ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Flow Injection Determination of Mebeverine Hydrochloride Using Fluorescence Resonance Energy Transfer (Fret) Via Isnag-Fluorimeter

Nagam S. Turkie[1], Hayder Q. Munshid[2*]

[1.] *Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.*

[2.] *Department of Pharmacy, Al-Esraa University Collage, Baghdad, Iraq.*

***Corresponding Author: Hayder Q. Munshid**

Abstract

A new approach was used for the determination of Mebeverine hydrochloride (MIP-HCl) via the fluorescence resonance energy transfer (FRET) from erythrosine B (Erth-B) which used as a carrier stream. The method was applied using flow injection system of a new homemade ISNAG fluorimeter with fluorescence measurements at ± 90◦ via 2×4 solar cell. The linear range for the new developed methodology was 0.01 – 0.6 mmol/L with correlation coefficient r = 0.9577. While the L.O.D was 0.699 µg/sample (sample volume 250 µL) from the stepwise dilution for the minimum concentration in the linear dynamic ranged of the calibration graph. The method was successfully applied to the determination of Mebeverine hydrochloride (MIP-HCl) in three different pharmaceutical drugs. A comparison was made between the newly developed method analysis and the classical method using t-test. It was noticed that there was no significant difference between the two methods at 95 % confidence level.

Keywords: *Mebeverine hydrochloride, Erythrosine B, Flow injection analysis, Fluorescence Resonance Energy Transfer (FRET).*

Introduction

Mebeverine hydrochloride (MIP-HCl) is R and S enantiomers of 4-[ethyl (4-methoxy-a-methylphenethyl) amino] butyl veratrate hydrochloride (Fig. 1-a) with an empirical formula of ($C_{25}H_{35}NO_5$.HCl). It is a white or almost white, crystalline powder, which is very soluble in water; freely soluble in ethanol (96%); practically insoluble in ether [1]. Mebeverine hydrochloride is a potent direct antispasmodic drug which is acting mainly on the smooth muscles of the gastrointestinal tract and it is particularly effective against colonic spasms [2]. There are several analytical methods have been reported in literatures for the determination of mebeverine hydrochloride in its different forms and preparations, some of these methods are spectrophotometric methods [3, 4], Spectrofluorometric [5], electrochemical [6, 7], chromatographic [8-13], and flow injection analysis (FIA) method [14]. Erythrosin B (Erth-B); tetra-iodofluorescein, also known as Spiro [isobenzofuran-1(3H), 90-[9H] xanthen]-3-one, 30, 60-dihydroxy-20, 40, 50, 70-tetraiodo-, sodium salt. Its molecular formula $C_{20}H_6I_4Na_2O_5$ and molecular weight of 876.86 g/mol (Fig. 1-b), red to brown powder that's soluble in water and ethanol [15]. It is an organoiodine compound and it is cherry-pink synthetic, primarily used for food coloring [16], Erythrosin B probe is used for the molecular mobility of water-soluble and membrane-bound proteins [17].

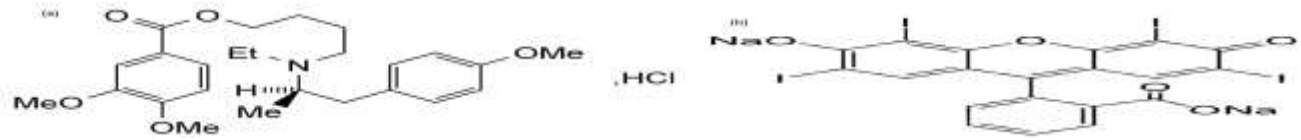


Figure 1: (a) Chemical structures of Mebeverine hydrochloride (MIP-HCl), (b) Chemical structure of erythrosine B (Erth-B)

This study aimed to develop a new method for the determination of mebeverine hydrochloride (MIP-HCl) via the fluorescence resonance energy transfer (FRET) from erythrosine B which used as a carrier stream using the new homemade ISNAG-fluorimeter.

Materials and Methods

Apparatus and Reagents

A homemade ISNAG fluorimeter [18] was used with 4-channels peristaltic pump (Ismatec, Switzerland) and Six-port medium pressure injection valve (I D E X corporation, USA) with sample loop (1 mm i.d. Teflon, variable length). Potentiometric recorder to estimate the output signals (Siemens, Germany (1- 5 V). Spectrophotometer (UV-1800, shimadzu, Japan) was also used for classical spectrofluorometric methods. All chemicals were used of analytical-reagent and distilled water was used to prepare all the solutions. A standard solution of 1 mmol/L of MIP-HCl and Erth-B, molecular weight 466.015 and 879.86 g/mole respectively, were prepared by dissolving 0.2330 g and 0.4399 g of MIP-HCl and Erth-B in 500 mL of distilled water. A pH range of 2.2–8.0 buffers were prepared according to McIlvaine citric acid–phosphate buffer systems [19].

Sample Preparation

Twenty tablets of three different kinds of pharmaceuticals drugs (Duspatalin Abbott France, Meva Jamjoom Pharma Saudi Arabia, Colospasmin Eipico Egypt) were weight and then crushed and grinded. A solution of 0.1 mmol/L were prepared by weighting 0.0070, 0.0068 and 0.0053 g (equivalent to 0.0023 g of active ingredient) from Duspatalin, Meva and Colospasmin respectively. Each one from these kinds of sample dissolved in distilled water. The solution was filtered to get rid of undissolved materials; the residue was washed with distilled water and completed the volume to 50 ml with the same solvent (distilled water).

Methodology

Erythrosine B (fluorescent molecule) was injected on distilled water which was used as a carrier stream through a single line manifold design using ISNAG-fluorimeter in addition to MIP-HCl treated as the same. It was noticed that the use of Erth-B gave a negative response (Fig. 2-A) which indicate the absorbance of light from LP-mercury lamp of ISNAG-fluorimeter, but the solar cells cannot detect the fluorescent light because it may be not within its spectral range (i.e.; 410-1150 nm), also the use of MIP-HCl didn't gave a suitable response which could be used for the determination of this molecule (Fig. 2-B). It probably might be attributed that using MIP-HCl as an acceptor molecule and an absorber for LP-Hg lamp, no profile shown in Fig. 2-C i.e.; shows that no signal which indicate either does not give fluorescence or short-lived fluorescent species and the fluorescence emission is not within detector response region i.e.<410 nm.

So, a new approach was used for the determination of MIP-HCl via the fluorescence resonance energy transfer (FRET) from Erth-B which used as a carrier stream (Fig. 2-C). Scheme 1 shows the possible mechanism.

