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Research Article

Fluorometric Determination of Drugs Containing α-Methylene Sulfoxide Functional Groups Using N^1- Methylnicotinamide Chloride as a Fluorogenic Agent

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A simple fluorometric method, using N^1-methylnicotinamide chloride (NMNCl) as a fluorogenic reagent, has been developed, adapted, and validated for the quantitative estimation of drugs containing α-methylene sulfoxide functional groups. The proposed method has been applied successfully to the determination of sulindac (**1**), omeprazole (**2**), lansoprazole (**3**), pantoprazole (**4**), and rabeprazole (**5**) in the pure form, laboratory-prepared mixtures, pharmaceutical dosage forms, spiked human plasma samples, and in hospitalized patient's or volunteer's blood. For the standard solutions of **1**, **2**, **3**, **4**, and **5**, the method showed linearity over concentration ranging between 1–50 μg/mL, 50–1200 ng/mL, 100–1500 ng/mL, 10–1500 ng/mL, and 20–2200 ng/mL, respectively. For the spiked human plasma of **1**, **2**, **3**, **4**, and **5**, the linearity was shown over concentration ranging between 1–50 μg/mL, 75–1200 ng/mL, 100–1400 ng/mL, 10–1500 ng/mL, and 50–2100 ng/mL, respectively. The method showed good accuracy, specificity, and precision in both laboratory-prepared mixtures and spiked human plasma samples. The proposed method is simple, does not need sophisticated instrumentation, suitable for quality control application, bioavailability, and bioequivalency studies. Besides, the sensitivity and detection limits are comparable to sophisticated chromatographic methods.

1. Introduction

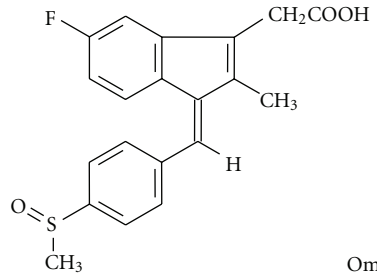
Nakamura described the application of a fluorometric method of the reaction of N^1-methylnicotinamide chloride (NMNCl) for the analysis of various compounds containing α-methylene carbonyl functional groups [1]. Nakamura also made qualitative tests on the reaction mechanism and identified the cyclized α-adduct fluorophore [1]. This reaction was not tested before for compounds containing α-methylene adjacent to other functional groups.

Encouraged by the successful application of the NMNCl methodology to the determination of similar to α-methylene carbonyl functional group-containing drugs, namely, warfarin [2], pentoxifylline, propafenone hydrochloride, and acebutolol hydrochloride [3] and the almost isosteric α-methylene sulfone/sulfonamide group, such as methyl sulfonyl methane (MSM), tinidazole, and rofecoxib [4], we began to adapt, validate, and extend the applicability of this methodology for the analysis of sterically hindered cyclic ketones such as ketamine hydrochloride, griseofulvin, and levonorgestrel (unpublished results).

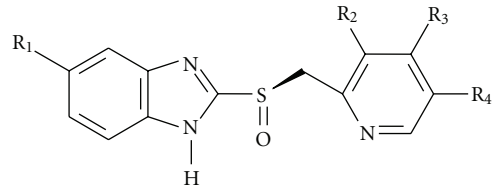
In this paper, we report the application of this methodology for determination of α-methylene sulfoxide functional group-containing drugs such as the nonsteroidal anti-inflammatory drug (NSAID) sulindac (**1**) and the proton pump inhibitors (PPIs) omeprazole (**2**), lansoprazole (**3**), pantoprazole (**4**), and rabeprazole (**5**).

Compound **1** is a nonsteroidal anti-inflammatory indene derivative [5]. Compounds **2**–**5** are proton pump inhibitors (PPIs) [6].

Several methods have been reported for the analysis of **1** that may include UV spectrophotometry [7], reversed-phase HPLC as a stability-indicating assay [8], other HPLC methods [9, 10], enzyme immunoassay [11], and capillary electrophoresis [12, 13].



Sulindac (**1**)



	R_1	R_2	R_3	R_4
Omeprazole (**2**)	OCH_3	CH_3	OCH_3	CH_3
Lansoprazole (**3**)	OCH_3	CH_3	OCH_2CH_3	H
Pantoprazole (**4**)	OCH_2F	OCH_3	OCH_3	H
Rabeprazole (**5**)	H	CH_3	$O(CH_2)_3OCH_3$	H

Figure 1: Chemical structures of **1–5**.

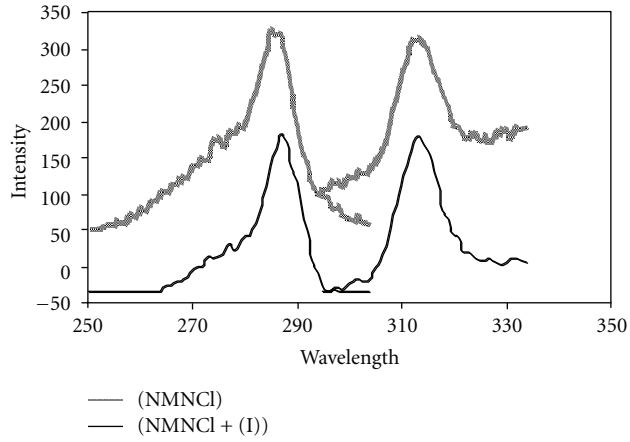


Figure 2: Excitation and emission spectra of the reaction product of **1** with NMNCl.

