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Sensitive spectrophotometric estimation of thiol drugs (Mesna) in pure, pharmaceutics and serum samples via a new method of FI system using Iron (III) nitrate nonahydrate as oxidizing agent

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Abstract

A simple, automated and sensitive continuous flow-injection analysis merging zones technique (CFIA/MZ) method was developed to determine Mesna in pure, pharmaceutical formulations and serum samples. This method included a reaction between the reagent N,N-dimethyl-*p*-phenylenediamine (DMPD) and Fe^{3+} to produce $DMPD^{\cdot+}$ radical cation and then $DMPD^{\cdot+}$ react with Mesna (MES) to produce a brawn colored product which has maximum absorbance at the wave length 428 nm. FIA/MZ was cheap, economical, accurate and precise which the detection limit was 4.0518 $\mu g.mL^{-1}$ and RSD% percent about 1.1061 % and the recovery is 99.93 %. The reaction was studied under a number of chemical and physical parameters. concentrations ranging from 10 to 500 $\mu g.mL^{-1}$, the calibration curve was rectilinear with a sample throughput of 103 $sample.hour^{-1}$. The proposed method has been applied to the estimation of Mesna in pharmaceutics and serum samples, and the gained results compared favorably to those gotten using a United States Pharmacopeia standard technique, with no significant difference in terms of accuracy and precision at the 95 percent confidence level.

Keywords: Mesna, CFIA/Spectrophotometric system, Modified detection unit, Pharmaceutics, N,N-dimethyl-*p*-phenylenediamine;

Introduction

Mesna (MES) ($C_2H_5NaO_3S_2$), M.wt = 164.181 $g.mol^{-1}$, is a thiol compound that is important. The chemical IUPAC name of MES is sodium 2-mercapto ethane sulfonate. Figure 1 shows the chemical structure of MES, utilized as an antioxidant to reduce urothelial damage in individuals receiving anticancer drugs, cyclophosphamide or ifosfamide. It has lately been employed as an antioxidant against acetaminophen toxicity in the kidney by neutralizing the highly reactive urotoxic metabolites of oxazaphosphorines locally in the urine. It oxidized to disulfide and was stabilized in medicinal formulations with EDTA, NaOH, and an inert gas environment. The reducing nature of mesna should be taken into account while developing any analytical procedures [1]. MES is officially in U.S. Pharmacopeia [2], The reference method for mesna was a titration method using 0.1N Iodine solution and 0.1 N sodium thiosulphate. There are many techniques can be determination of MES in the dosage form of pharmaceutical including Liquid Chromatographic [3], Flow Injection [4], HPLC [5-11], Spectrophotometric [12-18], SERS [19], Spectrofluorimetric [20,21] and Quantitative Estimation [22]. Although the processes are precise, many of the approaches outlined are time intensive and require multistage extraction operations. The reported spectrophotometric techniques suffer from one or more drawbacks, such as low sensitivity, by use of organic solvents, extraction issues, meticulous control of experimental variables and expensive equipment, or narrow ranges. The proposed method of Flow Injection analysis (CFIA/MZ) technique used to indirectly determine of MES by Fe^{3+}- DMPD system [23-27].

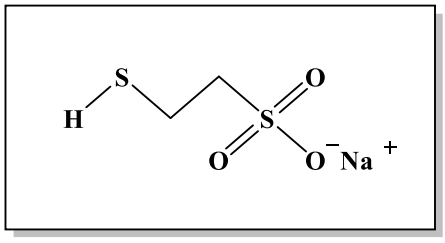


Figure 1: formula structure of Mesna.

Apparatus and FIA Manifold

The spectrophotometer with flow cell (quartz silica, 1cm) with 80 L internal volume is inside the detection unit and 1cm an optical path length is used for the absorbances such as average peak height expressed in mV (n=3) on the optima VIS 9200 by

recording the spectrophotometer with flow cell (quartz silica, 1cm) with 80 L internal volume is inside the detection unit and 1cm an optical path length is used for the absorbance. A one-channel manifold with (CFIA/MZ) for spectrophotometer MES estimation is working. A Peristaltic pump (Master flexC/L, two channel, USA) was used to pump a carrier stream in injection (distilled water) and solutions through a home-made injection valve using a power supply (Yaxun, 1501AD, China) (seven-three-way injection valve with three loops). chemicals and reagents solutions which are based on merging zones version [23,24]. The injection valve that was utilized to provide appropriate quantities of samples and reference solutions.

For peristaltic pump, flexible vinyl tubes with radius of 0.22 mm were used; the mixing and the reaction coil was constructed of glass with a diameter of 2 mm (I.D). All of the parts of the CIFA as shown in Figure 2 with details. Distilled water served as a carrier stream that was mixed with DMPD in L1, $Fe(NO_3)_3.9H_2O$ in L2 and MES in L3. Then mixed all together in a mixing coil that has length of 50 cm and the carrier flow rate is 12.8 mL.min^{-1}. The maximum absorbance was found at 428 nm for the brawn colored product.

