INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

NEW APPROACH FOR DETERMINATION OF MEBEVERINE BY QUENCHED FLUORESCENCE OF ANALYTICALLY INTERESTED SPECIES USING CONTINUOUS FLOW INJECTION LASER DIODE FLUORIMETER ANALYSER

Issam MA Shakir* and Raed F Hassan

Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.

ABSTRACT

The proposed method is sensitive, simple , fast for the determination of mebeverine hydrochloride in pure form or in pharmaceutical dosage . Using Homemade instrument fluorimeter continuous flow injection analyser with solid state laser (405 nm) as a source. Where it is based upon the fluorescence of fluorescein sodium salt and quenching effect of fluorescence by mebeverine in aqueous medium. The calibration graph was linear in the concentration range 0.05 to10 mMol.L^{-1} (r= 0.9629) with relative standard deviation (RSD%) for 1 mMol.L^{-1}mebeverine solution was lower than 3% (n=6). Three pharmaceutical drugs were used as an application for the determination of mebeverine. A comparison was made between the newly developed method of analysis with the quoted value using the standard addition method. It can be noticed that there was no significant differences between the newly developed method and the quoted value by the manufacturers companies. It indicates clearly that the new method can be used for the assessment as well as the method adopted by the manufactures companies that can be described.

Keywords: Mebeverinehydrochloride, Flow injection, Fluoresceinsodium salt.

INTRODUCTION

Mebeverine hydrochloride (MH), 3, 4-dimethoxybenzoic acid 4-[ethyl-2-(4-methoxyphenyl)-1-aminobutylveratrate hydrochloride(Figure 1), is a nonspecific antispasmodic agentwhich acts directly on the smooth muscleof the gastrointestinal tract. Mebeverinehydrochloride is widely used as a relaxant agent for the treatment of gastrointestinalspasmodic disorders such as irritable bowelsyndrome [1]. It is having molecular formula $C_{25}H_{35}NO_5HCl$, molecular weight 466 and melting point 105-107 ºC. It is white or almost white,crystalline powder, freely soluble in water and ethanol (96%), while practically insoluble in diethyl ether[2].

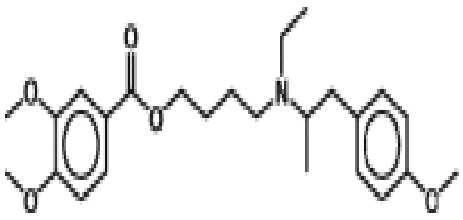

Fig. 1: Chemical structure of mebeverineHCl

Using UV- spectrophotometryfor determination of mebeverine hydrochloride [3,4,5,6],High-performance liquid chromatographic method is described for the determination of mebeverine[7,8,9,10]. Conductometricand spectroscopic method for the determination of mebeverine[11],potentiometric determination of mebeverinium ion in pharmaceutical preparations, serum and urine in steady state and flow injection (FI)[12]. This work describes the development of a new, sensitive, accurate, simple,rapid and selective method to determine the mebeverineconcentration in pharmaceuticals by quenching fluorescence.

Chemicals

Stock solution of mebeverine($C_{25}H_{35}NO_5HCl$)(0.1M) was prepared by dissolving 4.46 g in 100mL distilled water, and fluorescein sodium salt uranine ($C_{20}H_{10}Na_2O_5$)(0.01M) was prepared by dissolving 3.7627 g in 1 litter ofdistilledwater

Apparatus and manifold

A Homemade instrumentfluorimeter-CFI analyser using laser (405 nm) as a source.One channel of peristaltic pump was used (Ismatec type ISM 796), A rotary 6-port injection valve (Rheodyne, U.S.A) with a sample loop(id 0.5 mm , Teflon, Variable length) used for sample injection, coil (id 2mm, 50cm length) to complete the quenching process . The output signals was recorded by x-t potentiometric recorder(KOMPENSO GRAPH C-1032) Siemens (Germany). Peak height was measured for each signal. Figure (2) shows the flow gram that was used for the determination the mebeverine.