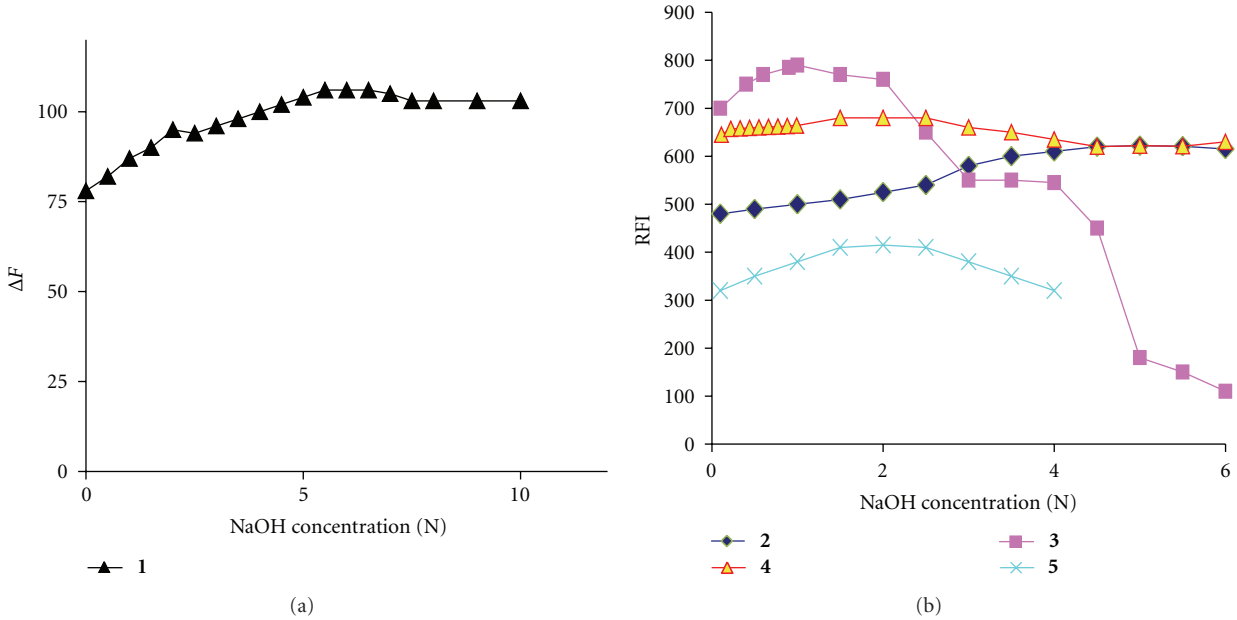


Figure 3: Effect of NaOH concentration (N) on fluorescence intensity of the reaction products of **1–5** with NMNCl. The variation of NaOH concentration is made at constant volume.

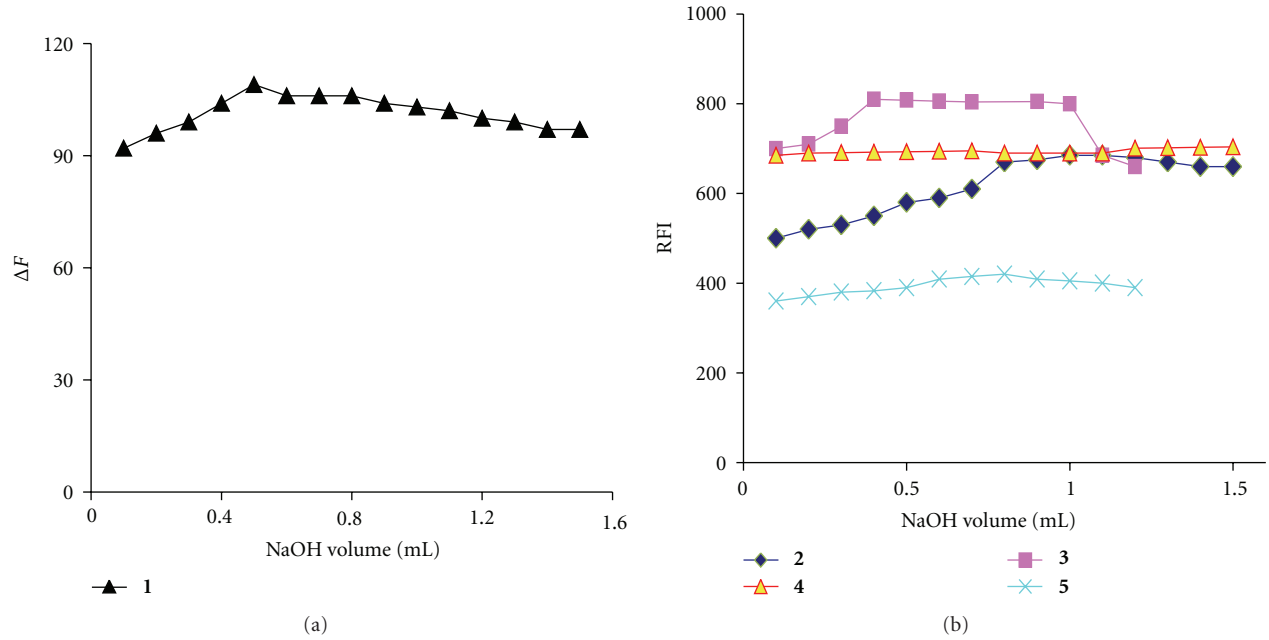


Figure 4: Effect of NaOH volume (mL) on fluorescence intensity of reaction product of **1–5** with NMNCl. The variation of NaOH volume is made at constant concentration.

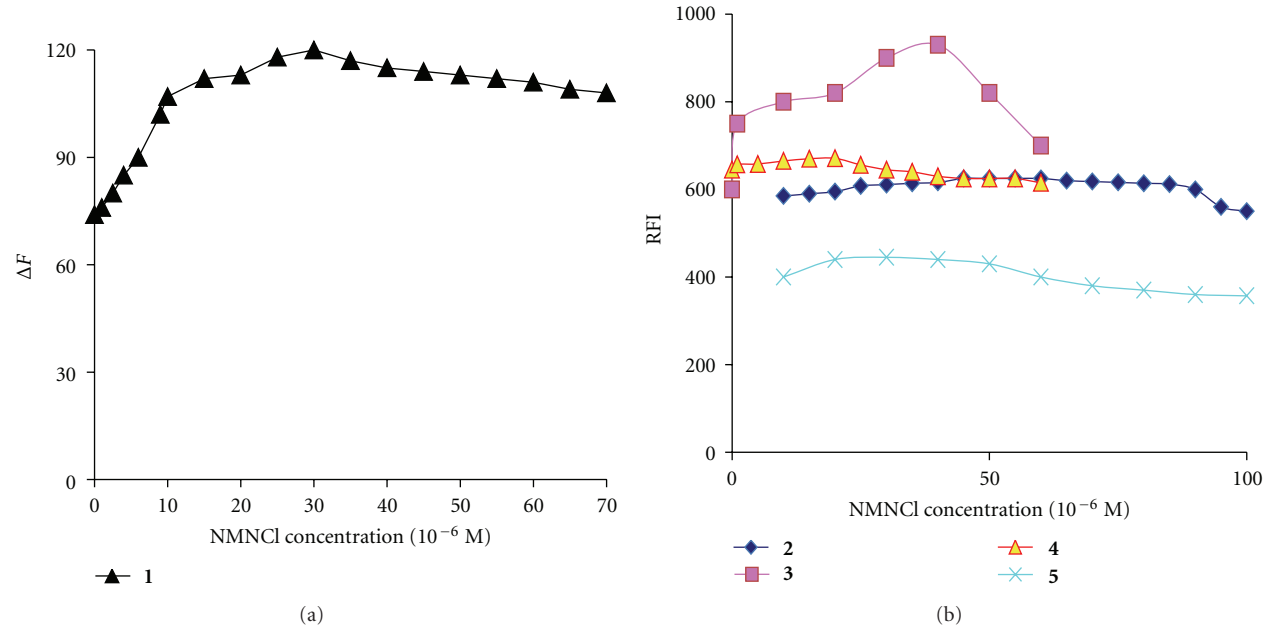


Figure 5: Effect of NMNCl concentration (mM) on fluorescence intensity of reaction product of **1–5** with NMNCl. The variation of NMNCl concentration is made at constant volume.