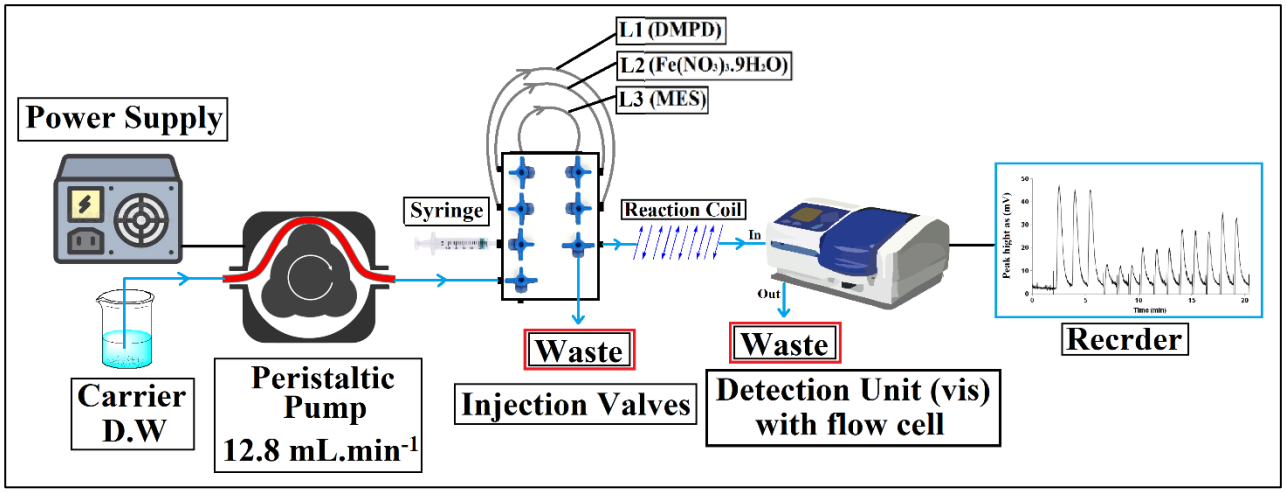


Figure 2: The developed CFIA system.

Reagents and Chemicals

All of the chemicals and reagents used were analytical grade, and they have been used to prepare all of the solutions.

- **Mesna (MES) stock solution** (M.wt=164.181g.mol^{-1}, Merck, Germany): (1000 µg.mL^{-1} = 6.1×10^{-3}M): MES (100 mg) was dissolved in distilled water and then consumed in a standard flask with distilled water to 100 mL. The diluted solutions are made by diluting the stock standard solution with distilled water to the desired concentration.
- **N,N-dimethyl-*p*-phenylenediamine (DMPD) solution** (M.wt = 362.42 g.mol^{-1}, Merck) (1.5 × 10^{-2} M) : DMPD (0.5107 g) was dissolved in 5 mL dilute HCl then the volume was made to 250mL using distilled water in standard flask and farther dilution to these solutions to obtain desired concentrations.
- **Ferric nitrate nonahydrate ($Fe(NO_3)_3.9H_2O$) solution** (M.wt= 404 g.mol^{-1}) (2 × 10^{-2} M) : $Fe(NO_3)_3.9H_2O$ (2.02 g) was dissolved in 5 mL dilute H_2SO_4 then the volume was made to 250 mL using distilled water in standard flask and farther dilution to these solutions to obtain desired concentrations.

Preparations of MES Pharmaceutical (1000 µg. mL^{-1})

The trading sources for gained pharmaceutical formulation obtainable injection from fore kinds companies were assayed by the procedure proposing. the various companies for different providers were including:

1. Mesna (200 mg) (Cipla), Cipla Limited, Injection
2. Mesna (200 mg) (Cytomed), Alkem Labratories, Injection
3. Mesna Inj (200 mg), Dabur India Ltd, Injection
4. Mistabron (300 mg), UCB India Ltd, Injection

Further solutions were diluted to prepare the concentration inside of the linearity of the calibration graph. Recovery experiment was performed by applying the standard-addition technique [28].

Preparation of Serum samples

The sample was taken from a healthy volunteer and kept at 20°C until usage after gently thawing. For serum sample preparation 100 µg.mL^{-1} was tested for accuracy and precision and analyzed thrice [26].

Result and discussion

Batch method

A 0.5 mL of 1.5 × 10^{-2} M DMPD was transferred with 0.5 mL of 2 × 10^{-2} M $Fe(NO_3)_3.9H_2O$ then increasing volumes (1-7) mL of 100 µg.mL^{-1} MES were added into a set of 10 mL standard flask and consummate the volume of the solutions to the mark with distilled water. The maximum absorbance of the brawn-colored product was found at λ_{max} 428 nm opposite the blank solution.