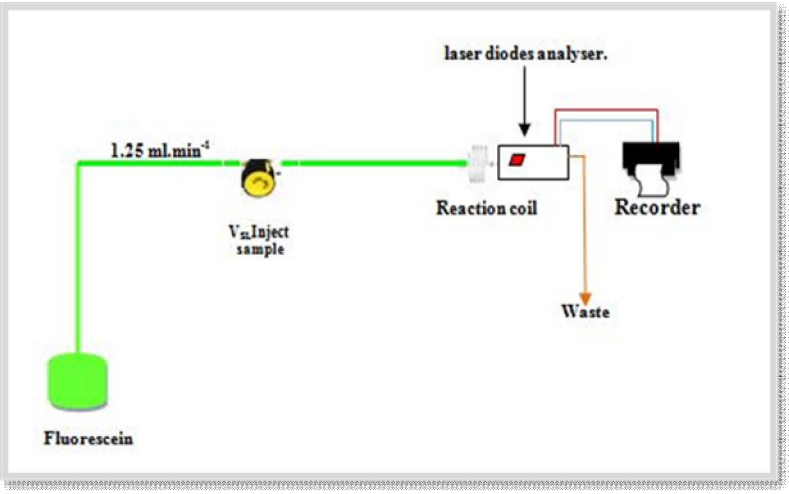

Fig. 2: A schematic diagram of flow injection analysis system for determinationof mebeverine using laser diodes CFIA analyser

Methodology

The flow diagram shown in figure (2) for determination of mebeverine by the reaction with fluorescein sodium salt to quench fluorescence which is formed in the first line.The carrier stream (fluorescein sodium salt uranine) (1 $mMol.L^{-1}$) at 1.25 $mL.min^{-1}$ flow rate to reaction with injection sample volum (35µl)mebeverine and carry the mixture to complete the reaction , with an outlet for reactants product to pass through Homemade instrument Fluorimeter-CFI analyser . The responses were recorded on x-t potentiometric recorder which measure the quenched fluorescence .

Chemical variables

1- Manifold reaction design coupled with laser diodefluorimeter CFIA analyser

The study carried out using experimental conditions, 10 $mMol.L^{-1}$of mebeverinewith 35 µL as a sample volume, (0.01,0.05,0.1,0.5,1,5) $mMol.L^{-1}$ fluorescein sodium salt and flow rate 0.95, 1.3 $ml.min^{-1}$ for the carrier (H_2O) and fluorescein respectively. This study was carried out to optimize the best manifold that can be used.

The first manifold design

One line system was used. Figure(3)shows the flow gram . Fluorescein sodium salt of various concentration (0.01,0.05,0.1,0.5,1,5) mMol.L^{-1} was used at flow rate 0.95 ml.min^{-1} and then passes to the injection valve for carrying mebeverineleading to quenching fluorescence as shown the response profile in figure(4)

The second manifold design

The manifold system is composed of two lines figure(5)The first line supplies distilled water as a carrier stream at 0.95 ml.min^{-1} which leads to the injection valve for carrying mebeverine sample. The second line supplied fluorescein sodium salt at 1.3 ml.min^{-1}. Both of lines met at Y- junctionwith an outlet for reactants product passes through laser diode fluorimeterCFIAanalyser .The experimentleading to quench fluorescence as shown in the response profile in figure(6).

As a final conclusion one line system gave regular and smooth response profile at flourescein concentration (1 mMol.L^{-1}).

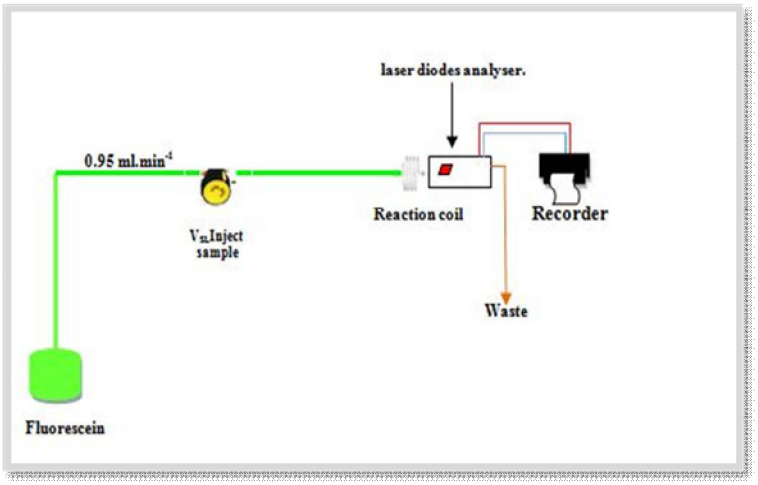

Fig. 3: A schematic diagram of flow injection analysis system for determination of mebeverine using laser diodes CFIA analyser. (Single line mode)