Several methods have been developed for determination, chiral separation, stability, or pharmacokinetic studies of **2** in bulk form, pharmaceuticals or biological samples, and these methods may include different spectrophotometric methods via formation of metal chelates [14], UV-derivative spectrophotometry [15] and HPLC methods for its determination [16], separation of enantiomers [17], for stability studies [18], or for the study of its pharmacokinetic profile [19].

Several methods have been reported for the analysis of **3** that may include UV/visible spectrophotometry [20] and HPLC for its determination [21], enantiomeric separation [22], or pharmacokinetic study [23].

Several methods have been reported for the analysis of **4** that may include kinetic spectrophotometry using 1-fluoro-2,4-dinitrobenzene [24], UV spectrophotometry [20], and different HPLC methods for its determination [25] or for its metabolites [26].

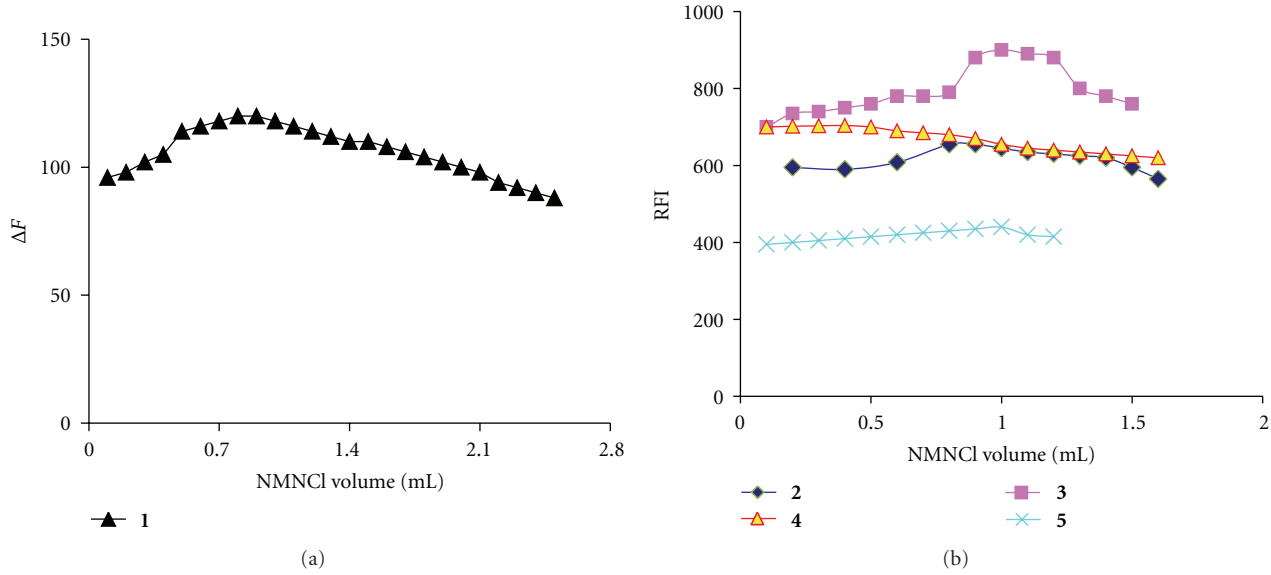


Figure 6: Effect of NMNCl volume (mL) on fluorescence intensity of reaction product of **1–5** with NMNCl. The variation of NMNCl volume is made at constant concentration.

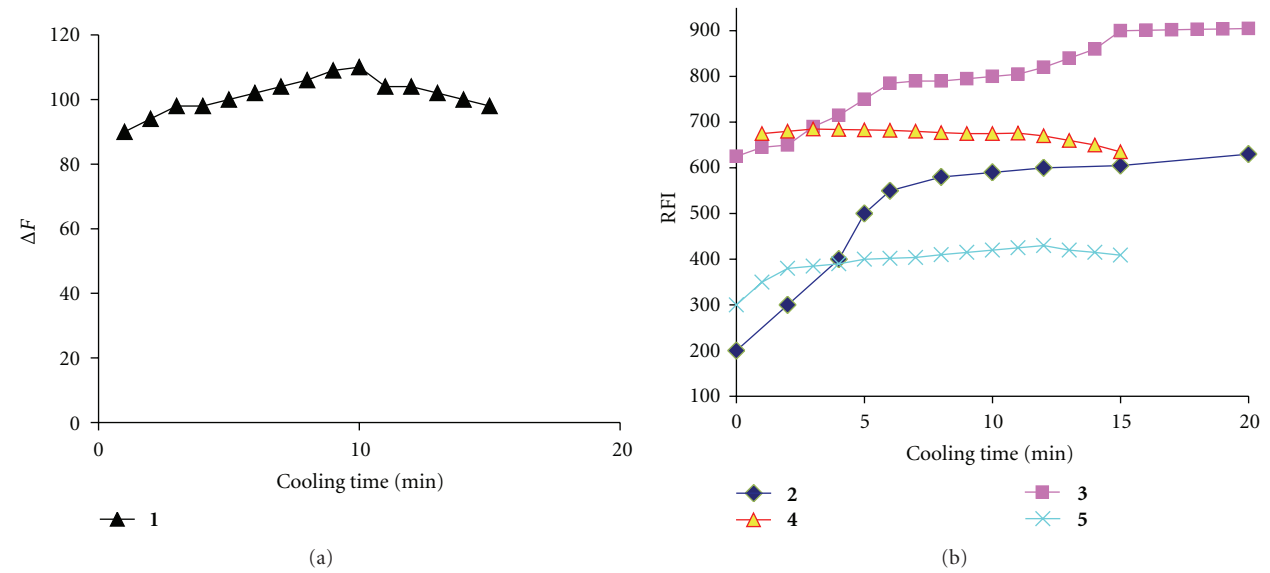


Figure 7: Effect of cooling time on fluorescence intensity of reaction product of **1–5** with NMNCl.

Several methods have been reported for the analysis of **5** that may include HPLC [27] and HPLC with NMR detection [28].

Recently published methods described the simultaneous estimation of **2–5** are also reported [29, 30].

The objective of this work is to develop, adapt, and validate a simple fluorometric method that can be applied in the determination of α-methylene sulfoxide group-containing drugs, namely, drugs **1–5** in pure form, dosage forms, spiked human plasma samples and in patient's blood. The proposed method has minimal instrumentation and chemical requirements; nevertheless, its sensitivity and specificity are comparable to other elaborated chromatographic techniques. In addition, it is a versatile method that can find applications on a wide range of pharmaceutical preparations and biological fluids.