Absorption spectra

The last concentration of 7.5×10^{-4} M DMPD was reacted with 1×10^{-3} M of $Fe(NO_3)_3.9H_2O$ and 50 µg.mL^{-1} MES to give the colored product which was examined under visible spectrum (from 350-650) in order to determine the maximum absorbance for the complex and it was clear that the λ_{max} was 428 nm for the brawn-colored product as shown in Figure 3.

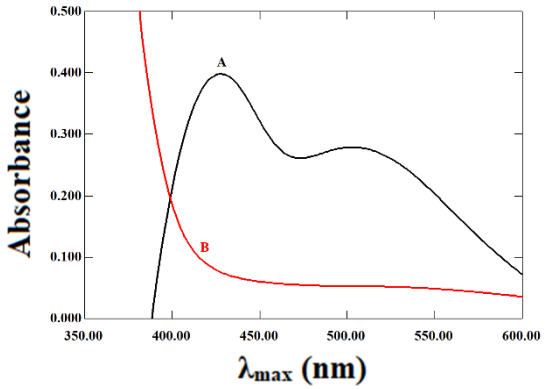
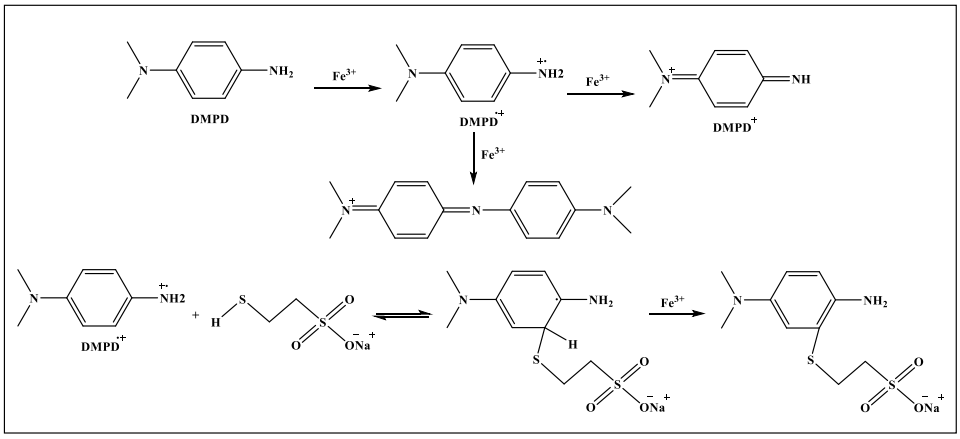


Figure 3: The Absorption spectrum of: A\ brawn colored product against blank solution (DMPD and $Fe(NO_3)_3.9H_2O$), B\ blank solution against distilled water.

The proposed mechanism of the reaction

The spectrophotometric determination of MES was based on reaction between the reagent DMPD and Fe^{3+} to produce $DMPD^{\cdot+}$ radical cation and then $DMPD^{\cdot+}$ react with MES to produce a brawn colored product (Scheme 1) [12].



Scheme 1: Mechanism of reaction between DMPD and MES. The ratio of reactions that happened through the reagent and the drug by two ways was preceded by mole ratio and continuous variation techniques (Job's method) and 1:1 ratio was for reagent and the drug as shown in Figure 4.

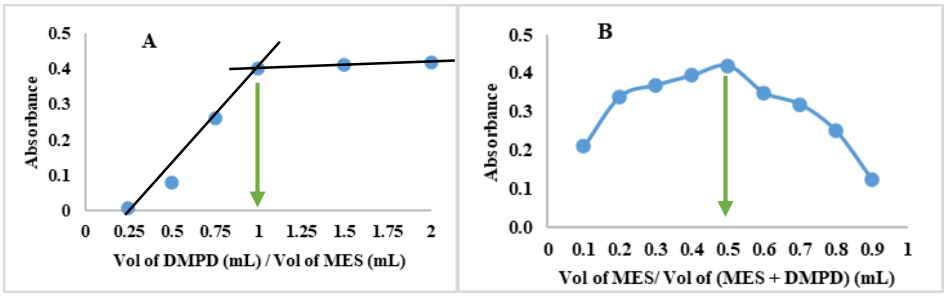


Figure 4: The complexation ratio between a reagent with drug, A\ mole ratio for the complex, B\ job's method for the complex.

Preliminary investigation

The effect of DMPD volume was examined with 50 µg.mL^{-1} MES. It has been monitored that the volume that gives the highest absorbance was 0.5 mL of 1.5×10^{-2} M DMPD and this volume was chosen for later adventures, as shown in Figure 5-A. The oxidative agent examined with MES and the best volume was 0.5 mL of 2×10^{-2} M ($Fe(NO_3)_3.9H_2O$) as shown in Figure 5-B.