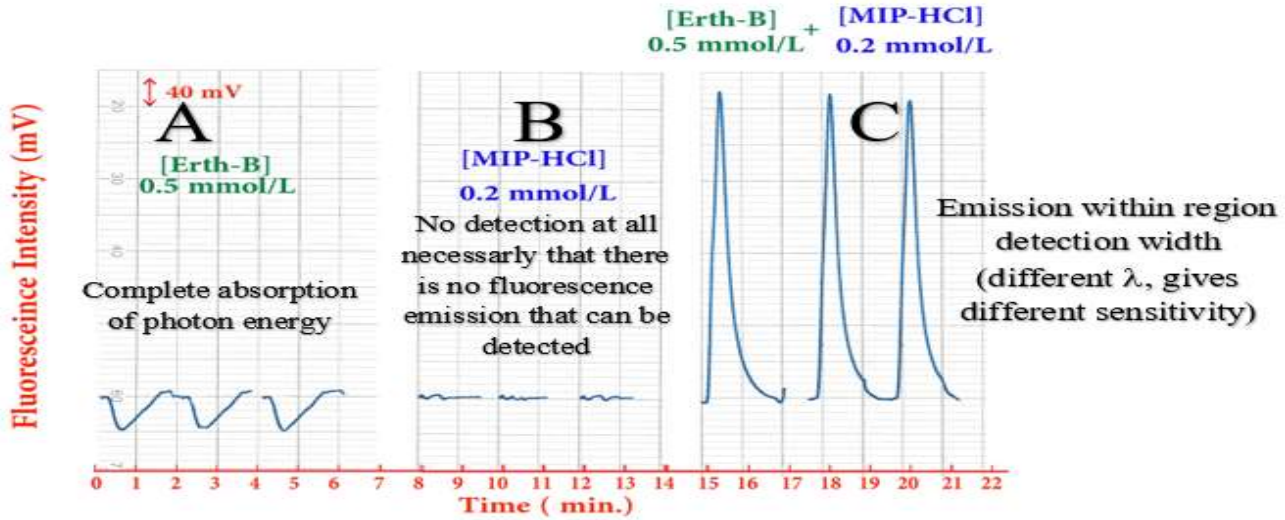
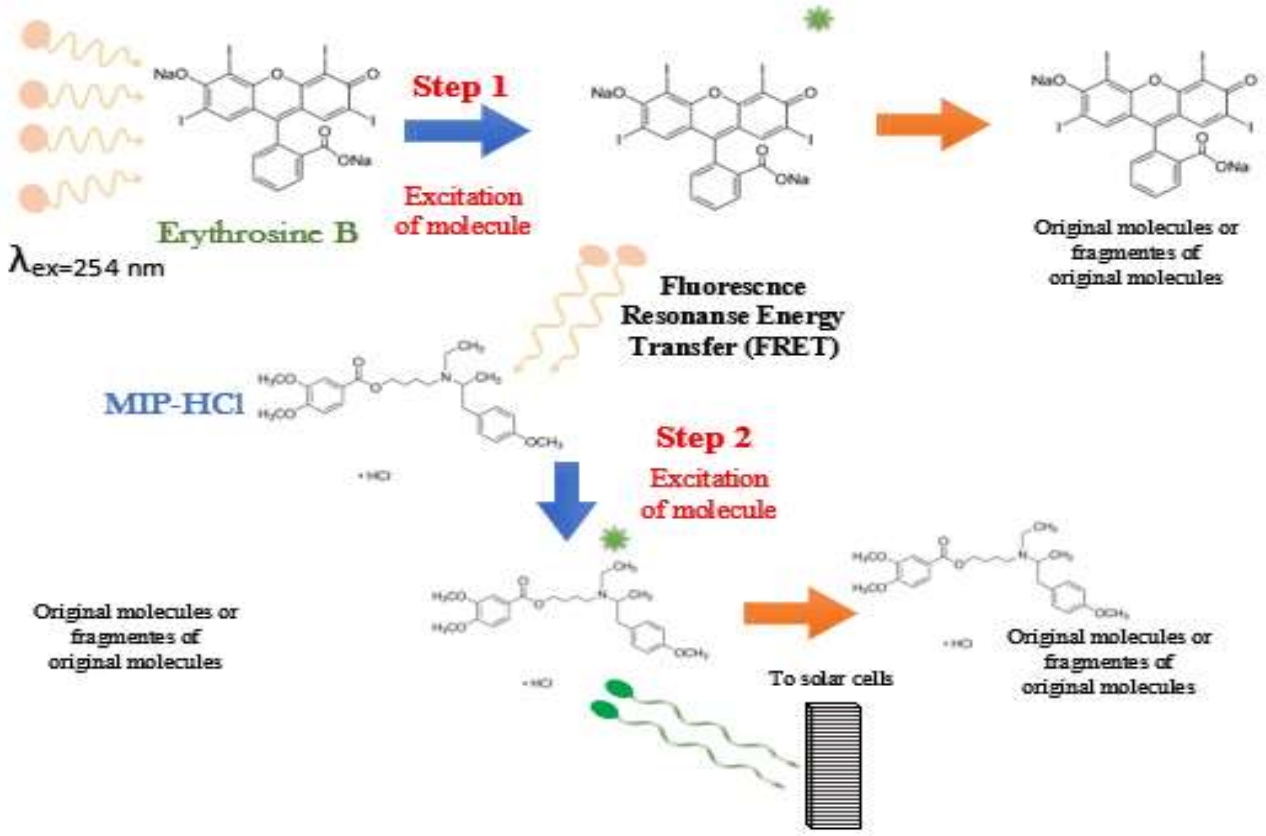


Figure 2: Response profile for the fluorescence intensity of Erth-B and MIP-HCl using ISNAG-fluorimeter and 0.5 mmol/L of Erth-B



Scheme 1: Proposed mechanism for the fluorescence resonance energy transfer (FRET)

Results and Discussion

Study of the Optimum Parameters

Variation of Erythrosine B Concentration

A series of Erth-B concentrations (5×10^{-3} – 1 mmol/L) were used as a carrier stream at a flowrate of 2.2 mL/min and 250 µL of sample segment (0.2 mmol/L of MIP-HCl) were injected. An increase in responses of the fluorescence resonance energy transfer (FRET) depend on the concentration of Erth-B up to 1 mmol/L tried as shown in fig.3. The selected of highest concentration of Erth-B molecule as it is regarded an inner irradiation source for drugs molecules and stimulate fluorescence. In order to enhance and smoothing the obtained response signal obtained from ISNAG-fluorimeter, a set (0.1632-3.774 sec. time constant) of RC-low band pass electronic filter were used. It was noticed that 0.3169 sec. (any response below this number ((time constant)) will not be measured which is in this case a fraction of seconds) is the most appropriate electronic filter for both molecules (Fig. 4).

