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Sensitive spectrofluorimetric determination of metoclopramide, itopride, mosapride, and trimebutine by Fluorescence Quenching Reaction with Eosin Y in Pharmaceutical Formulations and Spiked Human Plasma

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ABSTRACT

In order to determine the levels of metoclopramide hydrochloride (MTC), itopride hydrochloride (ITP), mosapride citrate (MR), and trimebutine maleate (TRI) in their pharmaceutical formulations and spiked human plasma, a new straightforward, accurate, highly sensitive, non-extractable, economical, and quick spectrofluorimetric method was developed. The technique was based on the reaction between the drug's tertiary amino group and eosin Y (EY), which causes quantitative fluorescence quenching of EY, which was detected at 542 nm after excitation at 520 nm. For MTC, ITP, and MS, the quenching in fluorescence intensity was directly proportional to the drug concentrations, while for TRI, it was between 100 and 1000 ng mL^{-1}. And with a quantitation limit of 24, 40, 4, and 25 ng mL^{-1} for MTC, ITP, MR, and TRI, as well as detection limits of 8 ng mL^{-1}, 13.5 ng mL^{-1}, 12.1 ng mL^{-1}, and 8.77 ng mL^{-1}. According to ICH requirements, the proposed analytical method was validated, and the outcomes were satisfactory. The researched medicines' presence in pharmaceutical formulations and spiked human plasma has been successfully determined using the suggested analytical method, which also provides an excellent percent recovery. The outcomes are quite good. agreement with the reported method with respect to precision and accuracy.

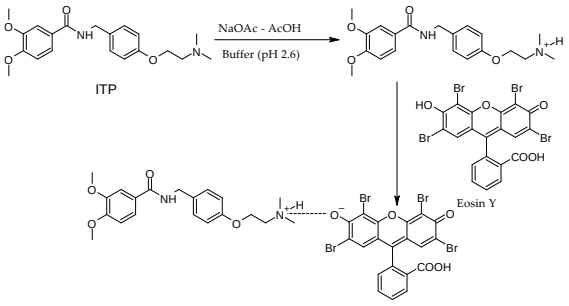
1. Introduction

The molecular name for metoclopramide (MTC) is 4-Amino-5-chloro-N-(2-diethylamino-ethyl)-2-methoxy-benzamide hydrochloride. MTC is widely used as an antiemetic medicine by stimulating the motility of the upper gastrointestinal tract [1]. Different analytical techniques, including, spectrophotometry [2, 3], HPLC attached to UV detector [4, 5], HPLC with fluorescence detector [6], HPLC with mass detector [7, 8], RP UPLC attached to UV detector [9], spectrofluorimetry [10-12], an electrochemical method [13, 14], and HPTLC[15] were proposed for the analysis of MTC in its pharmaceutical formulations and various biological fluids. Chemically speaking, itopride hydrochloride (ITP) is N-[4-(2-Dimethylamino-ethoxy)-benzyl]. ITP (-3, 4-dimethoxy-benzamide hydrochloride), a powerful gastroprokinetic drug, is used to treat various gastrointestinal symptoms brought on by decreased gastrointestinal motility, such as the sensation of being full after eating, heartburn, upper abdominal pain, nausea, and anorexia. It also has a dopamine antagonist effect similar to that of MTC [16]. Numerous analytical techniques, such as spectrophotometry [17, 18], electrochemical methods [19], HPLC attached to UV detector [20, 21], RP-HPLC with fluorescence detector[22], HPLC with mass detector [23, 24], HPLC with chemiluminescence detection [25], HPTLC [26-28], spectrofluorimetry [29, 30] have been proposed. Mosapride citrate (MR), a powerful gastroprokinetic medication that is a selective serotonin 5HT4 agonist, is used to treat gastroesophageal reflux disease and gastrointestinal motility impairment associated with no ulcer dyspepsia and esophagitis[31]. Different analytical techniques, including spectrophotometry [32, 33], HPLC attached to UV detector [34, 35], HPLC with mass detector [36-38], spectrofluorimetry [39, 40] have all been proposed for the measurement of MR in its pharmaceutical formulations and other biological fluids. The chemical name for trimebutine maleate (TRI) is 3, 4, 5-Trimethoxy-Benzoic Acid 2-Dimethylamino-2-phenyl-Butyl Ester Maleate; TRI has an agonist effect on peripheral k, k, and opiate receptors; TRI speeds up gastric emptying by releasing gastrointestinal peptides, and TRI is clinically used to treat a variety of conditions. Irritable bowel syndrome, post-operative ileus, and dyspepsia are just a few of the gastrointestinal motility diseases that TRI is clinically utilized to treat and improve [1, 41]. Different analytical methods were proposed for determination of TRI in its pharmaceutical formulations and different biological fluids, including, spectrophotometry [42, 43], capillary zone electrophoresis [44], conductometry [45], HPLC with UV detector [42, 46, 47], HPLC connected to mass spectrophotometer [48]. The major goal of this research is to create a quick, easy, affordable, and sensitive spectrofluorimetric method for precisely and accurately measuring the four medications for the gastrointestinal tract under study in their pharmaceutical formulations and spiked human plasma. There are a few spectrofluorimetric techniques for examining the researched pharmaceuticals that have been used from the previous analytical evaluation up to this point. The advantages of the suggested approach are that it is as quick, non-extractable, economical, and sensitive as existing spectrofluorometric methods [49-53]. The interaction between the four drugs under study and eosin Y (EY) was examined by measuring the quenching effect of these drugs on the fluorescence intensity of EY as a result of this interaction, and this interaction was the basis for this spectrofluorimetric determination of MTC, ITP, MR, and TRI. Based on the idea that the fluorescence quenching was proportional to the drug concentration, the spectrofluorimetric quenching method was created to identify MTC, ITP, MR, and TRI in human plasma spiked with pharmaceutical formulations. Following ICH recommendations, the reaction conditions were optimized and validated.

