: Accuracy and precision data for the determination of MRZ by the proposed method.

Amount taken (ng/mL)	% found	% RSD	% error
Intraday			
20	103.26 ± 1.161	1.124	−0.033
100	99.47 ± 1.577	1.585	0.005
200	97.28 ± 2.366	2.432	0.027
400	98.81 ± 1.003	1.015	0.012
Interday			
20	96.43 ± 1.947	2.019	−3.572
100	100.2 ± 1.125	1.123	0.195
200	99.19 ± 0.637	0.642	−0.81
400	100.39 ± 1.742	1.736	0.393

Table 4: Robustness of the proposed spectrofluorimetric method.

Experimental parameter variation	Recovery (%) ± SD[a]
No variation[b]	99.68 ± 0.88
SLS volume (μL)	
280	96.19 ± 1.23
320	101.51 ± 1.34
Acid concentration (N)	
0.08	97.81 ± 1.26
0.12	99.19 ± 1.51
Acid volume (μL)	
180	99.07 ± 0.61
220	102.05 ± 1.15
Temperature (C°)	
20	104.04 ± 1.67
30	97.22 ± 1.08

[a]Mean of three determinations, [b]following the general calibration procedures.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Table 5: Results of weight variation testing of MRZ tablets using the proposed method.

Parameter	Tablet number	Percentage of the label claim
Data	1	99.19 ± 1.51
	2	96.09 ± 1.21
	3	97.81 ± 2.26
	4	95.56 ± 0.89
	5	101.2 ± 2.32
	6	96.66 ± 1.32
	7	97.81 ± 0.84
	8	97.81 ± 0.96
	9	98.37 ± 1.05
	10	97.75 ± 0.77
Mean	97.83	
SD	1.31	
% RSD	1.34	
Acceptance value (AV) [20]	3.81	
Max. allowed AV (L1) [20]	15	

Acknowledgments

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at the King Saud University for funding this work through the Research Group Project no. RGP-322.

References

[1] P. K. Gillman, "A systematic review of the serotonergic effects of Mirtazapine in humans: implications for its dual action status," *Human Psychopharmacology*, vol. 21, no. 2, pp. 117–125, 2006.

[2] P. J. Goodnick, A. Puig, C. L. DeVane, and B. V. Freund, "Mirtazapine in major depression with comorbid generalized anxiety disorder," *Journal of Clinical Psychiatry*, vol. 60, no. 7, pp. 446–448, 1999.

[3] L. M. Koran, N. N. Gamel, H. W. Choung, E. H. Smith, and E. N. Aboujaoude, "Mirtazapine for obsessive-compulsive disorder: an open trial followed by double-blind discontinuation," *The Journal of Clinical Psychiatry*, vol. 66, no. 4, pp. 515–520, 2005.

[4] D. S. Baldwin, I. M. Anderson, D. J. Nutt et al., "Evidence-based guidelines for the pharmacological treatment of anxiety disorders: recommendations from the British Association for Psychopharmacology," *Journal of Psychopharmacology*, vol. 19, no. 6, pp. 567–596, 2005.

[5] R. E. Kast and K. F. Foley, "Cancer chemotherapy and cachexia: mirtazapine and olanzapine are 5-HT3 antagonists with good antinausea effects: Research in brief," *European Journal of Cancer Care*, vol. 16, no. 4, pp. 351–354, 2007.

[6] R. Mandrioli, L. Mercolini, N. Ghedini, C. Bartoletti, S. Fanali, and M. A. Raggi, "Determination of the antidepressant mirtazapine and its two main metabolites in human plasma by liquid chromatography with fluorescence detection," *Analytica Chimica Acta*, vol. 556, no. 2, pp. 281–288, 2006.

[7] N. Karaşen and S. Altinöz, "Determination of mirtazapine in tablets by UV spectrophotometric and derivative spectrophotometric methods," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 24, no. 1, pp. 11–17, 2000.

[8] L. Labat, P. Dallet, E. Kummer, and J. P. Dubost, "Spectrophotometric, spectrofluorimetric, HPLC and CZE determination of mirtazapine in pharmaceutical tablets," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 28, no. 2, pp. 365–371, 2002.

[9] R. M. Youssef, "Determination of mirtazapine in spiked human plasma and tablets by first derivative spectrofluorimetric method," *Saudi Pharmaceutical Journal*, vol. 18, no. 1, pp. 45–49, 2010.

[10] Z.-D. Zhai, Y.-P. Shi, and T. Wang, "Development and validation of HPLC methods for enantioseparation of mirtazapine enantiomers at analytical and semipreparative scale using polysaccharide chiral stationary phases," *Analytica Chimica Acta*, vol. 550, no. 1-2, pp. 123–129, 2005.

[11] F. J. M. de Santana and P. S. Bonato, "Enantioselective analysis of mirtazapine and its two major metabolites in human plasma by liquid chromatography-mass spectrometry after three-phase liquid-phase microextraction," *Analytica Chimica Acta*, vol. 606, no. 1, pp. 80–91, 2008.