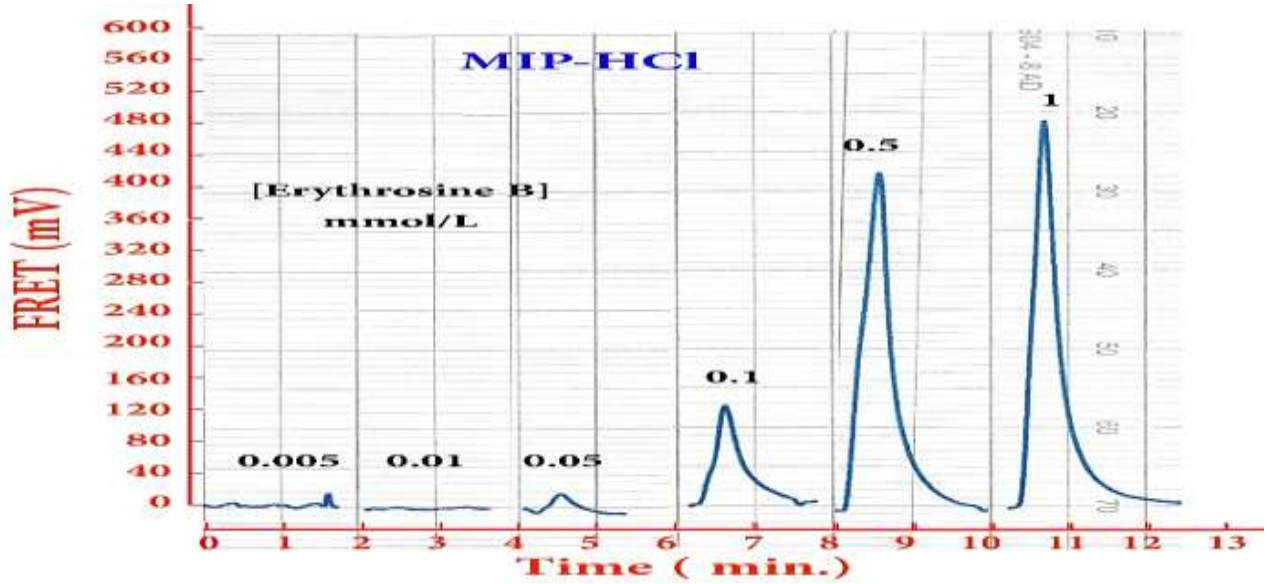


Figure 3: Variation of Erth-B concentration effect on response profile-time

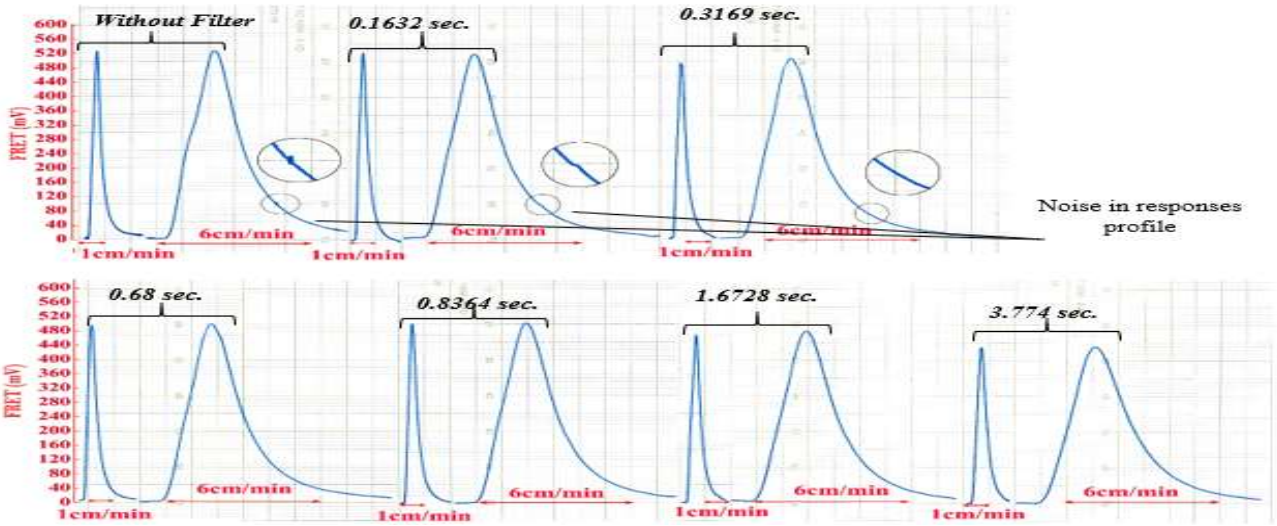


Figure 4: Response profile versus variable electronic filters using MIP-HCl

Effect of pH on Fluorescence Resonance Energy Transfer

A series of buffer solutions were used to prepare 1 mmol/L of Erth-B which was used as a carrier stream with a flow rate of 2.2 mL/min. and a sample of 250 µL of MIP-HCl was injected in an open valve mode. It was noticed (Fig. 5-A) that the use of buffers causes a decrease in the response and followed by a slight increase and gave a minimum response at pH 8 (Fig. 5-B). An increase in pH might probably leads to the precipitation of fluorophore acceptor molecule from the energy released from Erth-B molecules or quenching the inner fluorescence in the form of non-radiative thermal energy or internal convention between electronic levels of all fluorescent molecules and losing fluorescence energy. The Erth-B prepared in distilled water gave the best response, so it was used as the optimum for this study.