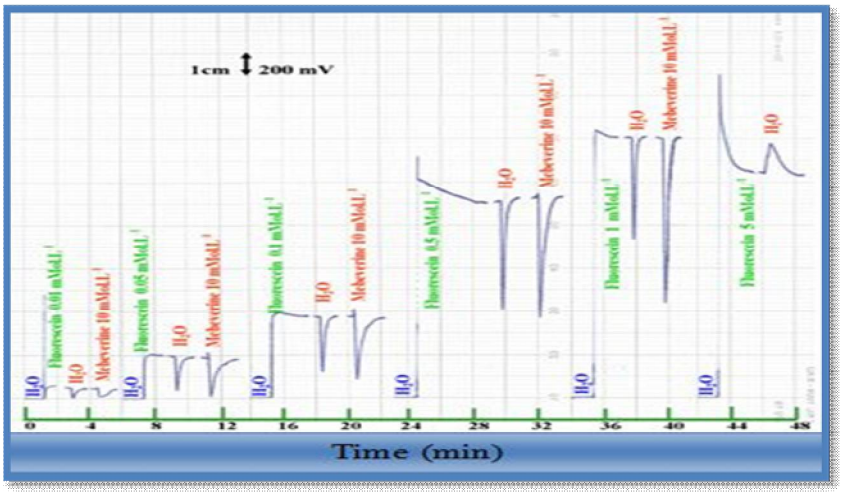

Fig. 4: Effect of the variation of fluorescein concentrationon response

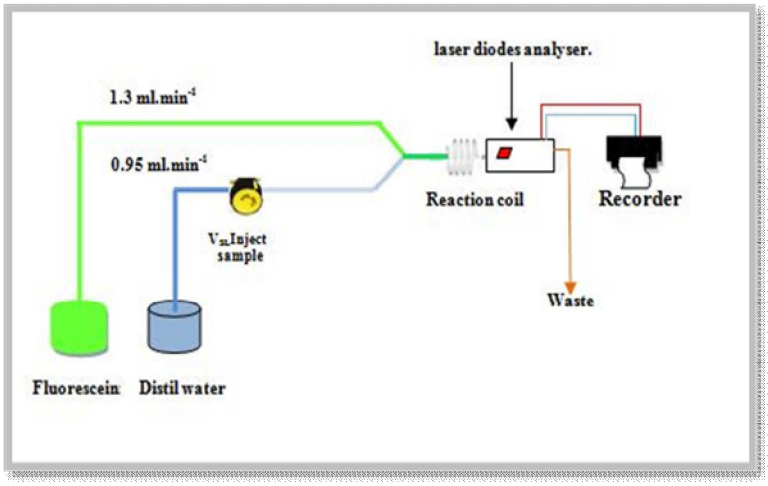

Fig.5: A schematic diagram of flow injection analysis system for determination ofmebeverine using laser diodes CFIA analyser. (Two lines system)

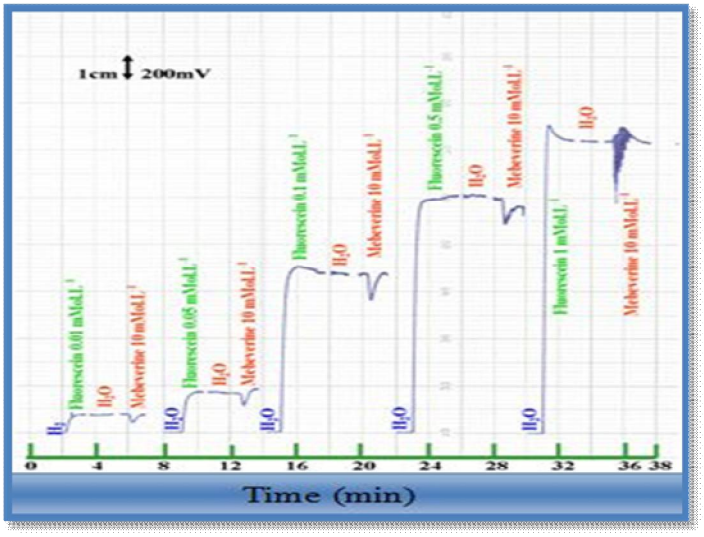

Fig. 6: Effect of the variation of fluorescein concentration on response

Variable Optimization

Physical parameters (flow rate, sample volume , purge times and volume of coil) were studied using one line manifold system figure (2).

physical variables

Flow rate

Conducting a flow rate study on fluorescence and quenched fluorescence using 10 $mMol.L^{-1}$ ofmebeverine with a carrier stream of fluorescein reveals kinds of energy transducer response output *vs*. time profile as shown in figure (7) the obtained results were tabulated in table (1). A flow rate of 0.2 to 0.9 $ml.min^{-1}$ gave a distorted profile with a time consuming measurements and difficulty in restoring the background . While a flow rate of 1.25 $ml.min^{-1}$ is an acceptable flow as it can be seen from figure (7). Taking into account measurement time, response profile and returning to the background.