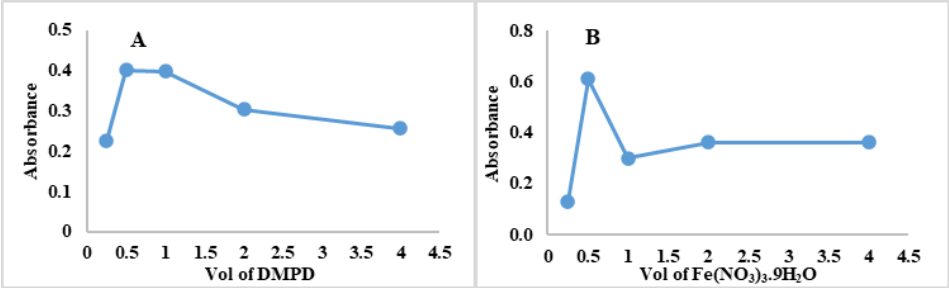


Figure 5: Chemical parameter for batch A/ volume of DMPD, B/volume of ($Fe(NO_3)_3.9H_2O$).

Calibration curve and (Precision and Accuracy) of classical method

The standard curve was constructed with a linear range (10-70) µg.mL^{-1} for the estimation of MES, as shown in Figure 6. based on the ideal conditions explained in established method, these measuring were by two different levels of MES for precision and accuracy, these results, which showed in Table 1, that the suggested method does have good precision and accuracy, and these measurements were done five times.

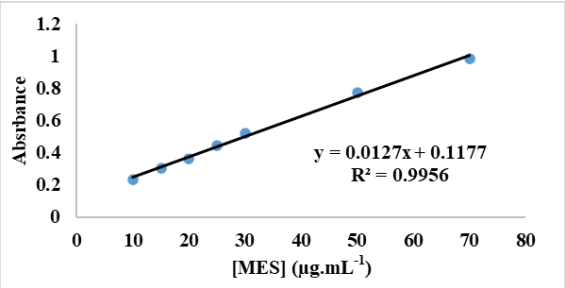


Figure 6: Linear calibration curve for determination of MES drug using Batch method.

Table 1: Precision and accuracy.

MES (µg. mL^{-1})		E	Rec%	Erel %	RSD %
Present µ	***Found $\bar{x}$**				
15	14.906	-0.094	99.37	-0.630	1.6768
30	30.449	0.449	101.50	1.496	0.6810

Average of five determinations

Calculations of stability constant

An observed stability constant [29-31] for the proposed interaction (MES: DMPD) was determined using two groups of solutions: first one does include a stoichiometric amount of MES to DMPD, while the second includes a two-fold excess of DMPD. According to the suggested mechanism and drug-to-reagent stoichiometry ratio (1:1). The reaction between MES and DMPD proceeds according to the equation:

$$\underset{\alpha C}{D} + \underset{\alpha C}{R} \rightleftharpoons \underset{(1-\alpha)C}{DR} \qquad K = \frac{[DR]}{[D][R]} \qquad K = \frac{(1-\alpha)}{\alpha\, C} \qquad \alpha = \frac{Am - As}{Am}$$

While K is the stability constant, C is the product's molar concentration (M), which is the same as the concentration of MES (1×10^{-4} M), (α) is the degree of dissociation. Where Am; As are the absorbance values of the aqueous solution, which includes a sufficient and stoichiometric quantity of reagent. The spontaneous of complex formation reaction (ΔG value) was estimated based on K evaluation as in Table 2 and the equation: ΔG = -RT lnK

where ΔG: Gibbs free energy, R: general constant of gases (8.314 J. mol^{-1}. K^{-1}), T: absolute temperature (298.15 K).

Table 2: stability constants and Gibbs free energy of the reaction.

	Am	As	α	K (L.mol^{-1})	ΔG (J.mol^{-1})
1	0.449	0.401	0.10690	781463	-33635
2	0.445	0.406	0.08764	1187837	-34673
Average				984650	-34154

CFIA/ MZ) spectrophotometric determination

After using the classical spectrophotometric technique to find the best conditions for the reaction of MES with DMPD. To examine the optimum practical settings and get spectra automated with a technique to estimate MES, the spectrophotometric reaction was automated using the flow injection-merging zones technique. As a result, the batch technique for MES estimates was used to build flow injection analysis methods.

Manifold of FIA system

With the installation of the system and its connected components, the investigation of the best design of a Homemade FIA system began. Figure 2 shows the created system, which consists of one line that delivers distilled water to the injection valve and 3 loops (various loop lengths with 0.5mm I.D.) that fill with reagents, oxidizing agents, and drugs in the order DMPD in L1, ($Fe(NO_3)_3.9H_2O$) in L2, and MES in L3.

Optimization of the developed FIA system conditions

Chemical variables

The optimal concentration of the reagent DMPD was investigated by injecting various concentrations (7.5×10^{-4}–1.2×10^{-2}) M. The concentration 1.2×10^{-2} M produced the best value of absorbance expressed as peak height in mV (n = 3) and high repeatability, which is shown in Figure 7-A. The best concentration of the oxidized agent ($Fe(NO_3)_3.9H_2O$) was investigated by injecting several concentrations (1×10^{-3} – 1.6×10^{-2}) M into a handmade injection valve, the concentration 8×10^{-3} M produced the greatest value of absorbance expressed as peak height in mV (n=3) with high repeatability, as shown in Figure 7-B. The results in Figure 7-C indicated that the best sequence is (R in L1 + O in L2 + D in L3) where D is MES, R is DMPD and O is ($Fe(NO_3)_3.9H_2O$).