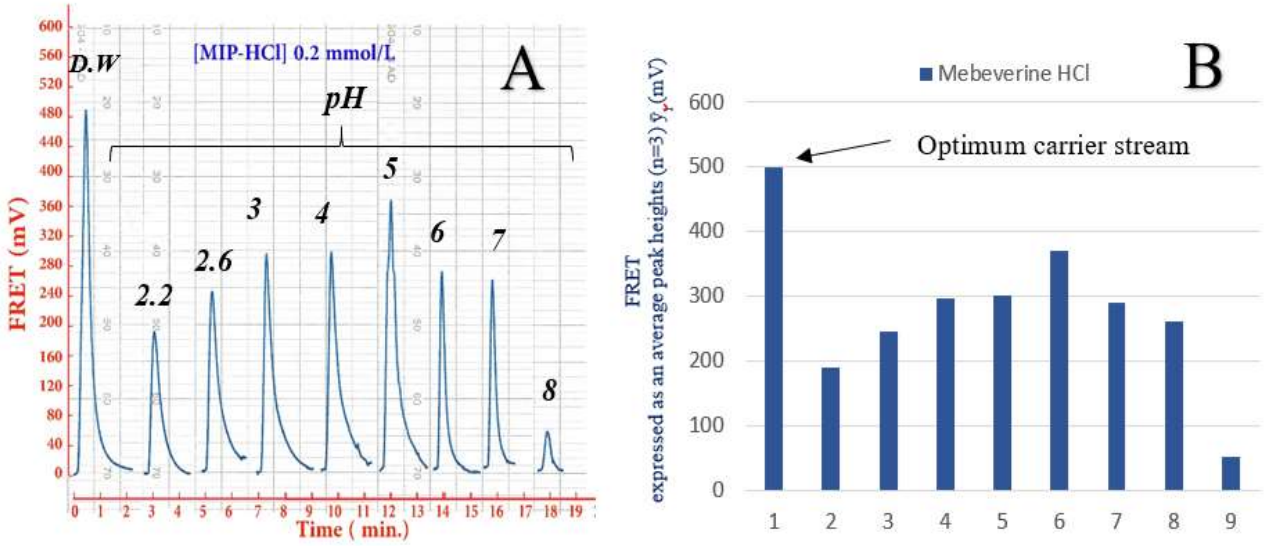


Figure 5: A; Effect of variable buffers on the response profile, B; Variation of buffers solutions on the fluorescence resonance energy transfer of 0.2 mmol/L of MIP-HCl at flowrate of 2.2 mL/min and 250 µL sample volume

Physical Parameters Optimization

Effect of Flow Rate

Using Erth-B prepared in distilled water as a carrier stream and 250 µL of 0.2 mmol/L of MIP-HCl as an injected sample volume. The flow rate effect was studied (0.575-4.3 mL/min.); it was noticed (Fig. 6) that there is a decrease in responses with increasing the flow rates and a decrease in peak base width, 2.2 mL/min was chosen as the optimum flow rate due to obtain high responses with sharp profile and less Δt_b; all this might be attributed to the convection effect of moving sample segment that increases and decrease of diffusion and dilution at optimum flow rates. The results obtained were summarized in Table 1.

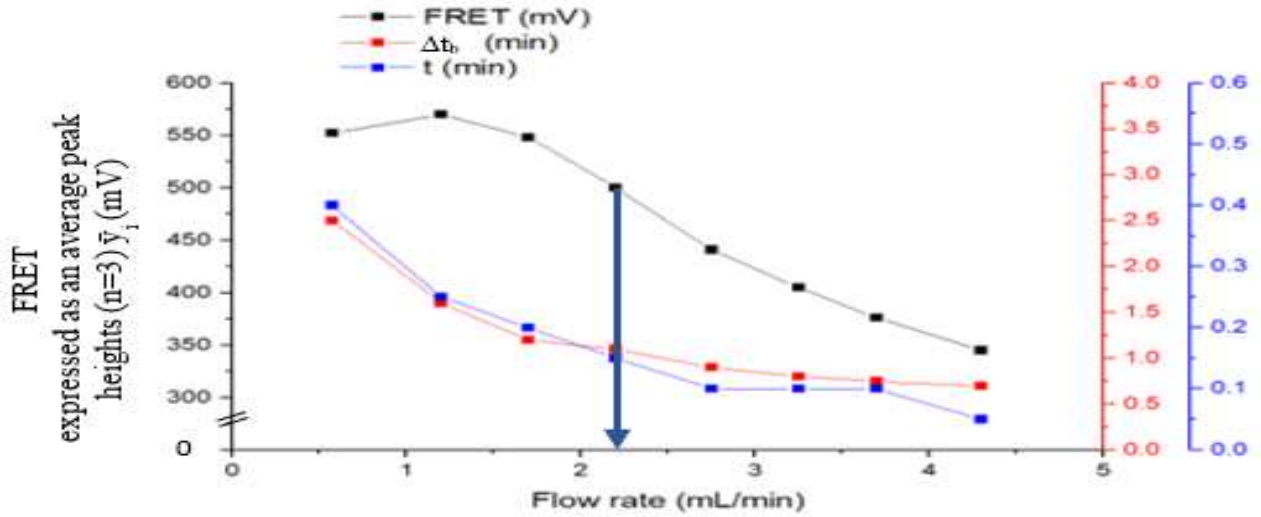


Figure 6: Flowrate variation on fluorescence resonance energy transfer, leaving time from injection valve to the measuring cell and peak base width for MIP-HCl using 250 µL of 0.2 mmol/L of MIP-HCl as an injected sample and Erth-B as a carrier stream

Table 1: Data summery of flowrate effect study using 250 µL of 0.2 mmol/L MIP-HCl using open valve mode

Speed of peristaltic pump (indication approximate)	Flow rate (mL/min)	Fluorescence resonance energy transfer (FRET) expressed as an average peak heights (n=3) ȳi (mV)	RSD %	Confidence interval of the average response (at 95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\, \sigma_{n-1}/\sqrt{n}$	Δt_b Peak base width (min)	t* (min)	V_{final} × (mL)	C_{final} × (mmol/L)	$Df = \frac{C_0}{C_{final}}$
5	0.575	552	0.15	552 ± 2.0372	2.5	0.4	1.688	0.0296	6.7568
10	1.200	570	0.15	570 ± 2.1117	1.6	0.25	2.170	0.0230	8.6957
15	1.700	548	0.14	548 ± 1.9626	1.2	0.2	2.290	0.0218	9.1743
20	2.200	500	0.22	500 ± 2.7825	1.1	0.15	2.670	0.0187	10.6952
25	2.750	441	0.21	441 ± 2.2856	0.9	0.1	2.725	0.0183	10.929
30	3.250	405	0.30	405 ± 3.0557	0.8	0.1	2.850	0.0175	11.4286
35	3.700	376	0.29	376 ± 2.7079	0.75	0.1	3.025	0.0165	12.1212
40	4.300	345	0.37	345 ± 3.2048	0.7	0.05	3.260	0.0153	13.0719

Effect of Sample volume

This study was carried out for the optimization of sample segment using flow rate 2.2 mL/min and different volumes of sample loop (i.e.; 50-250 µL) were used. It was noticed from fig. 7 that an increase of fluorescence intensity related with the increase of sample segment as well as increase in the peak width. Therefore, 250 µL was chosen as the best injected volume. This is due to the increased accepter molecules species of fluorescence light that is released from Erth-B (as an inner source of irradiation) and as a consequence increased fluorescence light that is emitted from drugs molecules. The released light is captured via the detector cells.