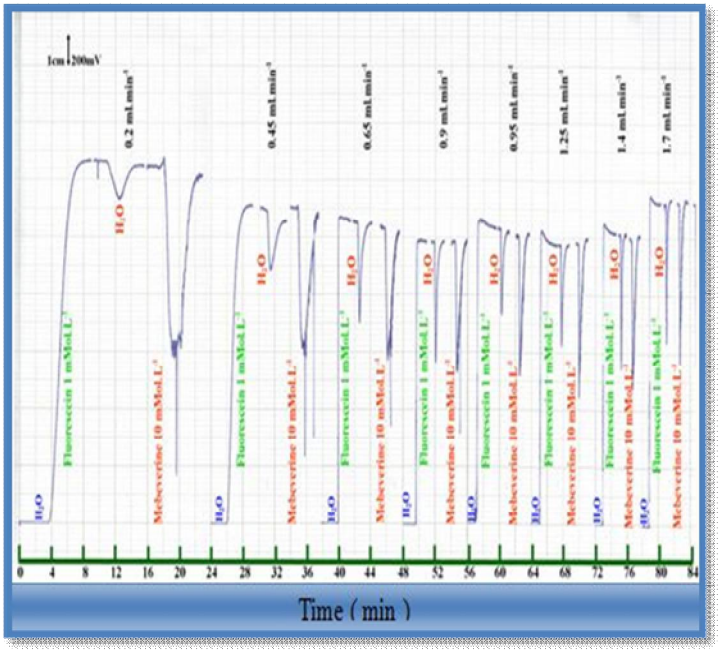

Fig. 7: Effect of the variation of the flow rate onquenched fluorescence response profile

Table 1: Effect of flow rate on the quenched fluorescence response

Flow rate $mL.min^{-1}$	Quenched fluorescence response of blank (H_2O)(mV)	Quenched fluorescence response expressed as an average peak heights (n=3) in $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y} \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$	Base width Δt_B (sec)
0.2	340	1360	2.94	99.37	420
0.45	540	760	0.00	0.00	228
0.65	900	280	0.00	0.00	168
0.9	1060	100	0.00	0.00	132
0.95	760	480	2.08	24.84	108
1.25	900	460	0.00	0.00	84
1.4	1180	160	3.13	12.42	84
1.7	1220	200	0.00	0.00	84

Sample volume

The injected volume of sample was varied from 18 to 39 µL by changing the length of the sample loop in the injection valve, while the other chemical and physical parameters were remained fixed. An increase in the injection volume led to a significant increase in sensitivity and more fluorescence that can be shown in figure (8) and table (2). That 35 µl can be described by the manifold system used.

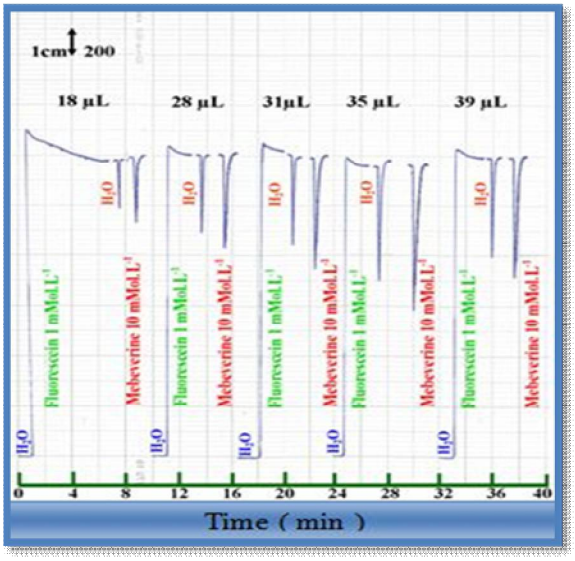

Fig. 8: Effect of the variation of the sample volume on quenched fluorescence response profile