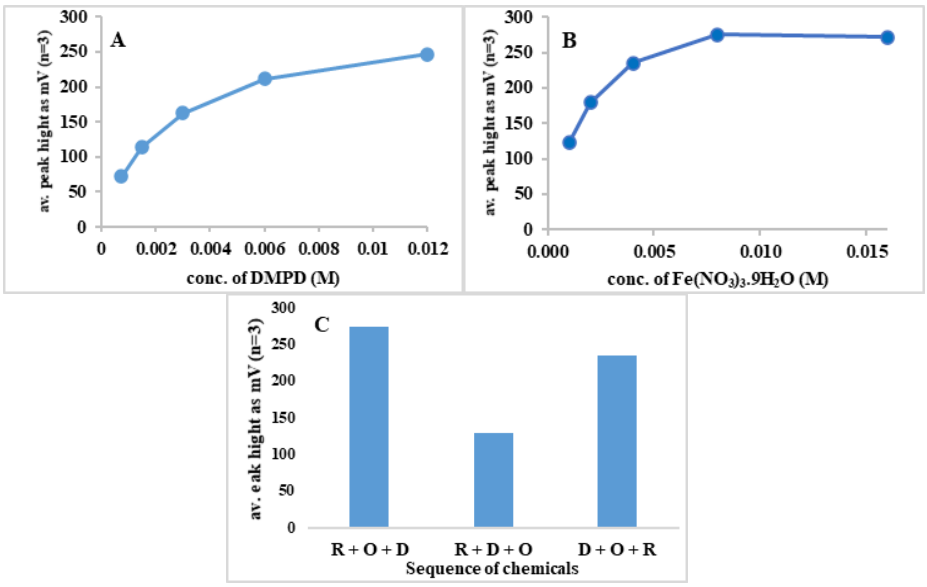
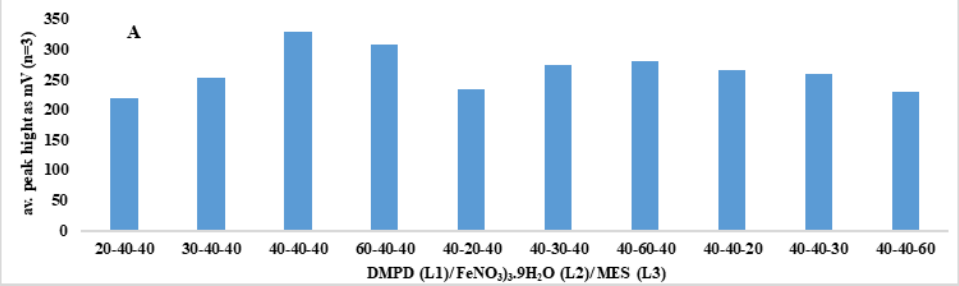


Figure 7: Effect of A\ DMPD concentration, B\ ($Fe(NO_3)_3.9H_2O$) concentration, C\ sequence of chemicals. physical variables

For the reaction, the best loop volume for reagent, oxidized agent and drug were (78.5-78.5-78.5) µL as shown in Figure 8-A and the best reaction coil length was 50 cm as shown in Figure 8-B. All available flow rates were studied for the system and that shows the best flow rate was 12.8 $mL.min^{-1}$ with sample through-put about 103 samples. $hour^{-1}$ as shown in Figure 8-C. The sampling rate was calculated based on the time it took to put the solutions into the seven three-way valve loops (15 sec.) plus the time required to maximum peak height appear (35 sec) so the sampling rate was 103 samples. $hour^{-1}$.



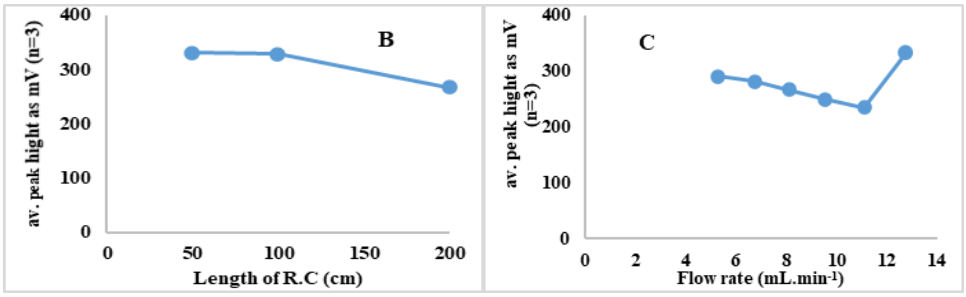


Figure 8: Effect of: A\ Injected volume, B\ Reaction coil, C \ Total flow rate.