2. Experimental

2.1. Apparatus. Shimadzu RF 5301 PC spectrofluorometer.

2.2. Materials

2.2.1. Authentic Drugs. Working standards of **1**, **2**, **3**, **4**, and **5** were supplied by Sigma Pharmaceutical Industries, Egypt,

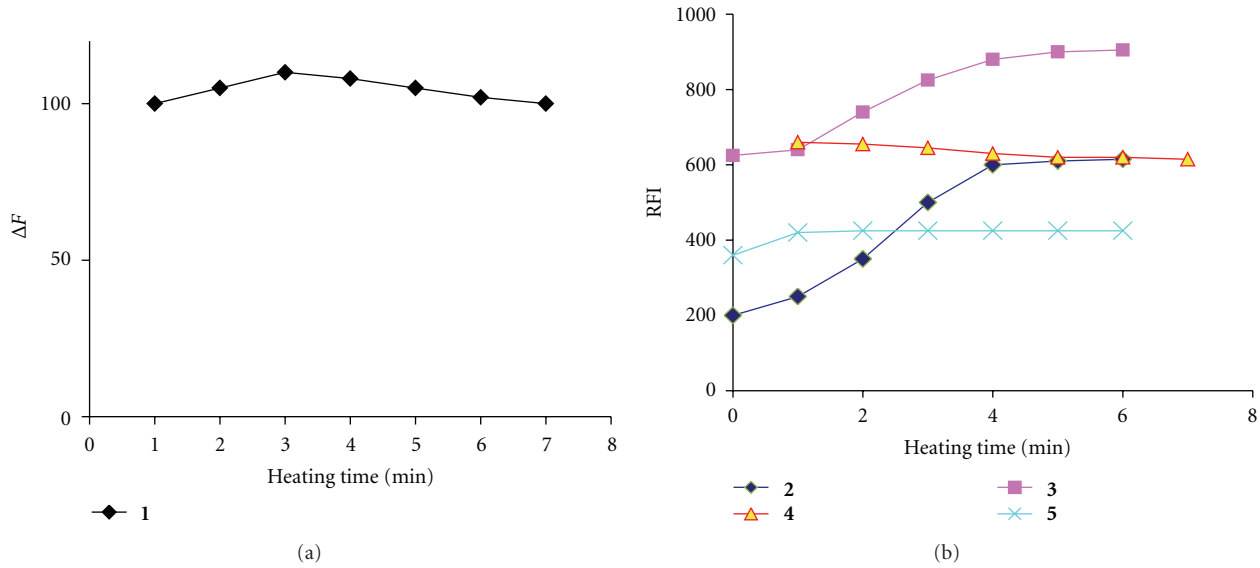


Figure 8: Effect of heating time on fluorescence intensity of reaction product of **1–5** with NMNCl.

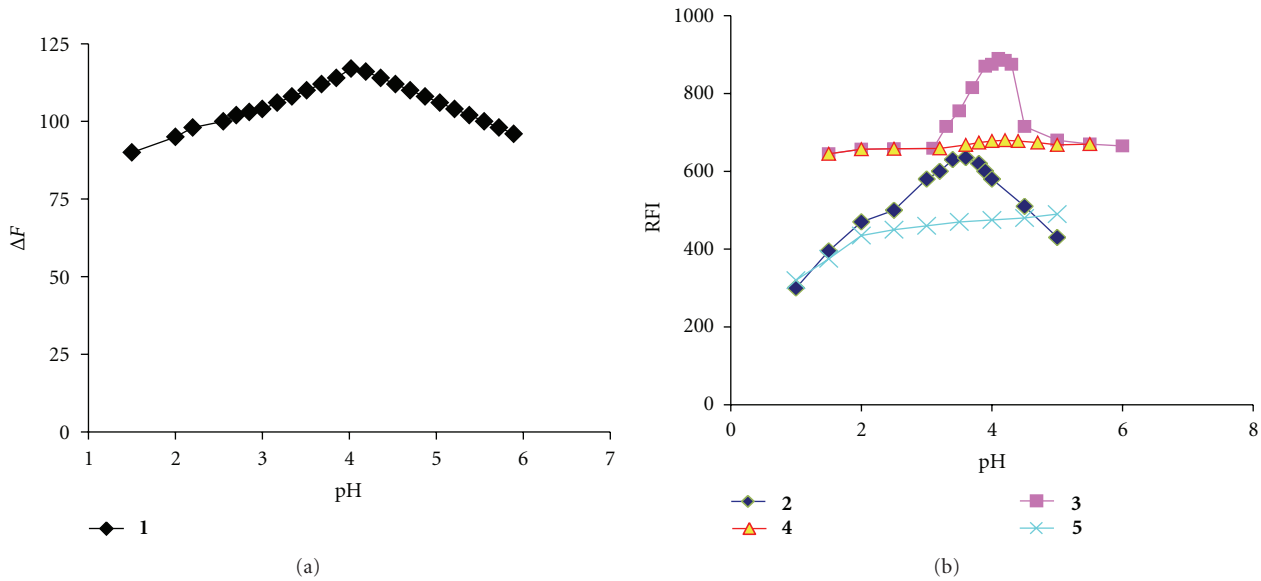


Figure 9: Effect of pH on fluorescence intensity of each reaction product of **1–5** with NMNCl.

Amyria Pharmaceutical Industries, Alexandria, Egypt, T_3A Pharma, Egypt, Medical Union Pharmaceuticals (MUP), Egypt, and Global Napi Pharmaceuticals (GNP), Egypt, respectively.

Plasma samples were purchased from the Central Blood Bank of Tanta University Hospital.

2.2.2. Other Chemicals. N^1-Methylnicotinamide chloride was purchased from Sigma Chemicals Co. Formic acid, sodium hydroxide, methanol, and all other chemicals were of analytical grade. Water used was doubly distilled.

2.3. Dosage Forms

2.3.1. Sulindac (1). Rudac tablet (Sigma Pharmaceutical Industries) was labeled to contain 150 and 200 mg, HiDac tablet (Hipharm for Manufactured Pharmaceuticals) labeled to contain 200 mg.