2. Results

The current study suggests a novel, straightforward, quick, affordable, and sensitive spectrofluorimetric approach for determining the concentrations of MTC, ITP, MR, and TRI in various pharmacological dosage forms and spiking human plasma. This study technique is based on quantifying the quantitative fluorescence quenching that occurs

when the examined medicines are added to EY. As seen in **Fig. 1**, when measured at e m =542 nm without adding the drug, EY has maximum fluorescence intensity (RFI0). However, when the studied drugs are added to EY, this causes a quantitative quenching in the fluorescence intensity that is proportional to the added drug concentration within the specific concentration range of the analytical method.



Scheme 1. The suggested mechanism for the reaction of MTC, ITP, MR, and TRI with EY in an ion-pair complex.

Table 1. Analytical parameters for the analysis of MTC, ITP, MR, and TRI by the proposed spectrofluorimetric method.

Parameter	MTC	ITP	MS	TRI
λ_{ex} (nm)	520	520	520	520
λ_{em} (nm)	542	542	542	542
Conc range (ng ml^{-1})	50 -1000	50 - 1000	50 - 1000	100 - 1000
r	0.9999	0.9999	0.9999	0.9999
r^2	0.9999	0.9998	0.9998	0.9999
Slope	4.4	2.3	2.8	6.1
intercept	315.4	749	1724	-365
SD of the intercept (S_a)	10.6	9.47	10.2	16.3
SD of slope (S_b)	0.017	0.0149	0.0177	0.028
LOD (ng ml^{-1})	8	13.5	12.1	8.8
LOQ (ng ml^{-1})	24	40.4	36.3	26.4

2.1. Mechanism of reaction

The aliphatic chain of the four medicines for the gastrointestinal tract under study has a tertiary amino group. By protonating their tertiary aliphatic nitrogen atom, which produces a positive center that can readily interact electrostatically with the negatively charged centers of dyes or reagents, these medicines can react with negatively charged dyes or reagents at acidic pH levels. There are two potential sites for ionization in the EY molecule: the hydroxyl group and the carboxylic group. The presence of two strong electron-withdrawing bromo groups in the ortho position to the hydroxyl group causes a decrease in the charge density at the hydroxyl group's oxygen atom, making the hydroxyl group easier to ionize than the carboxylic group in EY and resulting in the formation of EY monovalent anion. The drugs positively charged protonated tertiary amine and the negatively charged ionized hydroxyl group of EY monovalent anion combine to form an ion-pair complex (**Scheme 1**) 2.2. Stoichiometric relationship using equimolar solutions (510-5) of the medication and EY, the drug EY complex composition ratio was calculated using Job's method of continuous variation [54]. A total of 2 mL of two reactants were employed in complementary volumes. The RFI was measured under the ideal reaction conditions at a certain emission wavelength (542 nm), after a particular excitation wavelength (520 nm), as shown in **Fig. 2**. The results obtained using Job's approach were consistent with the proposed response mechanism (**Scheme 1**). The molar ratio of the investigated drugs: EY in the created complex as a result.

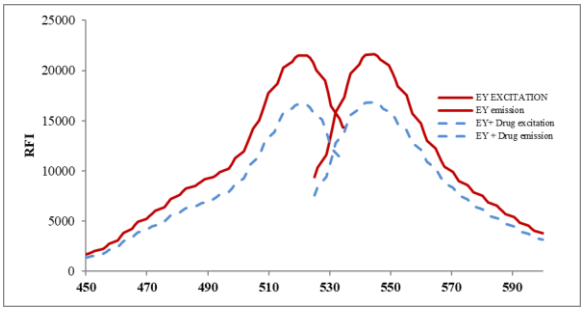


Figure 1. Excitation and emission spectra of eosin (1.45×10-3 Mol L-1), Excitation and emission spectra of the reaction product of eosin and drug at optimum reaction condition.

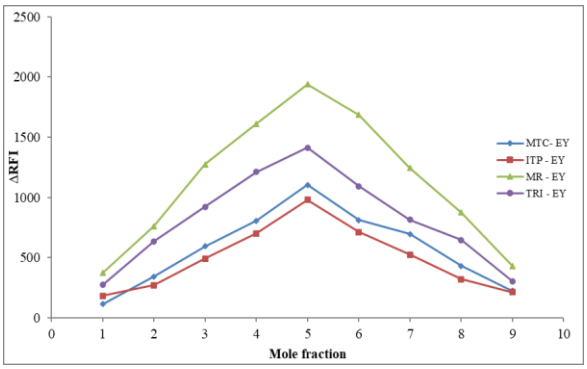


Figure 2. Job's method plot for the determination of the molar ratio of MTC-EY, ITP-EY, MR-EY, and TRI-EY ion-pair complex

2.3. Variable optimization

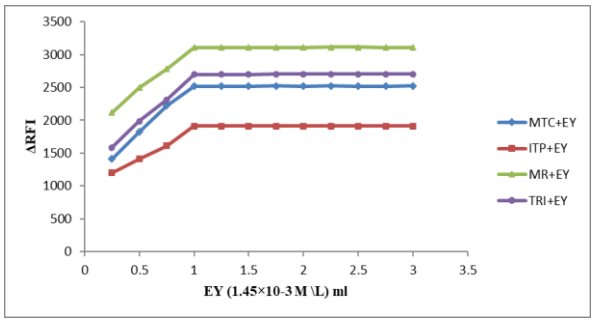
It took considerable consideration and optimization to achieve the best results with the many experimental factors impacting the development and stability of the drug EY binding complex. Each of these elements was altered independently while the others stayed the same to analyze and enhance them. The buffer type, pH, EY volume, diluting solvent, and reaction time were all parameters that were examined.

2.3.1 Effect of pH and buffer type, section

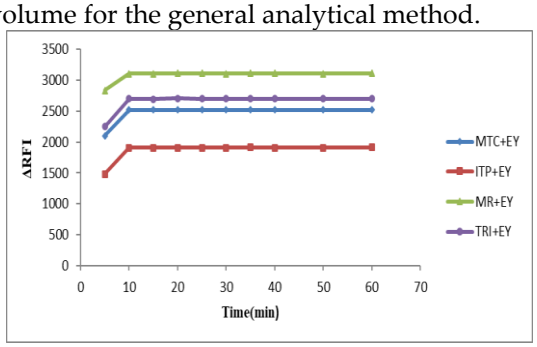
Different forms of a buffer with different pH ranges were examined in order to identify the best buffer type and pH for drug EY complex formation. In comparison to Teorell and Stenhang buffer and phosphate buffer with the same pH, it was discovered that the acetate buffer at pH 2.6 was the best-suited buffer and provided the highest level of fluorescence quenching.