Purge time

Using the optimal chemical and physical characteristics that were previously analyzed, the purge time for the sample segment that would be transferred into the carrier stream (distilled water) was evaluated [30]. For time intervals of 4, 8, 12, and 16 seconds, as well as an open valve, the purging time was more than 16 seconds, resulting in the maximum reaction intensity. For this reason, as indicated in Figure 9, the open valve was selected as the optimum purge time for complete sample transportation from the sample loop to the flow cell.

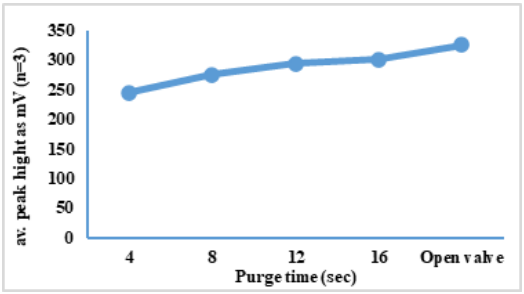


Figure 9: Effect of purge time.

Dispersion of zone

Dispersion is a physical phenomenon that occurs as a result of the interaction of various solutions with the sample in the FIA method, which is subsequently dispersed throughout the solution. FIA analytical technique success is based on three concepts [31]. (Control over the dispersion of the sample zone, repeatable injection time, and repeatable sample injection volume). The dispersion of the reaction was 1.6 as shown in Figure 10 and Table 3 The dispersion was calculated according to the law: $D = C_o/C$. the peak Without dilution (performing contact outside the flow injection system), is C_o, while the peak with dilution is C (conducting interaction inside the flow injection system). In the first experiment, all of the components were combined in an appropriate beaker, and the solution was then sent through the flow injection system (as carrier stream) to get a fixed response expressed (C_o). In the second experiment, DMPD into L1, ($Fe(NO_3)_3.9H_2O$) in L2 and MES in L3.

Distilled water is used as a carrier ($mL.min^{-1}$) in the system, and the component injected pushes the components to the reaction coil and subsequently to the detector, resulting in a response represented by (C).

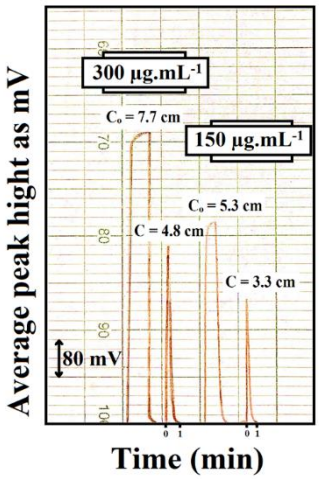


Figure 10: Dispersion of MES in CFIA system.

Table 3: Dispersion value of MES.

MES Conc. µg.mL^{-1}	C_o (cm)	C (cm)	D
150	5.3	3.3	1.6
300	7.7	4.8	1.6

Calibration curve

All ideal conditions after verbal and verified, a series of MES concentration (from 1 µg.mL^{-1} to 1000 µg.mL^{-1}) were prepared and inject to FIA system with DMPD and ($Fe(NO_3)_3.9H_2O$) in order to know the optimum range of MES concentration It can be used with this approach and it shows that the best concentration range extend (10-500) µg.mL^{-1} as shown in Figure 11 and Table 4.

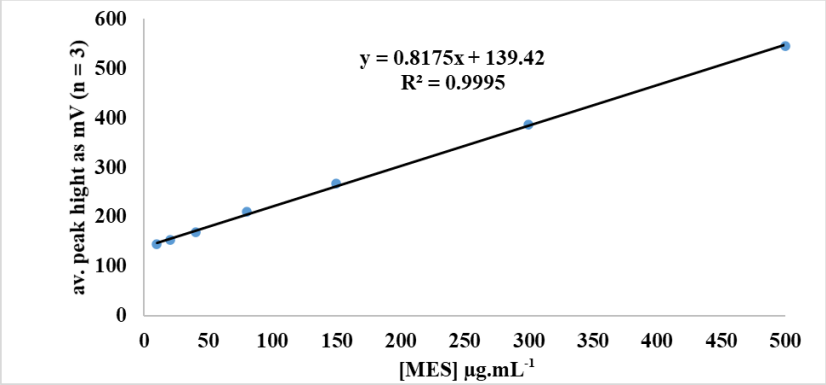


Figure 11: Linear dynamic range for determination of MES using the developed CFIA system.