2.3.2. Omeprazole (2). Gastrazole capsules, 20 mg (Amyria Pharmaceutical Industries), Epirazole capsules, 20 mg (Egyptian International Pharmaceutical Industries Co. (EIPICO)), Omepak capsules, 10 and 20 mg (Sedico Pharmaceutical Co.), Pepzole capsules, 40 mg (Alkan Pharma),

Gasec capsules, 20 mg (Mepha), Omez capsules, 10 mg (Pharaonia Pharmaceuticals PharoPharma), Gastrocure capsules, 20 mg (October Pharma), Napizole capsules, 20 mg (Global Napi Pharmaceuticals (GNP)), Ulstop capsules, 20 mg (Pharco Pharmaceuticals), Risek capsules, 20 mg (Julphar), Gastroloc capsules, 40 mg (Sigma), Omepral capsules, 20 mg (Memphis Co. for Pharmaceuticals and Chemical Industry), Trio capsules, 20 mg (Alkan Pharma), and Nexium tablets, 20 mg esomeprazole (Astra Pharmaceuticals).

*2.3.3. Lansoprazole (**3**).* Lansoprazole capsules, 15 mg (Rexcel), Zollipak capsules, 30 mg (Sedico), Peptazole capsules, 30 mg (Saudi Pharmaceutical Industries and Medical Appliances Corporation (Spimaco), Lanzor capsules, 15 and 30 mg (Aventis Pharma), and Lopral capsules, 30 mg (T_3A Pharma).

*2.3.4. Pantoprazole (**4**).* Controloc tablets, 40 mg (Byk Gulden Lomberg Chemische Fabrik GmbH), Pantoloc tablets, 20 and 40 mg (Medical Union Pharmaceuticals (MUP)), and Pantazole tablets, 40 mg (Sigma Pharmaceutical Industries).

*2.3.5. Rabeprazole (**5**).* Bepra tablets, 20 mg (GNP) and Pariet tablets, 20 mg (Janssen Cilag).

All these preparations were purchased from the local market of Egypt except for Nexium that was purchased from Saudi Arabia.

2.4. Reagents and Standard Solutions

2.4.1. Stock Standard Solutions of Drugs. Stock standard solutions were prepared in 0.1 N sodium hydroxide for **1**, **2**, and **4**; 50% aqueous methanol for **3** and methanol for **5** to contain 100 mg/mL for **1**, 150 μg/mL for **3**, **4**, and **5**, and 250 μg/mL for **5**.

2.4.2. Serial Standard Solutions of Drugs. Aliquots of the stock solutions were diluted quantitatively with the same solvent to obtain concentration ranging between 10–500 μg/mL for **1**, 0.1–15 μg/mL for **2**, **3**, and **4**, and 0.1–25 μg/mL for **5**.

2.4.3. Assay Solutions of Drugs in Synthetic Mixtures. Several synthetic mixtures were prepared for **1**, **4**, and **5** to contain different proportions of the possible interfering substances that may be present with the drug in its dosage form. Three synthetic mixtures containing **1** were prepared. The first mixture contained 200 mg **1**, 230 mg cellulose, 10 mg magnesium stearate, and 60 mg starch. The second mixture contained 200 mg **1**, 100 mg lactose, 60 mg starch, 60 mg gelatin, 8 mg magnesium stearate, and 72 mg talc. The third mixture contained 130 mg avicel instead of lactose and gelatin.

Three synthetic mixtures containing **4** along with various additives and inactive ingredients were prepared. The first mixture contained 20 mg **4**, 280 mg avicel, 60 mg maize starch, 10 mg magnesium stearate, 10 mg magnesium carbonate, 1 mg quinolone, 1 mg acidisol, 60 mg talc, 1 mg colloidal silicon, 6 mg polyethylene glycol 6000, 50 mg Eudragit L_{100-55}, and 1 mg titanium dioxide. The second mixture contained 20 mg **4**, 280 mg lactose, 60 mg starch, 60 mg gelatin, 8 mg magnesium stearate, and 72 mg talc. The third mixture contained 340 mg avicel instead of lactose and gelatin.

Three synthetic mixtures containing **5** were prepared. The first mixture contained 20 mg **5**, 300 mg croscarmellose sodium, 72 mg talc, 1 mg sodium hydroxide, 1 mg titanium dioxide, and 8 mg magnesium stearate. The second mixture contained 20 mg **5**, 180 mg lactose, 60 mg starch, 60 mg gelatin, 8 mg magnesium stearate, and 72 mg talc. The third mixture contained 240 mg avicel instead of lactose and gelatin.

Each synthetic mixture of **1** and **4** was extracted with 100 mL 0.1 N sodium hydroxide. The synthetic mixture of **5** was extracted with 100 mL methanol. The extracts were filtered, and the first 10 mL of the filtrate were rejected. Aliquots of the filtrate were diluted with the same solvent to obtain serial solutions in the concentration ranges 10–500 μg/mL, 0.1–15 μg/mL, and 0.1–25 μg/mL for **1**, **4**, and **5**, respectively.

2.4.4. Assay Solutions of Drugs in Their Pharmaceutical Preparations. A quantity of the mixed contents of 20 capsules or tablets equivalent to one capsule or tablet of **1–5** were finely powdered and transferred with the aid of several portions of 0.1 N sodium hydroxide solution (for **1**, **2**, and **4**), 50% aqueous methanol (for **3**), or methanol (for **5**) to a 100 mL volumetric flask, and the volume was completed with the same solvent. The resulting solution was filtered and the first 10 mL of the filtrate was rejected. Aliquots of the filtrate were diluted with the same solvents to obtain 200 μg/mL, 6 μg/mL, 3 μg/mL, 4 μg/mL, and 6 μg/mL solutions of **1–5**, respectively.

2.4.5. Assay Solutions of Drugs in Spiked Human Plasma Samples

Serial Standard Solutions of the Drugs. Serial standard solutions for **1**, **2**, **3**, and **4** were prepared in 0.1 N sodium hydroxide solution in concentrations ranging between 1–50 mg/mL for **1** and 50–1500 μg/mL for **2**, **3**, and **4**. Serial standard solutions of **5** were prepared in methanol in concentrations ranges of 1–2500 μg/mL.

Preparation of Spiked Human Plasma Samples. Two hundred μL of each **1**, **2**, **3**, and **4** serial standard solution were diluted with 1800 μL human plasma and vortex mixed to obtain concentrations ranging between 0.1–5 mg/mL for **1** and 5–150 μg/mL for **2**, **3**, and **4**. For **5** 200 μL of each serial standard solution were evaporated, the residue was dissolved in 1800 μL human plasma and vortex mixed, and 200 μL distilled water were added and vortex mixed to obtain 1–250 μg/mL.

Table 1: Optimum conditions for the fluorometric procedure.

Drug	pH	NaOH conc. (N)	NaOH volume (mL)	NMNCl conc. (mM)	NMNCl volume (mL)	Cooling time (min)	Heating time (min)	λ_{ex} (nm)	λ_{em} (nm)
1	4.0	6.0	0.7	3×10^{-2}	0.9	10	3	285	315
2	3.4	5.0	1.0	5×10^{-2}	1.0	20	5	284	315
3	4.1	1.0	0.4	4×10^{-2}	1.0	15	5	279	306
4	3.9	2.0	0.6	2×10^{-2}	0.8	10	4	285	315
5	4.0	2.0	0.8	3×10^{-2}	1.0	12	4	280	307

Table 2: Regression analysis parameters for the determination of **1–5** in standard solutions using the proposed method.