2.3.2. Volume of eosin Y has an impact

The maximum quenching in the fluorescence intensity of various EY volumes (1.4510-3 Mol L^{-1}) was observed in order to determine the best EY volume. The study's findings are shown in Fig. 3. It was discovered that an increase in EY volume up to 1ml EY resulted in a progressive increase in RFI. A negligible increase in the RFI was then noticed, leading to the selection of 1mL of EY (1.4510-3 Mol L-1) as the ideal EY volume for the general analytical method.



(**a**)



(**b**)

Figure 3. (a)Effect of EY(1.45×10-3 Mol L^{-1}) Volume on ΔRFI of MTC, (500ng ml^{-1}), ITP,(500ng mL^{-1}), MR, (500ng mL^{-1}), and TRI, (500ng mL^{-1}) (b) Effect of time of reaction of MTC, (500ng mL^{-1}), ITP,(500ng mL^{-1}), MR, (500ng ml^{-1}), and TRI, (500ng mL^{-1}) with EY(1.45×10-3 Mol L^{-1})

.2.3.3. The Impact of Solvent Diluting

Water, methanol, ethanol, acetone, and acetonitrile were among the diluting solvents that were investigated. It was discovered that water is the ideal diluting solvent with the highest quenching effect.

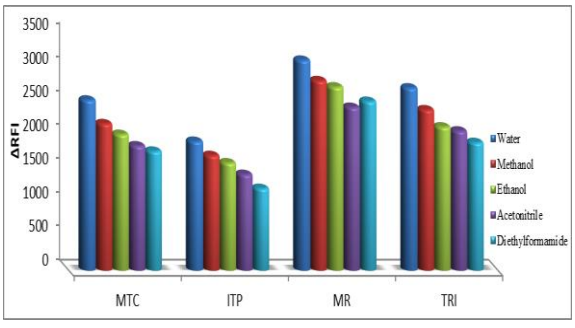


Figure 4. Effect of diluting solvent on the reaction of MTC, ITP, MR, and TRI, (500ng ml^{-1}) with EY (1.45×10-3 Mol L^{-1}).

2.3.4. Reaction time's impact

At 5-minute intervals, the fluorescence intensity was constantly measured for 60 minutes. **Fig. 3** presents a summary of the study's findings. Based on the data, 10 minutes was determined to be the ideal reaction time with the maximum RFI, following which a negligible rise was seen.

2.4. Validation of the Suggested Method

The suggested analytical technique was approved per ICH recommendations [55-63][61-69]. Precision, accuracy, robustness, selectivity, the limit of detection (LOD), limit of quantitation (LOQ), and linearity

2.4.1. linearity and range

The linearity of the proposed fluorometric method was evaluated by analyzing a series of standard solutions of the studied drugs, with concentrations ranging between 50 ng ml^{-1} to 1000 ng ml-1 for MTC, ITP, and MR and from 50 ng ml^{-1} to 1000 ng ml^{-1} for TRI. Under the optimum reaction conditions mentioned above, the calibration curves of the studied drugs were obtained by plotting the ΔRFI value against the concentration of the drugs within the specific method range. Each concentration was repeated three times. Statistical treatment of the data was carried out by using linear regression analysis and different analytical parameters were calculated in **Table 1**. The correlation coefficient (r) for the studied drugs was 0.9999, indicating excellent linearity.

2.4.2. Accuracy and precision

Within the defined concentration range of each tested medication, the suggested fluorometric method's accuracy was assessed at five different concentration levels. Three replications of each concentration were performed. The three measurements' average was determined using the formula found. The outcomes of the three measurements were displayed as percentages of recovery minus standard deviation; **Table 2** provides an overview of the results. The acquired findings are in excellent agreement with the genuine values, demonstrating the great accuracy of the suggested procedure.

Table 2: Evaluation of the accuracy of the proposed analytical procedure for determining MTC,ITP,MR, and TRI at five concentration levels within the specified range.

	MTC			**ITP**			**MR**			**TRI**		
#	Taken ($ng\ ml^{-1}$)	found[a] ($ng\ ml^{-1}$)	% recov.	Taken ($ng\ ml^{-1}$)	found ($ng\ ml^{-1}$)	% recov.	Taken ($ng\ ml^{-1}$)	found[a] ($ng\ ml^{-1}$)	% recov.	Taken ($ng\ ml^{-1}$)	found[a] ($ng\ ml^{-1}$)	% recov.
1	50	49.9	99.8	50	49.9	99.8	50	49.97	99.94	100	100.2	100.2
2	100	100	100	100	100.1	100.1	100	100.1	100.1	150	149.8	99.86
3	250	249.4	99.9	250	250.2	100.08	250	250.7	100.3	250	250.2	100.08
4	400	400.3	100.1	400	400.2	100.05	400	400.3	100.1	400	399.2	99.8
5	500	501.3	100.3	500	502	100.4	500	500.7	100.1	500	500.4	100.08
Mean		100.02			100.05			100.1			100	
SD		0.172			0.29			0.128			0.168	
RSD		0.172			0.29			0.128			0.168	

SD: Standard deviation; [a]: mean of three replicate measurements

Table 3: Evaluation of the intraday and interday precision of the proposed spectrofluorimetric method for determination of MTC, ITP, MR, and TRI in pure form.