[12] X. Hong, Y. Yao, S. Hong, and C. Lei, "LC-MS-MS analysis of mirtazapine in plasma, and determination of pharmacokinetic data for rats," *Chromatographia*, vol. 68, no. 1-2, pp. 65–70, 2008.

[13] C. Sánchez De La Torre, M. A. Martínez, and E. Almarza, "Determination of several psychiatric drugs in whole blood using capillary gas-liquid chromatography with nitrogen phosphorus detection: comparison of two solid phase extraction procedures," *Forensic Science International*, vol. 155, no. 2-3, pp. 193–204, 2005.

[14] Z. Aturki, V. Scotti, G. D'Orazio, A. Rocco, M. A. Raggi, and S. Fanali, "Enantioselective separation of the novel antidepressant mirtazapine and its main metabolites by CEC," *Electrophoresis*, vol. 28, no. 15, pp. 2717–2725, 2007.

[15] T. S. Reddy and P. S. Devi, "Simultaneous determination of mirtazapine and its three main impurities by a high performance thin layer chromatography/densitometry method," *Journal of Liquid Chromatography and Related Technologies*, vol. 31, no. 8, pp. 1204–1212, 2008.

[16] C. Salgado-Petinal, J. P. Lamas, C. Garcia-Jares, M. Llompart, and R. Cela, "Rapid screening of selective serotonin re-uptake inhibitors in urine samples using solid-phase microextraction gas chromatography-mass spectrometry," *Analytical and Bioanalytical Chemistry*, vol. 382, no. 6, pp. 1351–1359, 2005.

[17] P. Adamowicz and M. Kała, "Simultaneous screening for and determination of 128 date-rape drugs in urine by gas chromatography—electron ionization-mass spectrometry," *Forensic Science International*, vol. 198, no. 1–3, pp. 39–45, 2010.

[18] F. J. M. De Santana, V. A. P. Jabor, E. J. Cesarino, V. L. Lanchote, and P. S. Bonato, "Enantioselective analysis of mirtazapine, demethylmirtazapine and 8-hydroxy mirtazapine in human urine after solid-phase microextraction," *Journal of Separation Science*, vol. 33, no. 2, pp. 268–276, 2010.

[19] F. J. M. de Santana, V. L. Lanchote, and P. S. Bonato, "Capillary electrophoretic chiral determination of mirtazapine and its main metabolites in human urine after enzymatic hydrolysis," *Electrophoresis*, vol. 29, no. 18, pp. 3924–3932, 2008.

[20] M. Rockville, "The United States Pharmacopoeia 30, the National Formulary 25 US Pharmacopeial Convention," 2007.

[21] H. W. Darwish, A. S. Abdelhameed, A. H. Bakheit, and A. M. Alanazi, "A new method to determine the new C-Met inhibitor "Cabozantinib" in dosage form and human plasma via micelle-enhanced spectrofluorimetry," *RSC Advances*, vol. 5, no. 51, pp. 40484–40490, 2015.

[22] M. I. Walash, F. Belal, M. M. Tolba, and M. I. Halawa, "Micelle-enhanced spectrofluorimetric determination of amlexanox in bioadhesive buccal tablets: application to content uniformity testing," *Luminescence*, vol. 30, no. 6, pp. 823–829, 2015.

[23] J. B. Ghasemi and E. Zolfonoun, "Application of principal component analysis–multivariate adaptive regression splines for the simultaneous spectrofluorimetric determination of dialkyltins in micellar media," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 115, pp. 357–363, 2013.

[24] C. C. Wang, A. N. Masi, and L. Fernández, "On-line micellar-enhanced spectrofluorimetric determination of rhodamine dye in cosmetics," *Talanta*, vol. 75, no. 1, pp. 135–140, 2008.

[25] N. A. Alarfaj and M. F. El-Tohamy, "Determination of the anti-viral drug Ribavirin in dosage forms via micelle-enhanced spectrofluorimetric method," *Luminescence*, vol. 28, no. 2, pp. 190–194, 2013.

[26] H. W. Darwish and A. H. Bakheit, "Micellar enhanced synchronous spectrofluorimetric method for determination of dasatinib in tablets, human plasma and urine: application to in vitro drug release and content uniformity test," *RSC Advances*, vol. 5, no. 67, pp. 54471–54480, 2015.

[27] H. W. Darwish, A. H. Bakheit, A. S. Abdelhameed, and A. S. Alkhairallah, "Micellar enhanced spectrofluorimetric method for the determination of ponatinib in human plasma and urine via cremophor RH 40 as sensing agent," *International Journal of Analytical Chemistry*, vol. 2015, Article ID 210503, 9 pages, 2015.

[28] 2016, http://www.chemicalize.org/structure/#!mol=mirtazapine&source=fp.

[29] J. N. Miller and J. C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, Pearson Education, 2005.

[30] ICH, "Validation of analytical procedures," in *Methodology (Q2AR1), International Conference on Harmonization*, 1996, 2005.