Table 2: Effect of the variation of sample volume on the quenched fluorescence response

Sample volume µl	Quenched fluorescence response of blank (H2O)(mV)	Quenched fluorescence response expressed as an average peak heights (n=3) in ȳi (mV)	RSD%	Confidence interval at (95%) ȳ ±t0.05/2,n-1 σn-1/ $\sqrt{n}$	Base width ∆tB (sec)
18	500	160	3.13	12.42	84
28	780	180	0.00	0.00	84
31	920	200	0.00	0.00	84
35	1160	300	1.11	12.42	84
39	1000	220	0.00	0.00	84

Purge time

Variable purge times for the sample segment of 5 to 25 seconds were used in . Also 30 second was used which describe openvalve mode was used to allow enough time for the carrier solution to pass through the injection valve sample volume 35µl was used with concentration of mebeverineof 10 mMol.L^{-1}. Figure(9) shows the continuation of the increase in quenched response with increase of injection time. Complete purge of the sample from sample loop. The obtained results were tabulated in table (3) that can be described by the manifold system used.

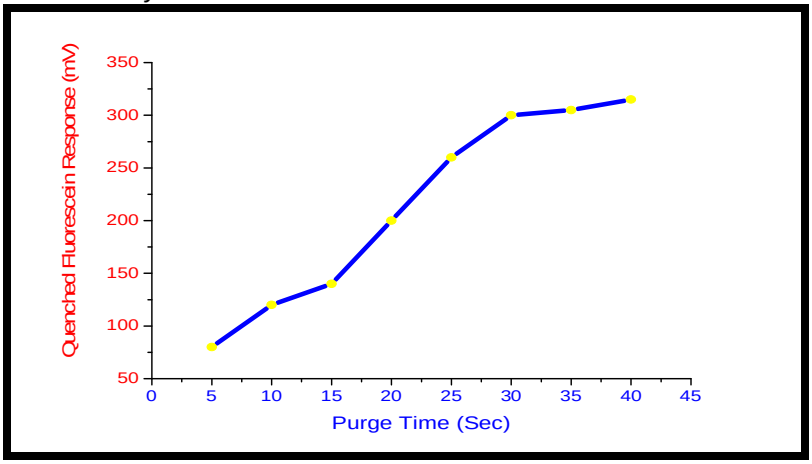

Fig. 9: Effect of the variation of the purge timeon fluorescence response profile showing open valve mode constant response

Table 3: Effect of the variation of purge time on the quenched fluorescence response

Purge time (Sec)	Quenched fluorescence response of blank (H_2O)(mV)	Quenched fluorescence response expressed as an average peak heights (n=3) in $\bar{y}_i$ (mV	RSD%	Confidence interval at (95%) $\bar{y} \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
5	360	80	6.25	80±12.42
10	580	120	4.17	120±12.42
15	720	140	3.57	140±12.42
20	880	200	0.00	200±0.00
25	880	260	1.92	260±12.42
30 Open Valve	880	300	0.00	300±0.00
35 Open Valve	880	305	2.10	300±12.42
40 Open Valve	880	315	1.80	300±12.42

Effect of coil length

Variable coil length 0 – 100 cm was studied, this range of length comprises a volume of 0.00 – 0.785 ml which is connected after injection valve directly in flow system .Optimum concentration of fluorescein sodium salt (1mMol.L^{-1}) and(10 mMol.L^{-1}) mebeverine. With sample volume (35 µL) were used. A study was noticed that the use of no coil gave a better response profile . Figure (10) describe the detailed profile . The use of no coil gave most acceptableresult . Therefore no coil was used .While maintaining the second reaction coil.