Precision level	Conc (ng ml^{-1})	% Recov.[a]	± SD	RSD	% Recov.[a]	± SD	RSD	% Recov.[a]	± SD	RSD	% Recov.[a]	± SD	RSD
Intraday	100	100.4	± 0.84	0.84	100.5	±1.3	1.3	99.9	± 1.02	1.02	100.4	± 1.5	1.5
	250	100.1	±0.38	0.38	100	±1.1	1.1	100.3	± 0.76	0.76	100.1	± 0.8	0.8
	500	99.86	±0.29	0.29	99.7	±0.61	0.61	100.3	± 0.42	0.42	100.1	± 0.61	0.61
Interday	100	99.7	± 0.45	0.45	100.4	±1.7	1.7	100.2	± 1.9	1.9	100.5	± 1.3	1.3
	250	100.47	±0.4	0.4	99.2	±0.4	0.4	99.6	± 0.42	0.42	99.5	± 0.5	0.5
	500	100	± 0.44	0.44	99.9	±0.31	0.31	99.9	± 0.42	0.42	99.8	± 0.2	0.2

SD: standard deviation, RSD: relative standard deviation; a Mean of three replicate measurement

2.4.3. Accuracy and precision

Within the defined concentration range of each tested medication, the suggested fluorometric method's accuracy was assessed at five different concentration levels. Three replications of each concentration were performed. The three measurements' average was determined using the formula found. The outcomes of the three measurements were displayed as percentages of recovery minus standard deviation; Table 2 provides an overview of the results. The acquired findings are in excellent agreement with the genuine values, demonstrating the great accuracy of the suggested procedure. Both the intra-day precision and the inter-day precision of the suggested analytical approach were assessed. By performing replication analyses on three different concentrations of each drug under study three times in a row, the intra-day precision was assessed. By doing a replication analysis on three different drug concentrations over the course of three days, the inter-day precision was also assessed. **Table 3** provided a summary of the intra-day and inter-day precision data. The suggested fluorometric approach has good precision at both repeatability and intermediate precision levels, as seen by the computed relative standard deviation of various measurements being less than 2 %.

2.4.5. Limit of detection (LOD) and limit of quantitation (LOQ)

Using the equations LOD= 3.3/S and LOQ= 10/, where is the standard deviation of intercept and is the slope of the calibration curve, the limit of quantitation and limit of detection determination were based on the standard deviation of response and slope of a calibration curve. The results were compiled in Table 1. For MTC, ITP, MR, and TRI, the limits of detection were 8, 13.5, 12.1, and 8.8 ng ml-1, whereas the limits of quantitation were 24, 40.4, 36.3, and 26.4 ng ml-1. The obtained results show that the suggested fluorometric approach is highly sensitive when compared to other published analytical methods.

2.4.6. Robustness

The analytical method's ability to stay unaffected by minute, unintentional changes in procedure parameters is known as robustness. One experimental variable was modified separately while the other variables stayed the same to test the robustness of the suggested fluorometric approach. The variables that were examined were time, EY volume, and buffer solution pH. **Table 4** provides a summary of the outcomes. Clearly show that minor modifications in any of these factors had no noticeable impact on how well the suggested

approach worked. This demonstrated the validity of the suggested strategy.

2.5. Application of the proposed method to different samples

2.5.1. Application to pharmaceutical dosage forms

Table 4: Robustness study of the proposed spectrofluorimetric method for determination of MTC, ITP, MR, and TRI (250 ng mL^{-1}) in pure form.

	MTC		ITP		MR		TRI	
Variation	**%Recov.[a]**	**± SD**	**%Recov[a]**	**± SD**	**%Recov.[a]**	**± SD**	**%Recov.[a]**	**± SD**
Optimum condition	100.1	± 0.38	100	± 1.1	100.3	± 0.76	100.1	± 0.8
Effect of pH (acetate buffer)								
pH = 2.2	98.4	± 0.4	98.1	± 0.29	98.3	± 0.21	98.7	± 0.75
pH =2.9	99	± 0.53	97.6	± 0.4	98.5	± 0.83	99.2	± 0.6
EY volume								
0.75 ml	98.1	± 0.4	98.1	± 0.8	97.9	± 0.46	97.8	± 0.6
1.25 ml	100.1	± 0.3	100.3	± 0.28	100.2	± 0.25	100.1	± 0.31
Effect of time								
5min	98.3	± 0.7	98.1	± 0.8	98	± 0.35	97.8	± 0.6
15 min	100.2	± 0.55	100.1	± 0.4	100.1	± 0.35	100	± 0.4

SD: standard deviation. [a] Mean of three replicate measurement

The suggested method was gradually used to identify the substances under study in their prescription dose forms. To assess the selectivity of the suggested fluorometric approach, the interaction of the tablet excipients was observed. Using the student's *t*-test and *F*-test, the suggested method's findings were contrasted with those obtained using other documented methods in terms of accuracy and precision. **Table 5** provides a summary of the outcomes. According to the table, which demonstrated that the calculated values did not, at a 95 percent confidence level, exceed the theoretical values, there is no significant difference between the results obtained using the proposed method and those obtained using the reported methods [5, 27, 42], as indicated by the student's *t*-test and F-test. This demonstrates how accurate and precise the suggested procedure is.

2.5.2. Application to spiked human plasma

utilized to determine its concentration. Standard solutions of the investigated medicines were spiked to human plasma to produce final concentrations of 100, 150, and 200ng ml-1. With a standard deviation of 0.33 to 0.74, the mean recovery rates for the three concentration levels in plasma ranged from 97.9 to 98.95. This suggests that the researched medications can be accurately and precisely tested in spiking human plasma without any influence. **Table 6** displays the outcomes that were attained. The suggested method's great sensitivity, it is possible that it might be used to analyze the studied drugs in actual human plasma samples after oral administration without significantly affecting the blood matrix.

Table 5: Comparison between the proposed spectrofluorimetric and reported methods for determination of MTC, ITP, MR, and TRI in its pharmaceutical dosage forms.