Drug	Linearity range	Slope*		Intercept*		R^2
		Mean	SE	Mean	SE	
1	1–50 μg/mL	4.078	0.02	42.75	0.39	0.998
2	50–1200 ng/mL	0.6694	0.0005	15.226	1.376	0.9997
3	100–1500 ng/mL	0.5104	0.01	99.826	3.376	0.9983
4	10–1500 ng/mL	0.6215	0.055	73.274	0.298	0.999
5	20–2200 ng/mL	0.436	0.02	13.31	1.55	0.9998

Average of triplicate analyses, 15 calibration data points.
* Three calibrations curves were performed for each drug.

Preparation of Assay Solutions of Drugs in Plasma Samples. Two hundred μL of each spiked human plasma samples were mixed with 1800 μL methanol and centrifuged for 15 minutes to separate the precipitated protein. The clear supernatant was filtered through Millipore filter (0.45 μm) to obtain solutions in concentration ranging between 10–500 μg/mL for **1**, 0.5–15 μg/mL for **2**, **3**, and **4**, and 0.1–25 μg/mL for **5**.

*Determination of **1–5** in Volunteer's Blood or Hospitalized Patient's Blood.* A blood sample was withdrawn in a test tube to which heparin was previously added and dried. The sample was centrifuged to separate plasma and then treated as previously mentioned under preparation of assay solutions of each drug in plasma samples.

2.4.6. N^1-Methylnicotinamide Chloride Reagent (NMNCl). One mM solution of NMNCl reagent was prepared by dissolving 1.7262 g NMNCl in one liter of distilled water. Aliquot of this solution was diluted with distilled water to obtain 5×10^{-2}, 4×10^{-2}, 2×10^{-2}, and 3×10^{-2} mM solutions.

2.4.7. Sodium Hydroxide Reagent. Sodium hydroxide solutions were prepared in distilled water to have concentration of 0.1 N, 1 N, 2 N, 5 N, and 6 N solutions.

2.5. General Fluorometric Procedure. One milliliter of each drug standard solution, assay solution of synthetic mixture, assay solution of pharmaceutical preparation, assay solution of plasma sample, or the assay solution of the volunteer's plasma was transferred to 10 mL screw capped test tube. Solutions of sodium hydroxide and NMNCl were added. The mixture was cooled in ice for the indicated time then the pH adjusted using formic acid and heated for the indicated time and then was cooled in ice for 5 minutes (optimum NaOH concentration and volume, volume and concentration of added NMNCl, reaction pH values and cooling, and heating times are shown in Table 1). The mixture was transferred to 10 mL volumetric flask and the resulting solution was completed using distilled water. The intensity of the resulting fluorescence was measured at the optimal wavelengths indicated in Table 1.

The fluorometric measurements were performed against reagent blank experiments. Concentrations of the drugs were calculated from the corresponding calibration graphs prepared simultaneously.

3. Results and Discussion

The reaction of NMNCl, with drugs containing active methylene sulfoxide groups, was not previously studied and this is the first application of this reaction to compounds containing this functional group. When **1–5** (for chemical structures, cf. Figure 1 and for plausible chemical pathway, cf. [4]) were allowed to react with NMNCl under the optimal conditions specified for each, strong fluorescent products were produced except for **1** that was found to have a quenching effect on the fluorescence of the reagent blank used at excitation and emission wavelengths of 285 nm and 315 nm, respectively; compare Figure 2. The optimal wavelengths of excitation and emission of the reaction product were determined using synchronous wavelength search compare Table 1.

Different variables affecting the reaction between the estimated drug and NMNCl, including sodium hydroxide concentration and volume, added NMNCl concentration and volume, cooling time, heating time, and pH variations, were studied to optimize the reaction conditions to give

Table 3: Regression analysis parameters for the determination of **1–5** in spiked human plasma samples using the proposed method.

Drug	Linearity range	Slope*		Intercept*		R^2
		Mean	SE	Mean	SE	
1	1–50 μg/mL	4.0771	0.023	42.674	0.78	0.994
2	75–1200 ng/mL	0.6673	0.006	16.3935	2.168	0.9949
3	100–1400 ng/mL	0.5084	0.0035	100.731	0.019	0.9971
4	10–1500 ng/mL	0.6283	0.12	67.419	0.39	0.9989
5	50–2100 ng/mL	0.466	0.01	10.3	1.25	0.999

Average of triplicate analyses, 15 calibration data points.
*Three calibrations curves were performed for each drug.

Table 4: Recovery data of **1–5** when assayed in standard solutions using the proposed method.

Drug	Claimed drug concentration	Recovered concentration*	% recovery	Mean % recovery ± S.D.	CV
1 μg/mL	1	0.99	99%	99.4 ± 0.44	0.45%
	3	3.01	100.3%		
	12	12.02	100.16%		
	15	14.99	99.9%		
	20	20.1	100.5%		
	30	30	100%		
	40	39.99	99.9%		
	50	49.89	99.8%		
2 ng/mL	50	50.1	100.2%	100.2 ± 0.75	0.755%
	75	74.4	99.2%		
	200	203	101.5%		
	500	500	100%		
	1000	1004	100.4%		
	1200	1199	99.9%		
3 ng/mL	100	98	98%	99.625 ± 1.16	1.166%
	300	301	100.3%		
	1200	1195	99.6%		
	1500	1509	100.6%		
4 ng/mL	100	99.5	99.5%	99.94 ± 0.46	0.455%
	300	301	100.3%		
	400	403	100.75%		
	600	597	99.5%		
	800	799	99.87%		
	1000	1002	100.2%		
	1200	1198	99.83%		
	1500	1493	99.53%		
5 ng/mL	20	20.2	101%	100.5 ± 0.92	0.92%
	200	202	101%		
	600	606	101%		
	800	795	99.4%		
	1000	990	99%		
	1500	1510	100.6%		
	2200	2185	99.3%		

* Average of triplicate analyses for each concentration.

maximum fluorescence intensity; compare Figures 3, 4, 5, 6, 7, 8, and 9.

After optimization of the different reaction parameters, linear relationships between fluorescence intensity and each drug concentration were obtained over the following concentration ranging between 50–1200 ng/mL, 100–1500 ng/mL, 100–1500 ng/mL, and 20–2200 ng/mL in the standard solutions of **2**, **3**, **4**, and **5**, respectively, and 75–1200 ng/mL, 100–1500 ng/mL, 100–1400 ng/mL and, 50–2100 ng/mL in plasma samples for **2**, **3**, **4**, and **5**, respectively. The linearity range was 1–50 μg/mL in both standard solutions and spiked human plasma samples for the observed fluorescence quenching produced by **1**.