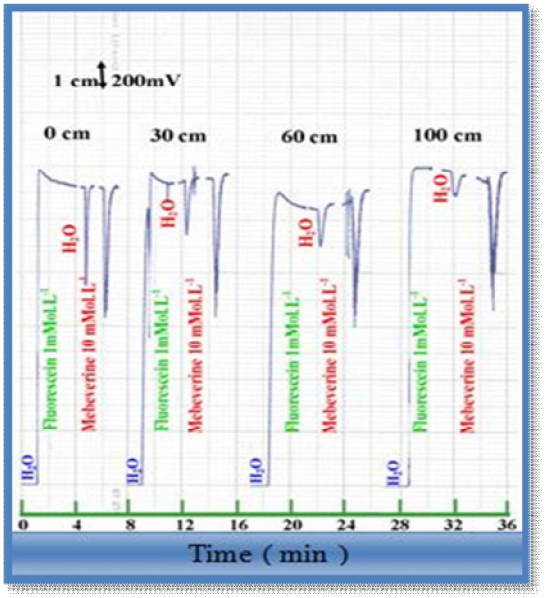

Fig. 10: Effect of the variation coil length on fluorescence response profile

Calibration graph

A series of variable concentration ranging from 0.007 to 100 mMol.L^{-1} for mebeverin were prepared and injected at the established optimum condition. Tables no. (4) tabulates The results obtained and variation of mebeverine concentration at 35 µL sample volume .Alsoquenched response of laser diode fluorimeteranalyser as shown in figure (11). While a scatter plot diagram was constructed between the variations of the quenched responses *vs*. concentration of mebeverine showing a linear dynamic range from 0.05to 10 mMol.L^{-1} figure (12). The tables no.(5) tabulates The correlation coefficient, linear percentage, straight line equation and the calculated t-value at 95% confidence; which is larger than tabulated t-value indicating clearly that the linearity against non-linearity is accepted to quenched response of laser diode fluorimeteranalyser, When using(1 mMol.L^{-1}) fluorescein sodium salt.

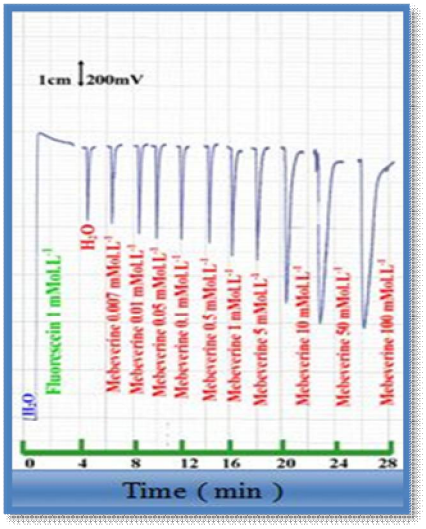

Fig. 11: Effect of the variation of mebeverine concentration on fluorescence response

Table 4: Effect of the variation of mebeverine concentration on the measurement of quenched fluorescence response
Energytransducer response of fluorescein = 2880 mV
Measurement of quenched fluorescence response of blank= 780mV

Concentration ($mMol.L^{-1}$)	Quenched fluorescence response expressed as an average peak heights (n=3) in $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y} \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
0.007	40	0.00	40±0.00
0.01	160	3.13	160±12.42
0.05	200	0.00	200±0.00
0.1	220	2.27	220±12.42
0.5	260	0.00	260±0.0
1	380	2.63	380±24.84
5	420	0.00	420±0.00
10	860	1.16	860±24.84
50	1060	0.94	1060±24.84
100	1020	0.98	1020±24.84

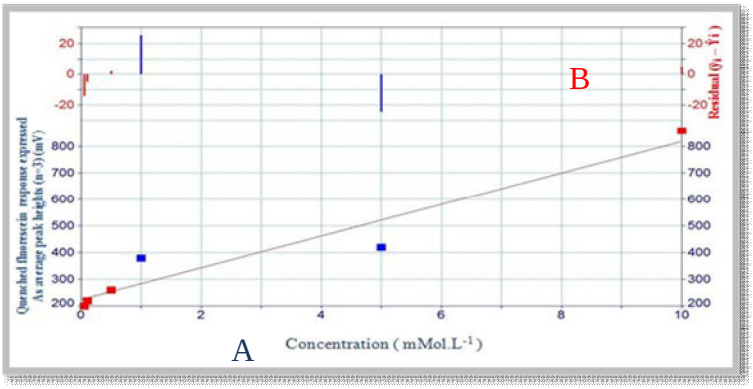

Fig. 12: Calibration graph for the variation of mebeverine concentration on: -
A: Quenched fluorescence response expressed by linear equation using laserdiode fluorimeteranalyser
B: Residual ($\bar{y}_i - \hat{Y}i$) , $\bar{y}$: practical value , $\hat{Y}i$: estimated value

1δ , 2δ

Table 5: Summary of linear regression for the variation of quenched fluorescence response with mebeverineconcentration using simple regression line of the from (Ŷ = a+bx) at optimum conditions