Dosage form	%Recovery[a] ± SD		t-value[b]	f-value[b]
	Proposed	Reported[c]		
Primpran®tablet 10mg MTC/tab	99.96± 0.0.74	99.26± 0.79	1.44	1.127
meclopram®tablet 10mg MTC/tab	100± 0.89	99.1± 0.81	1.61	1.21
Ganaton®tablet 50mg ITP/tab	100.16± 0.56	99.64± 0.33	1.79	2.89
Itopride®tablet 50mg ITP/tab	100.6± 0.87	99.5± 0.43	2.4	3.96
Fluxopride®tablet 10mg MTC/tab	100.46± 1.1	99.5± 0.61	1.67	3.3
Mosabride®tablet 10mg MTC/tab	99.8± 0.68	99.4± 0.55	0.92	1.52
Gast-Reg® tablets 100mg TRI/tablet	99.9± 0.77	99.2± 0.78	1.56	1.03
Tritone®tablet 100mg TRI/tab	99.56± 0.64	99.1± 0.79	1.01	1.5

[a] The values are the mean of five determinations. [b] The tabulated t- and F-values at 95% confidence limit are 2.78 and 6.39, respectively, [c] Reported methods [5, 27, 42]

Table 6: Application of the proposed spectrofluorometric method for determination of MTC, ITP, MR, and TRI in spiked human plasma.

MTC			ITP		MR			TRI	
Added conc ($ng\ ml^{-1}$)	**Found conc[a] ($ng\ ml^{-1}$)**	**% Recov. ±SD**	**Found conc[a] ($ng\ ml^{-1}$)**	**% Recov. ±SD**	**Found conc[a] ($ng\ ml^{-1}$)**	**% Recov.**	**±SD**	**Found conc[a] ($ng\ ml^{-1}$)**	**% Recov. ±SD**
100	98.46	98.46± 0.61	98.4	98.4± 0.53	98.16	98.2	±0.72	97.9	97.9± 0.7
150	147.6	98.36± 0.71	147.4	98.5± 0.6	147.5	98.2	±0.74	147.4	98.8± 0.54
200	197.9	98.95± 0.4	197.46	98.7± 0.59	196.3	98.2	±0.38	196.2	98.1± 0.33

[a] Mean of five determinations.

3. Experimental

3.1. Instrumentation

Fluorescence spectrometer Scinco model FS-2 (Korea), connected to an HP PC, with a quartz cuvette of 1 cm in diameter, both the excitation and emission monochromators' slit widths set to 5 nm, and a PMT voltage of 400 V. Hungary-made Adwa pH meter type AD111, 18,659 g-forces on the Bremsen laboratory centrifuge (Germany)

3.2 Materials and reagents

The study's materials and reagents were all of the analytical grades. Amoun Pharmaceutical Company generously gave metoclopramide hydrochloride (El Obour city, Cairo, Egypt). The Borg pharmaceutical business generously donated itopride hydrochloride (Borg Arab, Alex, Egypt). The pharmaceutical manufacturer Marcyrl kindly offered mosapride citrate (El Obour city, Cairo, Egypt). The Amoun pharmaceutical firm graciously offered trimebutine maleate (El Obour city, Cairo, Egypt). El Nasr Chemical Co. provided the Eosin Y that was bought (Abo Zaabal, Cairo, Egypt). El Nasr Chemical Co. provided all additional chemicals, including acetic acid, sodium acetate, acetonitrile, citric acid, phosphoric acid, sodium hydroxide, and hydrochloric acid (Abo Zaabal, Cairo, Egypt). Distilled water was utilized throughout the investigation to create various buffer solutions with various pH levels. Until analysis after mild thawing, human plasma samples were graciously donated by Zagazig University Hospital, Zagazig city, El Sharkia, Egypt. They were maintained frozen at -20 C.2.3. Pharmaceutical dosage forms. The following pharmaceutical dosage forms were examined: primperan® tablets (batch No. 5EG003), made by Sanofi-Aventis Egypt and advertised as containing 10 mg of metoclopramide/tablet. Meclopram® tablets (batch No. 3159014), made by Alexandria Co. for the pharmaceutical industry, are marked as containing 10 mg of metoclopramide per tablet. Egypt's Alexandria. Produced by Kahira pharm. And Chem. Ind. Co., Cairo, Egypt, Ganaton® tablets (batch No. 473037/3j), with a label stating that each tablet contains 50 mg of itopride. Produced by the Borg pharmaceutical company, Itopride® tablets (batch No. 041027) are marked as containing 50 mg of Itopride per tablet (Borg Arab, Alex, Egypt). The Marcyrl pharmaceutical company's Fluxopride® pills (batch No. 1340444), bear a 5 mg Mosapride/tablet label (El Obour city, Cairo, Egypt). Produced by a business in the Western pharmaceutical industries, Mosabride® pills (batch No. 15423), with a label stating that each tablet contains 5 mg of Mosapride (El Obour city, Cairo, Egypt). The Amoun pharmaceutical firm manufactures Gast-Reg® tablets (batch No. 133619), which are stated to contain 100mg of trimebutine per pill (El Obour city, Cairo, Egypt). The manufacturer of Tritone® tablets, Global Napi Pharmaceuticals, 6th October City, Cairo, Egypt, states on the label that each tablet contains 100mg of trimebutine. Batch number 24303. All medication dosage forms were bought from a nearby drugstore.

3.4. Preparation of the standard drug solution

Stock standard solutions (100 g mL^{-1}) of MTC and ITP were made by weighing 10 mg of the drug powder into a 100 mL volumetric flask, diluting it with approximately 75 mL of distilled water, thoroughly dissolving it, and then sonicating it for five minutes. The solution was then diluted to the proper concentration with distilled water. Stock standard solutions (100 g ml^{-1}) of MR and TRI were made by weighing 10 mg of the drug powder into a 100 mL volumetric flask, diluting it with approximately 75 mL of methanol, thoroughly dissolving it, and then sonicating it for five minutes before

adding the remaining methanol to the mark. To create the working solutions right before usage, each prepared stock solution was further diluted with distilled water. EY (1.4510-3 Mol L^{-1}) solution was made by adding 9.4 mg of precisely weighed EY powder to a 100 mL volumetric flask, diluting it with approximately 75 mL of pure water, and thoroughly dissolving it. Distilled water was used to fill the volume to the mark.