These results reveal a good and dynamic linearity ranges of the proposed method with different drugs. The good linearity of these relations was indicated by the corresponding

Table 5: Recovery data of **1–5** when assayed in spiked human plasma samples using the proposed method.

Drug	Claimed drug concentration	Recovered concentration*	% recovery	Mean % recovery ± S.D.	CV
1 μg/mL	1	1.01	101%	99.98 ± 1.04	1.05%
	3	2.9	97.5%		
	12	12.02	100.15%		
	15	15.1	100.6%		
	20	19.89	99.45%		
	30	30.15	100.5%		
	40	40.09	100.3%		
	50	49.9	99.8%		
2 ng/mL	75	73.5	98%	104±3.21	3.20%
	200	210	105%		
	500	500	100%		
	1000	9700	97%		
	1200	1224	102%		
3 ng/mL	100	103	103%	100.26 ± 1.71	1.72%
	300	295	98.3%		
	1200	1204	100.3%		
	1500	1498	99.8%		
4 ng/mL	100	101	101%	100.11 ± 0.61	0.62%
	300	302	100.67%		
	400	399	99.75%		
	600	603	100.5%		
	800	795	99.375%		
	1000	995	99.5%		
	1200	1204	100.33%		
	1400	1392	99.4%		
5 ng/mL	50	51	102%	100.42±43	1.43%
	200	196	98%		
	600	602	100.3%		
	800	810	101.25%		
	1000	1012	101.2%		
	1500	1485	99%		
	2100	2125	101.2%		

* Average of triplicate analyses for each concentration.

regression equations shown in Tables 2 and 3, for standard solutions and spiked human plasma samples, respectively.

3.1. Detection Limit (DL). Detection limits were practically determined according to the ICH topic Q2B (R1) [31] and found to be 0.5 μg/mL, 10 ng/mL, 20 ng/mL, 20 ng/mL, and 1 ng/mL in standard solutions for **1**, **2**, **3**, **4**, and **5**, respectively, and 0.5 μg/mL, 25 ng/mL, 30 ng/mL, 50 ng/mL, and 10 ng/mL in plasma samples for **1**, **2**, **3**, **4**, and **5**, respectively.

3.2. Quantitation Limit (QL). Quantitation limits were practically determined according to the ICH topic Q2B (R1) [31] and found to be 1 μg/mL, 50 ng/mL, 100 ng/mL, 100 ng/mL, and 20 ng/mL in standard solutions for **1**, **2**, **3**, **4**, and **5**, respectively, and 1 μg/mL, 75 ng/mL, 100 ng/mL, 100 ng/mL and 50 ng/mL in plasma samples for **1**, **2**, **3**, **4** and **5**, respectively. These results show the high sensitivity of the proposed method.

3.3. Accuracy. The accuracy of the proposed method was studied according to the ICH topic Q2B (R1) [31], by preparing standard solutions and spiked human plasma samples containing various concentrations, lying within the linearity range of each drug, and analyzing them using the proposed method. The results, expressed as % recovery ± S.D., are shown in Tables 4 and 5 for standard solutions and spiked human plasma samples, respectively.

3.4. Precision. The precision of the method was judged by performing intraday and interday triplicate analyses of different concentrations covering the linearity range of each drug in both standard solutions and spiked human plasma samples. The results are reported as S.D. and C.V. in Tables 6 and 7 for standard solutions and spiked human plasma samples, respectively.

3.5. Specificity. To study the method specificity, three synthetic mixtures of **1**, **4**, and **5** were prepared to contain

Table 6: Intraday and interday precision data of 1–5 in standard solutions using the proposed method.

Drug	Claimed conc.	Intraday			Interday		
		Found conc.*	S.D.	C.V.	Found conc.*	S.D.	C.V.
1 μg/mL	1	0.995	0.035	0.354%	0.99	0.007	0.71%
	3	3.01	0.007	0.235%	2.98	0.014	0.473%
	12	11.99	0.007	0.058%	12.02	0.014	0.118%
	15	15.01	0.007	0.047%	15.025	0.018	0.118%
	20	19.98	0.014	0.071%	20.01	0.007	0.035%
	30	30.015	0.011	0.035%	29.99	0.007	0.024%
	40	39.95	0.035	0.088%	39.98	0.014	0.036%
	50	49.715	0.202	0.404%	50.01	0.007	0.014%
2 ng/mL	50	50.1	0.07	0.14%	49.8	0. 14	0.28%
	320	322	1.4	0.44%	320	0.0	0.0%
	600	600.1	0.07	0.012%	602	0.14	0.24%
	1150	1151	0.71	0.615%	1145	3.535	0.308%
	1200	1198	1.41	0.1179%	1203	2.121	0.176%
3 ng/mL	100	101	0.71	0.703%	99	0.71	1.4%
	300	299	0.71	0.24%	298	1.41	0.47%
	1200	1202	1.141	0.118%	1202	1.141	0.118%
	1300	1298	1.41	0.11%	1303	2.121	0.163%
	1500	1499	0.71	0.47%	1504	2.83	0.188%
4 ng/mL	100	101	0.707	0.704%	99	0.707	0.71%
	300	302	1.414	0.469%	303	2.12	0.703%
	400	399	0.707	0.177%	402	1.414	0.353%
	600	598	1.414	0.236%	597	2.12	0.354%
	800	798	1.414	0.176%	799	0.71	0.088%
	1000	1004	2.828	0.282%	1005	3.54	0.353%
	1200	1195	3.53	0.295%	1201	0.71	0.059%
	1500	1497	2.12	0.142%	1504	2.83	0.188%
5 ng/mL	20	20.2	0.14	0.7%	19.7	0.21	1.07%
	200	201	0.71	0.35%	202	1.41	0.71%
	600	605	3.5	0.6%	596	2.83	0.47%
	800	791	6.36	0.8%	806	4.3	0.53%
	1000	990	7.1	0.71%	1012	8.5	0.84%
	1500	1490	7.1	0.47	1518	12.8	0.84%
	2000	1985	10.6	0.53%	2009	6.4	0.32%
	2200	2220	14.14	0.64%	2190	7.1	0.15%

* Average of triplicate analyses for each concentration.

the possible interfering substances used during pharmaceutical formulations. These mixtures were analyzed using the proposed method and the results, expressed as % recovery ± S.D. No synthetic mixtures were prepared for **2** or **3** because these drugs are supplied as capsules containing the active ingredient without additives. The prepared mixtures were determined by the proposed method and the results, expressed as % recovery ± S.D., were found to be 99.58% ± 3.47, 99.3% ± 0.9, and 100.5% ± 0.67, for **1**, **4**, and **5**, respectively.