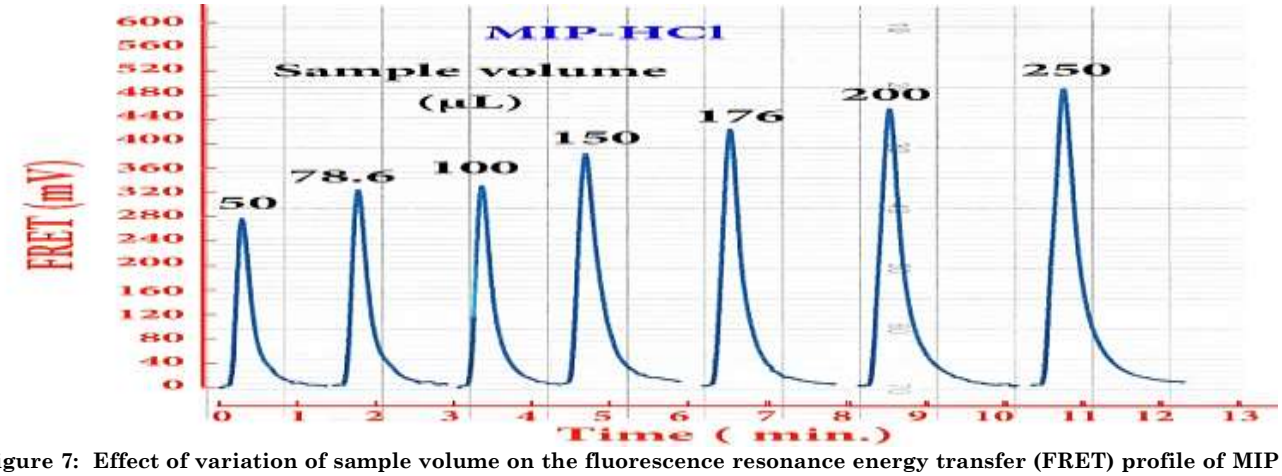


Figure 7: Effect of variation of sample volume on the fluorescence resonance energy transfer (FRET) profile of MIP HCl

Purge Time Effect

Purge time effect was made using variable purge time (2-25 sec. in addition to open valve mode) by injecting 250 µL of 0.2 mmol/L of MIP-HCl as a sample segment. It was noticed, there is an increased in response profile with increasing allowed permissible time (purge time). 15 second was chosen as an optimum due to the stability of response profile and there is no significant difference in sensitivity in case of using 15 and 20 second with open valve mode (i.e.; 25 sec) which means completely departure of sample segment from sample loop in load position until full complete response obtained. All the data tabulated in Table 2.

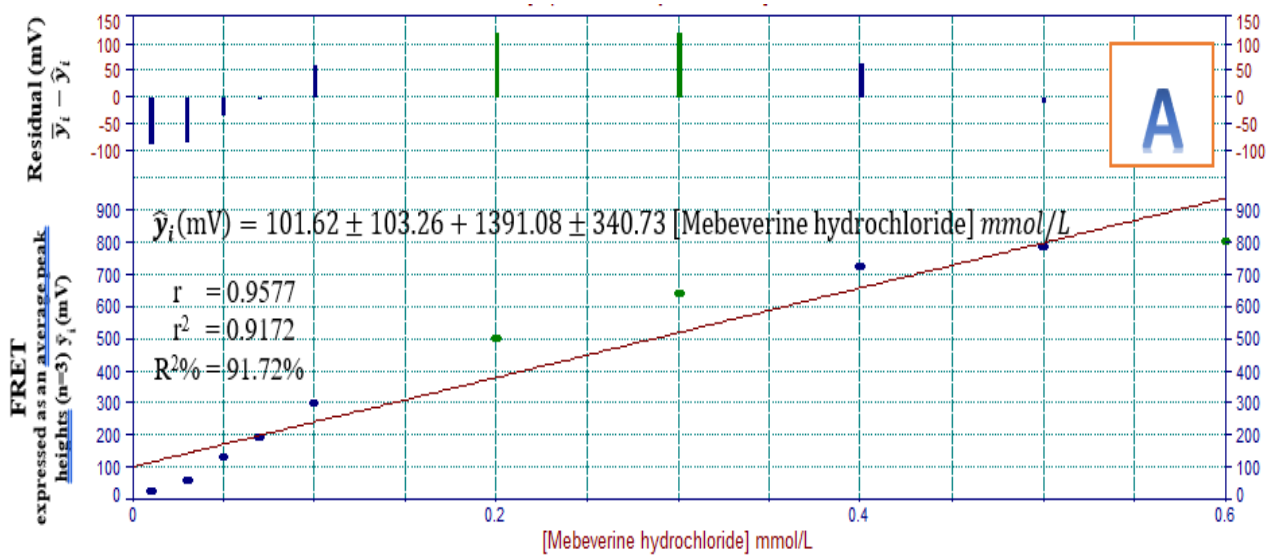
Table 2: Effect of purge time study on FRET using ISNAG-fluorimeter

Purge time (sec.)	Fluorescence Intensity expressed as an average peak heights (n=3) ȳi (mV)	RSD%	Confidence interval of the average response (at 95% confidence level) $\bar{y}_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$
2	151	0.87	151 ± 3.2545
5	312	0.39	312 ± 3.0309
10	409	0.28	409 ± 2.8321
15	508	0.24	508 ± 2.9812
20	505	0.23	505 ± 2.8818
25 (open valve)	503	0.19	503 ± 2.4347

*open valve: Injection valve in the open mode (injection mode) until departure of fluorescence species from measuring cell

Calibration Graph for the Variation of Fluorescence Resonance Energy Transfer (FRET) Versus MIP-HCl

Using all the optimum parameters that achieved in previous sections; a series of MIP-HCl (0.01-1 mmol/L) were prepared in distilled water and injected on a carrier stream of Erth-B at flow rate of 2.2 mL/min, the variation of this drug concentration with the fluorescence resonance energy transfer FRET in mV obtained by ISNAG-fluorimeter. A calibration graph was plotted with a linear range of 0.01-0.6 mmol/L (Fig.8-A). In which an increase of FRET due to the increased of acceptor molecules species i.e.; MIP-HCl that is absorbed the fluorescence light released from Erth-B (as inner source of irradiation) up to 0.6 mmol/L. A comparison was made with the classical spectrophotometric method through the measurements of absorbance for MIP-HCl at different concentrations. A calibration graph was plotted using λ_{max}= 219 & 262 nm (Fig. 8-B & C). All the data for the scatter plots for these two methods were listed in table no. 3. The repeatability of efficiency of homemade ISNAG-fluorimeter was studied at fix concentration (0.2 mmol/L) at optimum parameters. The repeated measurements for eight successive injections were measured and obtained results were tabulated in table 3 which shows that the RSD% less than 0.7%. The limit of detection was studied at three different approaches i.e.; gradual dilution of lowest concentration in the calibration graph or based on the value of slope and from the linear regression plot. Table 3 summed up all this calculation value of detection limit.