3.5. General analytical procedure

To give each flask a final concentration in the range of 0.05 -1 g/mL for MTC, ITP, and MR and from 0.1 -1 g/mL for TRI, various properly measured aliquots of the working solution were transferred into a succession of 10 mL volumetric flasks. The volume was completed to the mark of 10 mL with distilled water after adding 1 ml of EY solution (1.45 10-3 Mol L-1) and 1 mL of acetate buffer pH 2.6. After 520nm excitation and 542nm quenching were measured, a portion of the solution was transferred to a fluorescence cuvette. All observations were made in a 1 cm quartz cuvette at room temperature, with the emission and excitation monochromator slit widths both set to 5 nm. At ex/em = 520/542 nm, the fluorescence intensity of the (drug+EY) complex product (RFI) and the reagent blank (RFI0) were recorded. The fluorescence intensity quenching was estimated using the equation RFI= RFI0-RFI.

3.6. Procedure for pharmaceutical dosage forms

Itopride®, Fluxopride®, Mosabride®, Gast-Reg®, Tritone®, and Primperan® tablets were precisely weighed, finely powdered, and completely combined. The exact equivalent of 10 mg of MTC, ITP, MR, and TRI was weighed, transferred into a 100-mL volumetric flask, dissolved in roughly 75 mL of methanol, swirled, and then sonicated for five minutes before being diluted to the proper concentration with methanol. After thoroughly blending and filtering the contents, the first batch of the filtrate was discarded. To get the final solution concentration within the parameters of the suggested method, the filtrate solution was quantitatively further diluted before the general analytical procedure was carried out.

3.7. Procedure for spiked human plasma

According to the institutional policies, the plasma sample was graciously collected from healthy, human male volunteers at Zagazig University Hospital in Zagazig, El Sharkia, Egypt who do not use any medications. Five milliliters of drug-free human blood were drawn and placed in a heparinized tube. The tube was centrifuged for 30 minutes at 4000 rpm after being vortex blended for 60 seconds at 2000 rpm. One milliliter of drug-free human plasma (supernatant) was transferred to a 10-milliliter stoppered calibrated tube and spiked with one milliliter of stock standard drug solution. The tube's contents were then mixed with two milliliters of acetonitrile, which served as a protein precipitator, and the volume was diluted to the desired concentration with distilled water. The tube was then centrifuged at 4000 rpm for roughly 20 minutes. To create final solutions within the concentration range of MTC, ITP, MR, and TRI, a variable volume of the resultant supernatant was transferred to a succession of 10 ml volumetric flasks. The usual analytical process was then used to obtain the final solutions. A "blank" experiment was conducted by applying the general analytical process to a blood sample that had not been exposed to any drugs [55–59].

4. Conclusions

The MTC, ITP, MR, and TRI concentrations in pharmaceutical formulations and spiking human plasma were determined using a straightforward, affordable, highly sensitive, less time-consuming, and quick spectrofluorometric approach proposed in the current work. The present study has the advantage of not requiring time-consuming liquid-liquid extraction before analysis, critical and expensive chemical reagents, or expensive instrumentation as many other analytical methods do. As a result, the proposed method is more practical and straightforward, . It can be used for routine quality control analysis of the studied drugs because it is less complicated and requires less time and money. The suggested method can also calculate the examined

medicines' nanograms per milliliter. Therefore, it may be easily used to determine the presence of the researched medications in actual human interference.

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References

1. Sweetman, S., P. Blake, and J. McGlashan, *Martindale: the complete drug reference, ed 33, London, 2002*. The Pharmaceutical Press.
2. Jawad, A.A. and K.H. Kadhim, *Spectrophotometric determination of metoclopramide hydrochloride in bulk and pharmaceutical preparations by diazotization-coupling reaction. Inter.* J. of Pharmacy and Pharmaceutical Sciences, 2013. **5**: p. 294-299.
3. Bratulescu, G., *Determination of Metoclopramide with Chromium (III) Thiocyanate Complexes.* Asian Journal of Chemistry, 2016. **28**(1): p. 181.
4. Hegazy, A.M., et al., *Studying the compatibility of a metoclopramide-HCl–paracetamol mixture via IHCMC and establishing a validated RP-HPLC method for its determination in tablets.* Analytical Methods, 2013. **5**(15): p. 3714-3720.
5. Belal, F.F., et al., *Derivative spectrophotometric and liquid chromatographic methods for the simultaneous determination of metoclopramide hydrochloride and aspirin in pharmaceuticals.* Journal of chromatographic science, 2014. **52**(10): p. 1224-1232.
6. Walash, M., et al., *Simultaneous determination of metoclopramide hydrochloride and pyridoxine hydrochloride in syrup using HPLC method with fluorescence detection: application to human plasma.* Journal of Liquid Chromatography & Related Technologies, 2013. **36**(4): p. 439-453.
7. Hamamoto, K., et al., *Simple and sensitive method for measurement of metoclopramide in cattle plasma by LC-MS/MS using a multimode chromatography.* Journal of Veterinary Medical Science, 2013. **75**(4): p. 509-513.
8. Saraya, R.E., et al., *Mini Review: Insights On Instrumental Analysis Of Ombitasvir, Paritaprevir And Ritonavir.* International Journal of Chemistry Research, 2021: p. 1-4.
9. Sowjanya, P., et al., *Novel validated stability-indicating UPLC method for the determination of Metoclopramide and its degradation impurities in API and pharmaceutical dosage form.* Journal of pharmacy research, 2013. **6**(7): p. 765-773.
10. El-Enany, N., *Second derivative synchronous fluorescence spectroscopy for the simultaneous determination of metoclopramide and pyridoxine in syrup and human plasma.* Journal of AOAC International, 2008. **91**(3): p. 542-550.
11. Baeyens, W. and P. De Moerloose, *Fluorescence properties of metoclopramide and its determination in pharmaceutical dosage forms.* Analyst, 1978. **103**(1225): p. 359-367.
12. Elmansi, H., S.A.E.A. Mohamed, and M. Fathy, *Simultaneous determination of metoclopramide and aspirin by spectrofluorimetric technique: application to pharmaceutical formulations and human plasma.* Analytical Methods, 2016. **8**(6): p. 1281-1292.
13. Xx, L., et al., *Electrochemical determination of metoclopramide hydrochloride and its interaction with bovine serum albumin.* J Instrumental Anal, 2010. **11**: p. 005.
14. Saraya, R.E., et al., *Review article on Analytical Techniques of Lamivudine Determination in Different Matrices.* Journal of Advanced

Pharmaceutical Science And Technology, 2021. **2**(3): p. 37-46.