3.6. Assay of Pharmaceutical Preparations. All the pharmaceutical preparations available in the local market for each drug were analyzed using the proposed method. The results, expressed as % recovery ± S.D., are illustrated in Table 8.

*3.7. Determination of **1–5** in Hospitalized Patient's or Volunteer's Blood.* The success in the application of the highly sensitive proposed procedure for the determination of **1–5** in spiked human plasma samples with good accuracy and precision encouraged the investigators to study its application for monitoring the drug level in the blood of a volunteer or a hospitalized patient under **1–5** therapy.

The level of **2** was monitored in the blood of a hospitalized patient receiving it with other medications as paracetamol, bromohexine HCl, acephylline piperazine,

Table 7: Intraday and interday precision data of **1–5** in plasma samples using the proposed method.

Drug	Claimed conc.	Intraday			Interday		
		Found conc.*	S.D.	C.V.	Found conc.*	S.D.	C.V.
1 μg/mL	1	0.96	0.28	2.88%	0.96	0.028	2.88%
	2	1.98	0.014	0.71%	2.05	0.035	1.76%
	10	10.1	0.071	0.704%	9.95	0.035	0.35%
	13	13.15	0.106	0.811%	12.8	0.141	1.09%
	15	15.2	0.141	0.936%	14.8	0.141	0.949%
	20	19.9	0.071	0.354%	20.2	0.141	0.704%
	30	29.8	0.141	0.473%	30.2	0.141	0.47%
	40	40.2	0.141	0.353%	39.75	0.177	0.44%
	50	50.1	0.071	0.141%	49.7	0.212	0.43%
2 ng/mL	1200	1219	14	1.11%	1200	0.00	0.0%
	1000	988	9	0.86%	1020	1.4	1.4%
	500	49	7	1.43%	501	1	1.2%
	200	190	7	3.6%	206	4	2.1%
	75	74	1	0.95%	72.5	2	2.4%
3 ng/mL	100	102	1.414	1.4%	101	0.71	0.704%
	300	305	3.536	1.169%	298	1.141	0.47%
	1200	1195	3.536	0.245%	1206	4.25	0.353%
	1300	1301	0.71	0.0544%	1304	2.83	0.217%
	1500	1502	1.414	0.094%	1501	0.71	0.047%
4 ng/mL	100	100.5	0.354	0.353%	101	0.707	0.704%
	300	302	1.1414	0.469%	299	0.707	0.236%
	400	398	1.1414	0.354%	403	2.12	0.528%
	600	597	2.12	0.354%	601	0.707	0.118%
	800	804	2.828	0.353%	794	4.23	0.532%
	1000	1005	3.536	0.353%	995	3.536	0.354%
	1200	1194	4.24	0.354%	1205	3.536	0.294%
	1300	1304	2.828	2.17%	1304	2.828	2.17%
	1400	1408	5.65	0.402%	1396	2.83	0.202%
5 ng/mL	50	49	0.71	1.43%	50.2	0.14	0.28%
	200	199	0.71	0.35%	203	2.12	1.1%
	600	608	5.66	0.93%	595	3.53	0.6%
	800	810	7.1	0.9%	790	7.1	0.88%
	1000	1015	10.6	1.1%	990	7.1	0.71%
	1500	1520	14.1	0.94%	1515	10.6	0.71%
	1800	1785	10.6	0.6%	1781	13.4	0.75%
	2100	2115	10.6	0.5%	2095	3.5	0.16%

* Average of triplicate analyses for each concentration.

hyosine *N*-butylbromide, and captopril. The concentration of **2** in the patient's blood was found to be 0.31 μg/mL.

The level of each of **1**, **3**, **4**, and **5** was monitored in the blood of volunteers. The concentrations of **1**, **3**, **4**, and **5** in volunteers' blood were found to be 5.2 μg/mL, 1.1 μg/mL, 3.6 μg/mL, and 0.3 μg/mL, respectively.

4. Conclusion

The proposed method makes use of the high sensitivity and specificity of the fluorometric analysis to reach low limits of detection and quantitation for all the studied drugs in standard solutions, synthetic mixtures, pharmaceutical preparations, spiked human plasma samples, and patient's or volunteer's blood. The method is simple; it gives results comparable to those obtained by other techniques that require elaborate instrumentations and time-consuming sample preparation procedure.

The method showed good accuracy and precision suitable for quality assurance and could be recommended for bioequivalency and bioavailability studies as well as for validation of cleaning methodology prior to line clearance.

Table 8: Results of the proposed method recovery experiments of **1–5** of different pharmaceutical preparations.

Drug	Pharmaceutical preparations	% recovery ± S.D.*
1	*Rudac* 150 mg tablet	102±0.56%
	Rudac 200 mg tablet	101±2.1%
	HiDac 200 mg tablet	98.5 ± 1.55%
2	*Epirazole* 20 mg capsule	100.1 ± 1.37%
	Gastrozole 20 mg capsule	100±0.95%
	Gastrocure 20 mg capsule	99±1.22%
	Napizole 20 mg capsule	98.45 ± 0.88%
	Omepak 20 mg capsule	99.98 ± 1.56%
	Omepak 10 mg capsule	100±1.11%
	Ulstop 20 mg capsule	97.9 ± 0.99%
	Gasec 20 mg capsule	100.2 ± 1.52%
	Risek 20 mg capsule	98±1.63%
	Pepzole 40 mg capsule	97.88 ± 0.54%
	Gastroloc 40 mg capsule	97.2 ± 0.55%
	*Omepra*l 20 mg capsule	99±1.42%
	Omez 10 mg capsule	99.9 ± 0.78%
	Trio 20 mg capsule	98.1 ± 1.74%
	Nexium 20 mg (esomeprazole magnesium tablet)**	96.12 ± 0.56%
3	*Lansoprazole* 30 mg cap	100.6 ± 2.31%
	Zollipak 30 mg cap	101±1.51%
	Peptazole 30 mg cap	99±2.1%
	Lanzor 15 mg cap	100.1 ± 1.2%
	Lanzor 30 mg cap	99.8 ± 0.95%
	Lopral 30 mg cap	99±0.89%
4	*Controloc* 20 mg tablet	98±1.98%
	Pantoloc 20 mg tablet	97±0.51%
	Pantoloc 40 mg tablet	101±1.12%
	Pantazole 40 mg tablet	101.5 ± 0.52%
5	*Bepra* 20 mg tablet	102±1.35%
	Pariet 20 mg tablet	100±2.1%

* Average of triplicate analyses.
** The (*S*)-enantiomer of omeprazole; the pharmacologically active form.

The proposed method application could be extended to cover all available pharmaceutical preparations for each of the chosen drugs.

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