Measured [x] $mMol.L^{-1}$	Linear dynamic range [x]$mMol.L^{-1}$ n= 6	$\hat{y} = a \pm S_a t + b \pm S_b t$ [x]$mMol.L^{-1}$ at confidence interval at 95%, n-2	r r^2 r^2 %	$t_{table\ no.}$ at 95%, n-2	Calculated $t_{value} = \frac{\|r\|\sqrt{n-2}}{\sqrt{1-r_2}}$
0.007-100	0.05 - **10**	225.41±105.93 + 59.41±23.10 [x]	0.9629 0.9271 92.71 %	2.776<< 7.13	

Ŷ(mV) =Estimated response measurement (n=3) for each single concentration, r=correlation coefficient, r^2%:linearity percentage , x= mebeverine

Limit of Detection (L.O.D)

A study was carried out to determine the limit of detection of mebeverine via successive gradual dilution of the minimum concentration in the linear range. Table (6)shows the limit of detection conducted by linear range equation and corrected limit of detection(LOD) based on dilution factor(DF).

Table (6): Limit of detection of mebeverine at optimum parameters

Practically based on the gradual dilution for the minimum concentration	corrected limit of detection(LOD) based on dilution factor(DF).
114 ng/sample	ng/sample2.28

Repeatability

The repeatability of measurement and the efficiency of homemadelaser diode fluorimeteranalyser were studied at fixed concentrations for one of the used mebeverineconcentration (1$mMol.L^{-1}$) , Using the optimum parameters. Repeated measurements for six successive injections were measured the obtained results are tabulated in table (7) which shows that the percentage relative standard deviation indicate clearly the trustability of the adopted methodology usinglaser diode fluorimeteranalyser. Figure (13) shows a kind of response-time profile.

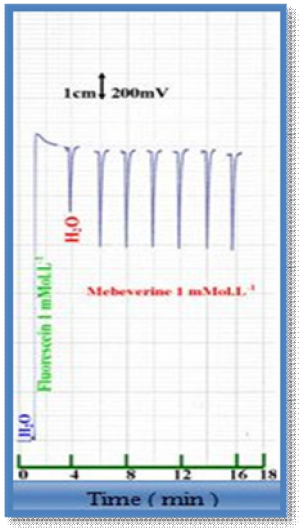

Fig. 13: A Profile of successive repeatability measurementsof mebeverine using laser diode fluorimeteranalyser

Table 7: Repeatability of successive measurements for mebeverine at (35 µL)

Energytransducer response of fluorescein = 3000 mV
Measurement of quenched fluorescence response of blank= 660 mV

Type of Measured	No. of measurements	Incident light response expressed as peak height (mV)	Average $\overline{y}_i$ mV	RSD %	confidence interval of the mean $\bar{y} \pm t_{0.05/2,\, n-1} \sigma n\text{-}1/\sqrt{n}$
mebeverine	6	320, 320, 320, 320, 340, 320	323.333	2.53	323.333 ±8.58

Application

Three different samples of pharmaceutical preparations (Colospasmin-Egypt, Meva – KSA andDuspalina- Syria) were used to determine of mebeverine. Continuous flow injection analysis using homemadelaser diode fluorimeteranalyser -Continuous flow injection analyser,.A series of solution were prepared of sample by transferring 10 mL to each of the five volumetric flask (50 ml), followed by the addition of (0,5,10,15,20,25) mL from 3 $mMol.L^{-1}$ standard solution of mebeverine in order satisfy the concentration range from 0 to1.5$mMol.L^{-1}$. Flask no.1 is the sample flask volume. Table(8)shows the summary of standard additions method results from the three samples with the amount of mebeverine in samples. Using paired t-test between the newly developed method and quoted value; as shown in Table (9). It can be noticed that there was no significant difference between the new developed method and the quoted value by the manufacturers companies. A calculated t-value which is less than critical t-value indicate clearly the new method can be used as well as the method adopted by the manufacture companies.