15. Huizing, G., A.H. Beckett, and J. Segura, *Rapid thin-layer chromatographic photodensitometric method for the determination of metoclopramide and clebopride in the presence of some of their metabolic products.* Journal of Chromatography A, 1979. **172**(1): p. 227-237.
16. Chiba, T., et al., *Effects of itopride hydrochloride and ranitidine in patients with functional dyspepsia: comparison between prokinetic and acid suppression therapies.* Hepato-gastroenterology, 2007. **54**(78): p. 1878-1881.
17. Pattanayak, P., R. Sharma, and S. Chaturvedi, *Simultaneous spectrophotometric estimation of rabeprazole sodium and itopride HCl.* Analytical letters, 2007. **40**(12): p. 2288-2294.
18. Sabnis, S.S., et al., *Spectrophotometric simultaneous determination of rabeprazole sodium and itopride hydrochloride in capsule dosage form.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2008. **69**(3): p. 849-852.
19. Hun, X. and Z. Zhang, *Electrogenerated chemiluminescence sensor for itopride with Ru (bpy) 32+-doped silica nanoparticles/chitosan composite films modified electrode.* Sensors and Actuators B: Chemical, 2008. **131**(2): p. 403-410.
20. Khan, A., et al., *Simultaneous determination of domperidone and Itopride in pharmaceuticals and human plasma using RP-HPLC/UV detection: Method development, validation and application of the method in in-vivo evaluation of fast dispersible tablets.* Journal of Pharmaceutical and Biomedical Analysis, 2016. **121**: p. 6-12.
21. Choudhary, B., A. Goyal, and S. Khokra, *High performance liquid chromatographic of Itopride hydrochloride in tablet dosage form.* Oriental Journal of Chemistry, 2009. **25**(1): p. 235.
22. Ma, J., et al., *Determination of itopride hydrochloride in human plasma by RP-HPLC with fluorescence detection and its use in bioequivalence study.* Pharmacological research, 2009. **59**(3): p. 189-193.
23. Lee, H.-W., et al., *Determination of itopride in human plasma by liquid chromatography coupled to tandem mass spectrometric detection: application to a bioequivalence study.* Analytica chimica acta, 2007. **583**(1): p. 118-123.
24. Bose, A., et al., *LC–MS simultaneous determination of itopride hydrochloride and domperidone in human plasma.* Chromatographia, 2009. **69**(11): p. 1233-1241.
25. Sun, Y., et al., *Determination of itopride hydrochloride by high-performance liquid chromatography with Ru (bpy) 32+ electrogenerated chemiluminescence detection.* Analytica chimica acta, 2009. **648**(2): p. 174-177.
26. Suganthi, A., S. John, and T. Ravi, *Simultaneous HPTLC determination of rabeprazole and itopride hydrochloride from their combined dosage form.* Indian Journal of Pharmaceutical Sciences, 2008. **70**(3): p. 366.
27. Saraya, R.E., R.A. Abdel Salam, and G.M. Hadad, *High-performance thin-layer chromatography method for the simultaneous determination of itopride, pantoprazole, and mosapride in their formulations and spiked human plasma.* JPC-Journal of Planar Chromatography-Modern TLC, 2017. **30**(4): p. 299-306.
28. Saraya, R.E., et al., *Instrumental Analysis of Sofosbuvir and Daclatasvir in Different Matrices.* Innovat International Journal Of Medical & Pharmaceutical Sciences, 2021: p. 1-8.
29. Ibrahim, F. and J.J. Nasr, *Fourth-derivative synchronous spectrofluorimetry and HPLC with*

fluorescence detection as two analytical techniques for the simultaneous determination of itopride and domperidone. Luminescence, 2016. **31**(1): p. 255-263.

30. Walash, M.I., et al., *Stability-indicating spectrofluorimetric method for determination of itopride hydrochloride in raw material and pharmaceutical formulations.* Journal of Fluorescence, 2013. **23**(6): p. 1293-1300.
31. Ruth, M., et al., *The effect of mosapride on oesophageal motor function and acid reflux in patients with gastro-oesophageal reflux disease.* European journal of gastroenterology & hepatology, 2003. **15**(10): p. 1115-1121.
32. Revanasiddappa, H. and M. Veena, *Spectrophotometric determination of mosapride in pure and pharmaceutical preparations.* Eclética Química, 2007. **32**: p. 71-75.
33. Birajdar, A.S., S. Meyyanathan, and B. Suresh, *Determination of mosapride and pantoprazole in a fixed-dose combination by UV-spectrophotometric methods and RP-HPLC.* International Journal of Pharmaceutical Studies and Research, 2011. **2**(2): p. 29-36.
34. Krishnaiah, Y.S., et al., *The determination of mosapride citrate in bulk drug samples and pharmaceutical dosage forms using HPLC.* Analytical sciences, 2002. **18**(11): p. 1269-1271.
35. Krishnaiah, Y., et al., *A validated RP-HPLC method for the determination of mosapride citrate in bulk drug samples and pharmaceutical formulations.* Die Pharmazie, 2002. **57**(12): p. 814-816.
36. Aoki, Y., et al., *Liquid chromatography–tandem mass spectrometric method for determination of mosapride citrate in equine tissues.* Journal of Chromatography B, 2007. **858**(1-2): p. 135-142.
37. Ramakrishna, N., et al., *Validation and application of a high-performance liquid chromatography–tandem mass spectrometry assay for mosapride in human plasma.* Biomedical Chromatography, 2005. **19**(7): p. 539-548.
38. Sebaiy, M.M., et al., *Analytical Methods for Determination of Ondansetron hydrochloride and Pantoprazole.* Journal of Medical Research and Health Sciences, 2021. **4**(2): p. 1175-1181.
39. Aleksandrova, D., Y. Scripinets, and A. Yegorova, *Indirect spectrofluorimetric determination of mosapride citrate in pharmaceutical formulations.* Acta Pol. Pharm.-Drug Res, 2009. **66**: p. 605-610.
40. Hegazy, M.A., A.M. Yehia, and A.A. Mostafa, *Stability-indicating methods for the determination of mosapride citrate in the presence of its degradation products according to ICH guidelines.* Drug testing and analysis, 2012. **4**(2): p. 104-115.
41. Delvaux, M. and D. Wingate, *Trimebutine: mechanism of action, effects on gastrointestinal function and clinical results.* Journal of international medical research, 1997. **25**(5): p. 225-246.
42. El-Gindy, A., S. Emara, and G.M. Hadad, *Spectrophotometric and liquid chromatographic determination of trimebutine maleate in the presence of its degradation products.* Journal of pharmaceutical and biomedical analysis, 2003. **33**(2): p. 231-241.
43. Abdel-Gawad, F.M., *Ion-pair formation of Bi (III)–iodide with some nitrogenous drugs and its application to pharmaceutical preparations.* Journal of pharmaceutical and biomedical analysis, 1998. **16**(5): p. 793-799.
44. Li, F. and L. Yu, *Determination of trimebutine maleate in rat plasma and tissues by using capillary zone electrophoresis.* Biomedical Chromatography, 2001. **15**(4): p. 248-251.
45. Elqudaby, H.M., G.G. Mohamed, and G.M. El Din, *Utilization of phosphotungestic acid in the conductometric determination of loperamide hydrochloride and trimebutine antidiarrhea*