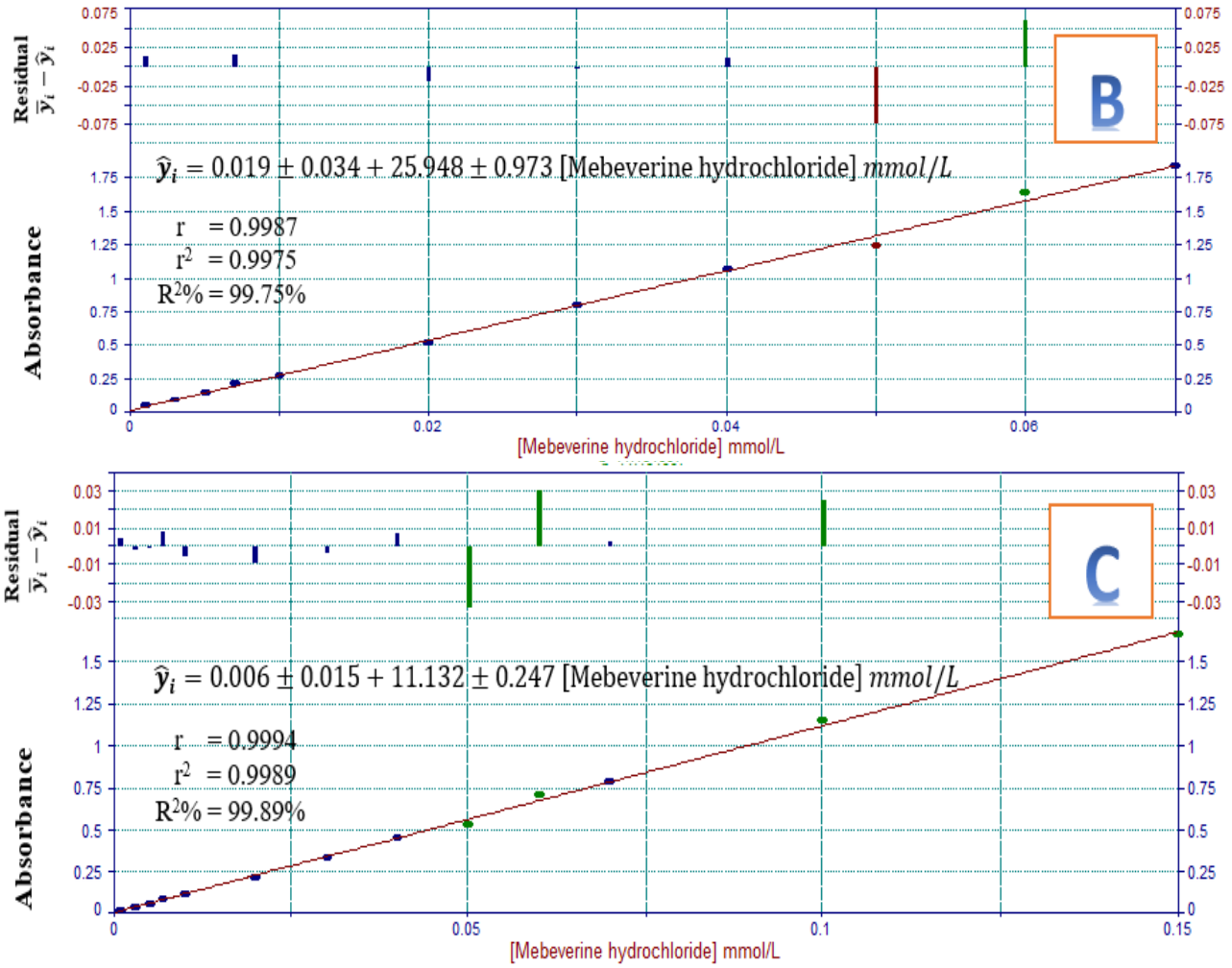


Figure 8: Linear calibration graph for MIP-HCl for; A- Newly developed methodology, B- Classical method at λmax=219 nm, C- Classical method at λmax=262 nm

$$Residual = \bar{y}_i(practical\ value) - \hat{y}_i(estimated\ value)$$

Color code of residual plot: Blue = 1σ, Green = 2σ, Red = 3σ.

Table 3: Summary of calibration graph results for determination of MIP-HCl using newly developed methodology (ISNAG-fluorimeter) and classical methods at 95% confidence level

Type of method		Range of calibration graph (mmol/L)	Equation of calibration graph $\hat{y}_i = a \pm S_a t + b \pm S_b t$ [X] mmol/L at confidence level 95%, n-2	r / r^2 / R^2 %	t_{tab} = $t_{0.025}$, n-2	$t_{cal} = \frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
Newly developed methodology		0.01 – 0.6 (n=10)	$\hat{y}_i$(mV) = 101.62 ± 103.26 + 1391.08 ± 340.73 [Mebeverine hydrochloride] $mmol/L$	0.9577 0.9172 91.72	2.306<<9.414	
Classical method	λex = 219 nm	0.001 – 0.07 (n=11)	$\hat{y}_i = 0.019 \pm 0.034 + 25.948 \pm 0.973$ [Mebeverine hydrochloride] $mmol/L$	0.9987 0.9975 99.75	2.262<<60.299	
	λex = 262 nm	0.001 – 0.15 (n=13)	$\hat{y}_i = 0.006 \pm 0.015 + 11.132 \pm 0.247$ [Mebeverine hydrochloride] $mmol/L$	0.9994 0.9989 99.89	2.201<<99.583	

Detection limit			
[Drug minimum concentration] mmol/L	**Practical based on the gradual dilution for the minimum concentration**	**Theoretical based on the value of slope X=3SB/slope**	**Theoretical (linear equation) based on the value of $\hat{Y} = Y_b + 3S_b$**
0.006	**2.796×10^{-3} g/L 0.699 µg/sample**	**6.030×10^{-4} g/L 0.151 µg/sample**	**0.095 g/L 23.703 µg/sample**

Repeatability			
[Drug concentration] mmol/L	**Fluorescence Resonance energy transfer (FRET) expressed as an average peak heights (n=8) ȳi (mV)**	**RSD %**	**Confidence interval of the average response (at 95% confidence level) $\bar{y}_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$**
0.2	**501.00**	**0.62**	**501.00 ± 4.2557**

[X]: Concentration of MIP-HCl in mmol/L, n: No. of measurements in calibration graph, r: correlation coefficient, r2: coefficient of determination, R2% (Percentage capital R Squared): explained variation as a percentage total variation. ttab : t0.025,11 = 2.201, t0.025,9 = 2.262, t0.025,8 = 2.306, t0.025,6 = 2.447, X: value of L.O.D based on slope, SB: Standard deviation of blank repeated for 13 times, Yb: average response for blank = intercept (a), Sb: standard deviation equal to Sy/x (residual)