Table 8: Results for determination of mebeverine in pharmaceutical preparations

Commercial content country	Confidence interval for average volume at 95%	Quoted content of active ingredient at 95% n= mMol.L^{-1}	Found content of active ingredient at 95 n= mMol.L^{-1}	Recovery %	Relative Error (RE %)
Colospasmin (Egypt)	0.326±0.004	135±1.66	136.05±9.00	100.78	0.78
Meva(KSA)	0.401±0.001	135±0.438	135.90±4.68	100.67	0.67
Duspalina (Syria)	0.367±0.002	135±0.663	136.95±7.43	101.44	1.44

Table 9: Summarize Paired t-test results for laser diode fluorimeteranalyser CFIA method with quoted value usingstandard addition method for the determination of mebeverine in pharmaceutical preparations

Type of Sample	Practical content (mMol.L^{-1}) .New method		Quoted value μ	$\bar{x}$- μ	$\bar{x}_d$	σ_{n-1}	Paired t-test $\frac{\bar{x}_d \sqrt{n}}{\sigma_{n-1}} \Downarrow$	confidence interval $t_{table.}$ at 95%,
	x	$\bar{x}$						
Colospasmin (Egypt)	135.90 145.13 127.13	136.05	135	1.05	1.3	0.57	3.95<< 4.303	
Meva(KSA)	141..30 133.20 133.20	135.90	135	0.9				
Duspalina (Syria)	144.45 129.60 136.80	136.95	135	1.95				

CONCLUSIONS

Thedeveloped method was simple, fast, accurate and precise. It could be applied for the determination of mebeverinehydrocloride in pure form and pharmaceutical formulations.It can be more useful for commercial applications and as an alternative analytical method.

REFERENCES

1. Souri E, NegahbanAghdamiA and Adib N. 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. 2014;9(3):199-206.
2. Farhan Ahmed Siddiqui, NajmulHasan, NawabSherAfridi and HinaShamshad.Spectrophotometric quantitation of mebeverine in bulk drug and pharmaceutical formulations using multivariate calibration technique. Pharmacovigilance& Clinical Trials. 2013;1(4):84.
3. El-Didamony and Akram M. Spectrophotometric determination of benzydamineHCl, levamisoleHCl and mebeverineHCl through ion-pair complex formation with methyl orange.Pergamon-elsevier science ltd. 2008;69:770-775.
4. Makwana DH and Patel PB. Development and validation of spectroscopic methods for simultaneous estimation of alprazolam and mebeverine hydrochloride in bulk drug and pharmaceutical dosage form.Indo American Journal of Pharm Research. 2013;3(6):4605-4616.
5. Farhan Ahmed Siddiqui, NawabSher, NajmulHasan, NighatShafi, HinaShamshad, Mansoor Ali Beg, Ali Akbar Sial and Alisha WafaSial. Spectrophotometric Multivariate Calibration Approach: Application in Quantitative Determination of Mebeverine in Bulk Drug and Pharmaceutical Formulations.World Applied Sciences Journal. 2014;32(7):1418-1422.
6. SafilaNaveed, NimraWaheed, SafeenaNazeer and HinaRehman. Method development of mebeverine hydrochloride by using UV spectrophotometric method.International Journal of Applied Science-Research and Review. 2015;2(1):1-5.
7. Mohamed I Walash, Mohie M KhSharaf El-din, Nahed M El-enany, Manal I Eid and Shereen M Shalan. Simultaneous determination of sulpiride and mebeverine by HPLC method using fluorescence detection: application to real human plasma. Chemistry Central Journal. 2012;6(13):1-12.
8. Ravi Kumar Reddy P, Krishna Reddy V, SasikiranGoud E and Ramachandra Reddy Y.Development and validation of aultra performance liquid chromatographic method for assay of mebeverine hydrochloride. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(2):442-445.
9. Sujana K, Hamuthal MZV, Murthy VSN and Shravani N. A Novel Validated Analytical Method Development for the Binary Mixture of Mebeverine and Chlordiazepoxide in Pharmaceutical Formulation andits Application to Stress Studies. PharmaceuticaAnalyticaActa. 2015;6(1):1-6.
10. Rania N. El-Shaheny and Fathalla F. Belal. Simultaneous HPLC Determination of Chlordiazepoxide and MebeverineHCl in the Presence of Their Degradation Products and Impurities. Hindawi Publishing Corporation. Journal of Chemistry.*2015*:1-9.
11. Marwa S. Elazazy, Manal S. Elmasry and Wafaa S. Hassan.Conductometric and Spectroscopic Determination of Mebeverine Hydrochloride and the Solubility Products of its Ion Recognition Species.International Journal of electrochemical Sciences. 2012;7:9781-9794.
12. Ibrahim H, Issa YM and Abu-Shawish HM.Potentiometric flow injection analysis of mebeverine hydrochloride in serum and urine.Journal of Pharmaceutical and Biomedical Analysis. 2005;36(5):1053-1061.