Table 4: Calibration table as S.E.M for MES-DMPD-($Fe(NO_3)_3.9H_2O$) system.

conc. of MES (µg.mL^{-1})	peak height (mV)			Average response ($\bar{y}$) (mV)	RSD %	S.E.M	*E/y %
10	144	145.6	145.6	145	0.64	145 ± 2.29	1.58
20	152	154.4	152.8	153	0.80	153 ± 3.03	1.98
40	168	168.8	171.2	169	0.98	169 ± 4.13	2.44
80	208	211.2	209.6	210	0.76	210 ± 3.97	1.90
150	264	265.6	269.6	266	1.08	266 ± 7.16	2.69
300	384	388	386.4	386	0.52	386 ± 5.00	1.29
500	544	546.4	546.4	546	0.25	546 ± 3.44	0.63

* $\frac{E}{y}\% = t_{tab}\frac{SD}{\sqrt{n}} \times \frac{100\%}{\bar{y}}$ **Analysis of variation and Repeatability**

Calculate the sum of squares of the difference between the values yi (response) and I (appraiser response) for (n2) degrees of freedom, (imply error), and call (about regression) [33,34]. From the average value, calculate the summation of squares of the variance of values yi (due to regression) and for one degrees of freedom to get sum of squares $(S_1)^2$, then divide the $(S_1)^2$ on $(S_o)^2$ to get (F), as shown in the Table 5. F_{crit}. (4.7472) << F (22.7370) As a result, it's possible to conclude that there's a direct relationship between MES concentrations and signal received.

Table 5: ANOVA for the developed FIA technique.

Source of Variation	Sum. of Squares (SS)	df	Mean of Squares (MS)	F ($\frac{S_1^2}{S_2^2}$)	F crit
Between Groups (Error) $\sum n_i(\bar{y}_i - \bar{y}_{GM})^2$	**251166.97**	**1**	**251166.97 = S^2_2**	**22.7370**	**4.7472**
Within Groups (Regression) $\sum(n_i - 1)\, S_i^2$	**132559.69**	**12**	**11046.64 = S^2_1**		
Total	**383726.65**	**13**			

The repeatability of the method was good as showed in Table 6.

Table 6: Repeatability of consecutive measurement of MES (n=8).

conc. of MES (µg.mL^{-1})	Found	Error	Rec%	Erel %	RSD%
80	79.853	-0.147	99.817	-0.183	1.527
300	300.159	0.159	100.053	0.053	0.685

Methods validation

At the optimized condition, the analytical characteristics of each technique, such as the detection limit, correlation coefficient (r), relative standard deviation, and linear range, were calculated [34,35] as shown in the Table 7. For a series of MES standard answers and the fundamental analytical figure of deserts proposed by the approach, a calibration curve was constructed (Figure 11). Standard deviation for residuals ($S_{y/x}$); slope (Sb); and intercept (Sa) within 95 percent confidence limits for (n-2) degrees of freedom were given in a statistical analysis of the regression line. The small subjects were shown the high repeatability of the results obtained with high reproducibility of the proposed CFIA technique compared with the batch method. Flow injection analysis/merging zones were easier and simpler because that was rapid in analysis (sample throughput of 63 samples. hour^{-1}); large linear scale of calibration curves was obtained.

Table 7: Analytical characteristic of calibration carve for the reaction between MES and DMPD using ($Fe(NO_3)_3.9H_2O$) as oxidated agent.

Parameters	FIA method	Batch method
λ_{max} (nm)	428	428
Regression equation; y = bx+ a; y = absorbance; x = concentration (µg. mL^{-1})	y = 0.8175x + 139.42	y = 0.0127x + 0.1177
Linear range (µg. mL^{-1})	10 - 500	10 - 70 ppm
Average of recovery (Rec%)	99.93	100.43
Average of Relative Error % (Erel%)	-0.0652	0.4331
Average of Relative standard deviation (RSD%)	1.1061	1.1789
Slope (b); (mL. µg^{-1}) $b = \frac{\sum_i (xi-\bar{x})(yi-\bar{y})}{\sum_i (xi-\bar{x})^2}$	0.8175	0.0127
Intercept (a); $a = y - bx$	139.4200	0.1177
Linearity R^2%	99.95	99.56
Correlation coefficient (r): $r = \sum_i [(xi-\bar{x})(yi-\bar{y})] \sqrt{(\sum_i (xi-\bar{x})^2)\,(\sum_i (yi-\bar{y})^2}$	0.9997	0.9978
Standard deviation of slope (Sb) $Sb = \frac{S_{\frac{y}{x}}}{\sqrt{\sum_i (xi-\bar{x})^2}}$	0.0085	0.0004
Standard deviation of intercept (Sa) $Sa = S_{\frac{y}{x}} \sqrt{n \sum_i (xi-\bar{x})^2}$	1.9630	0.0140
Limit of detection (LOD) : $LOD = \frac{3.3\,SD}{b}$ **and**	4.0518	1.0299
Limit of quantification (LOQ) $LOQ = \frac{10\,SD}{b}$	13.5061	3.4330
Molar absorptivity (Ɛ) (L/mol.cm) $\varepsilon = b \times M.Wt \times 1000$	---------	2085.10
Sandell s sensitivity (S) (µg.cm^{-2}) $S = \frac{M.Wt}{\varepsilon}$	---------	0.0787
Sample through put (h^{-1})	103	5
Standard deviation of the residuals; $S_{\frac{y}{x}} = \sqrt{\frac{\sum_i (yi-\hat{y}_i)^2}{n-2}}$ $\hat{y}_i = bxi + a$	3.7954	0.0197
Confidence limit of slope (b) $CL_b = b \pm t \times Sb$	0.8175 ± 0.0209	---------
Confidence limit of intercept (a) $CL_a = a \pm t \times Sa$	139.42 ± 4.8092	---------