Application of the use of ISNAG-fluorimeter for the determination of MIP-HCl in the pharmaceutical drugs

The newly developed methodology was used for the determination of MIP-HCl in three different pharmaceutical drugs from different companies (Duspatalin Abbott France, Meva Jamjoom Pharma Saudi Arabia, Colospasmin Eipico Egypt). The standard addition method was applied by preparing a series of solutions from MIP-HCl via transferring 5 mL of each sample (0.1 mmol/L) to five volumetric flasks (10 mL), followed by the addition of (0, 1, 2, 3 and 4 mL) from 0.5 mmol/L standard solution in order to have the concentration range from 0 – 0.2 mmol/L. Fig. 9 shows the response profile. The comparison of the obtained results was made with classical spectrophotometric method at λ_{max}=219 nm was chosen due to the highest slope which indicates high sensitivity of this method. Standard addition method was applied using a series of solutions via transferring 5 mL of MIP-HCl sample (0.01 mmol/L which prepared by transferring 5 mL of 0.1 mmol/L stock sample to 50 mL volumetric flask) to five volumetric flasks (10 mL), followed by the addition of (0, 1, 2, 3 and 4 mL) from 0.1 mmol/L standard solution to have the range of concentration from 0-0.04 mmol/L.

Table no. 4 explain the synopsis of standard addition graphs measurements. Values of % R^2 (which shows that all explained results from out of total values); in addition to practically values in term of concentration. The results were mathematically treated [20, 21] and tabulated in table 4 at confidence level of 95%.

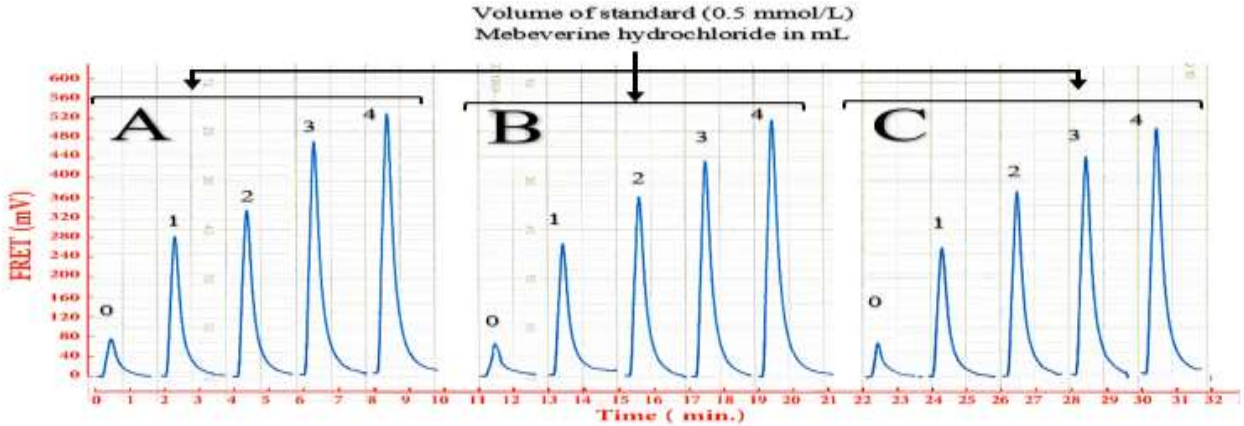


Figure 9: Response profile of standard addition method using developed method for the determination of MIP-HCl manufactured by different companies A- Duspatalin, Abbott France, B- Meva, Jamjoom Pharma Saudi Arabia, C- Colospasmin, Eipico Egypt

Table 4: Standard addition results for the determination of CIP-HCl in three different pharmaceuticals drugs using two methods

Commercial name, Company, Content, Country	Newly developed methodology using ISNAG-fluorimeter (mV)				
	UV- spectrophotometric classical method absorbance measurement at λmax = 219 nm				
	Confidence interval for the average weight of tablets $\bar{W}_i \pm 1.96\sigma_{n-1}/\sqrt{n}$ at 95% (g) (n=20)	Weight of sample equivalent to 0.0023 g (0.1 mmol/L) of active ingredient	Theoretical content for the active ingredient $W_i \pm 1.96\sigma_{n-1}/\sqrt{n}$ at 95% (g)	Equation of standard addition at 95% for n-2 $\hat{y}_i = a \pm S_a t + b \pm S_b t$ $[Mebeverine\ hydrochloride]$ $mmol/L$	r | r^2 | R^2%
Duspatalin, Abbott, 135 mg tab., France	0.4101 ± 0.0035	0.0070	0.135 ± 0.0012	$\hat{y}_i(mV) = 121.80 \pm 109.24 + 2216 \pm 891.93$ [mebeverine hydrochloride] mmol/L	0.9768 | 0.9542 | 95.42
				$\hat{y}_i = 0.117 \pm 0.127 + 22.710 \pm 5.174$ [mebeverine hydrochloride] mmol/L	0.9924 | 0.9849 | 98.49
Meva, Jamjoom Pharma, 135 mg tab., Saudi Arabia	0.3963 ± 0.0014	0.0068	0.135 ± 0.0005	$\hat{y}_i(mV) = 119.80 \pm 110.96 + 2150 \pm 906.0$ [mebeverine hydrochloride] mmol/L	0.9747 | 0.9500 | 95.00
				$\hat{y}_i = 0.118 \pm 0.116 + 23.060 \pm 4.748$ [mebeverine hydrochloride] mmol/L	0.9938 | 0.9876 | 98.76
Colospasmin, Eipico, 135 mg tab., Egypt	0.3090 ± 0.0037	0.0053	0.135 ± 0.0016	$\hat{y}_i(mV) = 116.0 \pm 121.67 + 2162 \pm 993.46$ [mebeverine hydrochloride] mmol/L	0.9701 | 0.9411 | 94.11
				$\hat{y}_i = 0.120 \pm 0.130 + 22.910 \pm 5.273$ [mebeverine hydrochloride] mmol/L	0.9923 | 0.9846 | 98.46
Commercial name, Company, Content, Country	Practical concentration (mmol/L) in 10 mL *	Weight of MIP-HCl in tablet $\bar{W}_{i(mg)} \pm 4.303\sigma_{n-1}/\sqrt{n}$	Efficiency of determination Recovery %	t-test: Individual t-test for compared between claim & practical value $(\bar{W}_i - \mu)\sqrt{n}/\sigma_{n-1}$	t-test: Paired t –test compared between two methods