drugs. journal of pharmacy research, 2013. **7**(8): p. 686-691.

46. Joo, E.-H., et al., *High-performance liquid chromatographic determination of trimebutine and its major metabolite, N-monodesmethyl trimebutine, in rat and human plasma.* Journal of Chromatography B: Biomedical Sciences and Applications, 1999. **723**(1-2): p. 239-246.
47. Ibrahim, A.E., et al., *Development and validation of eco-friendly micellar-HPLC and HPTLC-densitometry methods for the simultaneous determination of paritaprevir, ritonavir and ombitasvir in pharmaceutical dosage forms.* Heliyon, 2019. **5**(4): p. e01518.
48. Wang, H., et al., *Quantitative determination of trimebutine maleate and its three metabolites in human plasma by liquid chromatography-tandem mass spectrometry.* Journal of Chromatography B, 2002. **779**(2): p. 173-187.
49. Salman, B.I. and R.E. Saraya, *Bio-analytically fluorimetric method for estimation of ertapenem in real human plasma and commercial samples; application to pharmacokinetics study.* Luminescence, 2022.
50. Salman, B.I., et al., *Fabrication of novel quantum dots for the estimation of COVID-19 antiviral drug using green chemistry: application to real human plasma.* RSC Advances, 2022. **12**(26): p. 16624-16631.
51. Salman, B.I., et al., *Quantification of tyramine in different types of food using novel green synthesis of ficus carica quantum dots as fluorescent probe.* Luminescence, 2022.
52. Saraya, R.E., et al., *Ultra-Sensitive Fluorimetric Method for the First Estimation of Vonoprazan in Real Human Plasma and Content Uniformity Test.* Journal of Fluorescence, 2022: p. 1-8.
53. Salman, B.I., et al., *Ultra-sensitive and selective fluorescence approach for estimation of elagolix in real human plasma and content uniformity using boron-doped carbon quantum dots.* BMC chemistry, 2022. **16**(1): p. 1-9.
54. Renny, J.S., et al., *Method of continuous variations: applications of job plots to the study of molecular associations in organometallic chemistry.* Angewandte Chemie International Edition, 2013. **52**(46): p. 11998-12013.
55. Guideline, I.H.T., *Validation of analytical procedures: text and methodology.* Q2 (R1), 2005. **1**(20): p. 05.
56. Saraya, R.E., M. Elhenawee, and H. Saleh, *Development of a highly sensitive high-performance thin-layer chromatography method for the screening and simultaneous determination of sofosbuvir, daclatasvir, and ledipasvir in their pure forms and their different pharmaceutical formulations.* Journal of separation science, 2018. **41**(18): p. 3553-3560.
57. Saraya, R.E., M. Elhenawee, and H. Saleh, *Silver nanoparticles synthesis for sensitive spectrophotometric determination of sofosbuvir, lamivudine, and ritonavir in pure forms and pharmaceutical dosage forms.* Journal of AOAC International, 2020. **103**(1): p. 140-147.
58. Saraya, R.E., et al., *Highly sensitive high-performance thin-layer chromatography method for the simultaneous determination of Molnupiravir, Favipiravir, and Ritonavir in pure forms and pharmaceutical formulations.* Journal of Separation Science, 2022.
59. Saraya, R.E., M. Elhenawee, and H. Saleh, *Development of a high-performance thin-layer chromatographic method for the simultaneous determination of newly co-formulated antiviral drugs sofosbuvir and velpatasvir in their pure forms and tablet dosage form.* JPC-Journal of Planar Chromatography-Modern TLC, 2019. **32**(2): p. 141-147.
60. Saraya, R.E. and E.A. Abdel Hameed, *Eco-friendly micellar HPTLC technique for the simultaneous analysis of co-formulated antibiotic cefoperazone and sulbactam in pure*

form and vial pharmaceutical formulation. JPC–Journal of Planar Chromatography–Modern TLC, 2021. **34**(2): p. 121-129.

61. Saraya, R.E., R.A.A. Salam, and G.M. Hadad, *Stability-indicating high-performance thin-layer chromatographic determination of ondansetron in pure form and pharmaceutical formulations.* JPC-Journal of Planar Chromatography-Modern TLC, 2018. **31**(2): p. 122-128.
62. Abdel Hameed, E.A., et al., *Two New HPLC Methods, Assessed by GAPI, for Simultaneous Determination of Four Antipsychotics in Pharmaceutical Formulations: A Comparative Study*. Separations, 2022. **9**(8): p. 220.
63. Elhenawee, M., H. Saleh, and R.E. Saraya, *Simultaneous high-performance thin-layer chromatographic determination of ondansetron and pantoprazole in their pure forms and spiked human plasma.* JPC–Journal of Planar Chromatography–Modern TLC, 2019. **32**(2): p. 149-156.