Duspatalin, Abbott, 135 mg tab., France	**0.0550**	150.4428 ± 39.3713 (σ_{n-1}=15.8478)	111.44 %	1.688 < 4.303	$t_{cal} = \frac{\bar{X}d\sqrt{n}}{\sigma_{n-1}}$	t_{tab} at 95% confidence level (n-1)
	**** 5.1519×10^{-3} in 10mL 0.0515 in 50mL (diluted sample)**	140.8696 ± 48.1206 (σ_{n-1}=19.3696)	104.35 %			
Meva, Jamjoom Pharma, 135 mg tab., Saudi Arabia	**0.0557**	152.3563 ± 49.5787 (σ_{n-1}=19.9565)	112.86 %	1.506 < 4.303	$\bar{X}d$: 7.93275 σ_{n-1}: 6.18824 1.813 < 4.303	
	**** 5.1171×10^{-3} in 10mL 0.0512 in 50mL (diluted sample)**	140.0478 ± 40.8296 (σ_{n-1}=16.4348)	103.74 %			
Colospasmin, Eipico, 135 mg tab., Egypt	**0.0537**	146.8859 ± 46.6623 (σ_{n-1}=18.7826)	108.80 %	1.096 < 4.303		
	**** 5.2379×10^{-3} in 10mL 0.0524 in 50mL (diluted sample)**	143.3289 ± 36.4549 (σ_{n-1}=14.6739)	106.17 %			

$\hat{y}_i$: in mV for developed method and absorbance for classical method, r: correlation coefficient, r^2: coefficient of determination, R^2 %(Percentage capital R-squared): explained variation as a percentage total variation, $t_{0.025,\infty} = 1.96$ at 95% ,t_{tab}: $t_{0.025,3} = 3.182$ for n=5. $\sigma_{N-1}/\sqrt{n}$ = S.E.M standard Error of the mean, $t_{\frac{\alpha}{2}} = 4.303$ for n=3, μ: claim value (g). * Practical concentration (mmol/L) in 10 mL for newly developed methodology, ** in classical method the sample concentration diluted to 0.01 mmol/L before the addition by draw 5 mL from 0.1 mmol/L. In classical method: 5mL of 0.01 mmol/L sample, In developed method: 5mL of 0.1 mmol/L

Conclusion

The newly developed method was simple, sensitivities and rapid. The comparison between this works with classical spectrophotometric method via the t-test (the comparison tools) was shown that with no doubt that newly developed method (ISNAG procedure) is a good as the classical method. An alternative analytical method is found through this research work, which based on simple parameter conditions.

References

1. Cartwright AC (2016) The British pharmacopoeia, 1864 to 2014: medicines, international standards and the state Routledge.
2. Zayed S (2005) Simultaneous determination of mebeverine hydrochloride and sulpiride using the first derivatives of ratio spectra and chemometric methods. Analytical sciences, 21(8): 985-989.
3. Mahdi A, Z Abas (2018) Spectrophotometric Determination of Mebeverine hydrochloride in pharmaceutical preparation via Ion Association Reaction. in Journal of Physics: Conference Series IOP Publishing.
4. Lotfy HM, et al (2016) Simultaneous determination of mebeverine hydrochloride and chlordiazepoxide in their binary mixture using novel univariate spectrophotometric methods via different manipulation pathways. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 155: 11-20.
5. Derayea SM (2014) An application of eosin Y for the selective spectrophotometric and spectrofluorimetric determination of mebeverine hydrochloride. Analytical Methods, 6(7): 2270-2275.
6. El-Desoky HS, MM Ghoneim, FM El-Badawy (2017) Carbon Nanotubes Modified Electrode for Enhanced Voltammetric Sensing of Mebeverine Hydrochloride in Formulations and Human Serum Samples. Journal of The Electrochemical Society, 164(6): B212-B222.
7. Ali TA, et al (2015) Improved determination of mebeverine hydrochloride in urine, serum and pharmaceutical preparations utilizing a modified carbon paste electrode. Int. J. Electrochem. Sci., 10: 2439-2454.
8. Moskaleva NE, et al (2017) HPLC–MS/MS method for the simultaneous quantification of desmethylmebeverine acid, mebeverine acid and mebeverine alcohol in human plasma along with its application to a pharmacokinetics study. Journal of Pharmaceutical and Biomedical Analysis, 138: 118-125.
9. Chhalotiya UK, et al (2017) Thin-layer chromatography method for the simultaneous quantification and stability testing of alprazolam and mebeverine in their combined pharmaceutical dosage

form. Journal of Taibah University for Science, 11(1): 66-75.

10. Madhuri M, et al (2017) Selective and sensitive reverse phase high-performance liquid chromatography method for the simultaneous estimation of mebeverine and chlordiazepoxide in capsule dosage form. Journal of Pharmacy Research| 11(9): 1078.
11. Patel D, J Patel (2014) Development and validation of spectrophotometric, HPTLC and HPLC methods for the determination of mebeverine hydrochloride and chlordiazepoxide in pharmaceutical dosage forms.
12. Souri E, AN Aghdami, N Adib (2014) A stability indicating HPLC method for determination of mebeverine in the presence of its degradation products and kinetic study of its degradation in oxidative condition. Research in pharmaceutical sciences, 9(3): 199.
13. Jagadeesh C, R Chandan (2017) Method Development and Validation of Mebeverine HCl in Bulk Drugs by RP-HPLC Method. World Journal of Pharmacy and Pharmaceutical Sciences, 6(4): 1543-1551.
14. Shakir IM, RF Hassan (2015) New approach for determination of Mebeverine by quenched fluorescence of analytically interested species using Continuous Flow injection laser D-diode fluorimeter analyser. International Journal of Research In Pharmacy and Chemistry, 5(4):725-735.
15. Sabnis RW (2010) Handbook of biological dyes and stains: synthesis and industrial applications. John Wiley & Sons.
16. Lyday PA (2005) Iodine and iodine compounds. Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH, Weinheim.
17. Pravinata LC, Y You, RD Ludescher (2005) Erythrosin B phosphorescence monitors molecular mobility and dynamic site heterogeneity in amorphous sucrose. Biophysical journal, 88(5): 3551-3561.
18. Shakir IMA, NST (2014) Al-Awadie ISNAG Fluorimeter For Fluorescence Measurement At The Visible Region Of Electromagnetic Radiation Using Mercury Tube Lamp As A Source For Irradiation For Continuous Flow Analysis Using Solar Cells. Iraq.
19. McIlvaine T (1921) A buffer solution for colorimetric comparison. J. Biol. Chem., 49: 183-6.
20. Miller J, JC Miller (2018) Statistics and chemometrics for analytical chemistry, Pearson Education.
21. Skoog DA, FJ Holler, SR Crouch (2017) Principles of instrumental analysis. Cengage learning.