Effect of interferences

Excipients' potential for causing interference (such as sucrose, cellulose, lactose, glucose, and sodium citrate) was studied in order to check the accuracy of the suggested technique. A sample of pure 100 µg.mL^{-1} MES half, equal, and double fold excess concentrations of chosen interferences were spiked. The acceptable recovery values demonstrated that during the MES determination, there were no interfering factors. using new CFIA system, as shown in the Table (8).

Table (8): Interferences effect on the reaction.

Type of Interference	conc. of Interferences (µg.mL^{-1})	Average response ($\bar{y}$) (mV)	Erel%	Rec%
Standard	-----	221	0.0139	100.01
Sucrose	50	220.3733	-0.9745	99.03
	100	222.4000	1.5046	101.50
	200	220.2667	-1.1050	98.90
Cellulose	50	221.7333	0.6891	100.69
	100	221.0667	-0.1264	99.87
	200	221.6000	0.5260	100.53
Lactose	50	220.8000	-0.4526	99.55
	100	221.6267	0.5586	100.56
	200	221.7440	0.7021	100.70
Glucose	50	224.4187	3.9739	103.97
	100	221.1813	0.0139	100.01
	200	220.8867	-0.3466	99.65
Sodium citrate	50	221.6710	0.6129	100.61
	100	220.2640	-1.1083	98.89
	200	220.9240	-0.3010	99.70

Applications and assessment of suggested method

fore varieties of pharmaceuticals containing MES have been examined under the suggested approach, which are equipped with distinct sources, according to the conventional addition process. The statistical comparison [28,37] between the proposed method with official U.S. Pharmacopoeia titration method [2] using the student F-test and t-test [38,39] showed that the calculated F-test values were 0.8159 and 0.5883, t-test values were 0.516 and 0.3617 less than the theoretical F-test (9.28) and t-test (2.45) via CFIA/MZ. The FIA technique is also successfully used to estimate MES in a spiked human serum sample. The accuracy and precision of 100 g.mL^{-1} of MES were tested. Three times each concentration was examined. Table 10 shows that the serum samples have acceptable reproducibility.

Table (9): Application of the suggested techniques were compared to the official method for estimating MES in pharmaceutical formulations.

Dosage form	Proposed FIA method					Official method (theoretical)				
	conc. of MES (µg.mL^{-1})		Erel %	Rec %	RSD %	conc. of MES (µg.mL^{-1})		Erel %	Rec %	RSD %
	Present	Found				Present	Found			
Mesna (200 mg) (Cipla), Cipla Limited, Injection	80	80.12	0.1500	100.15	0.5511	80	80.02	0.0250	100.03	0.6109
	300	301.10	0.3667	100.37	0.1760	300	300.52	0.1733	100.17	0.2300
Mesna (200 mg) (Cytomed), Alkem Labratories, Injection	80	80.08	0.1000	100.10	0.5514	80	79.23	-0.9625	99.04	0.6170
	300	299.84	-0.0533	99.95	0.1768	300	300.4	0.1333	100.13	0.2300

Mesna Inj (200 mg), Dabur India Ltd, Injection	80	79.59	-0.5125	99.49	0.5548	80	80.02	0.0250	100.03	0.6109
	300	300.21	0.0700	100.07	0.1766	300	300.72	0.2400	100.24	0.2298
Mistabron (300 mg), UCB India Ltd, Injection	80	79.19	-1.0125	98.99	0.5576	80	80.39	0.4875	100.49	0.6081
	300	300.31	0.1033	100.10	0.1765	300	299.19	-0.2700	99.73	0.2310

$$t_{tab} = 2.45 \; for \; n_1 = n_2 = 4, n_1 + n_2 - 2 = 6, at \; 95\% \; confidence \; level$$

$$F_{tab} = 9.28 \; for \; n_1 - 1 = n_2 - 1 = 3, at \; 95\% \; confidence \; level$$

Table 10: Determination of MES in serum samples using suggest CFIA system.

Sample	Added Conc. (µ) µg.mL^{-1}	Found Conc. ($\bar{x}$) µg.mL^{-1}	Erel %	Rec. (%)	RSD (%)
1	100	100.04	0.0367	100.04	0.4176
2	100	98.94	-1.0642	98.94	0.5547
3	100	101.02	1.0153	101.02	0.7501
4	100	99.79	-0.2080	99.79	0.7385
5	100	100.40	0.4037	100.40	0.9862
6	100	101.63	1.6269	101.63	0.9048
7	100	100.65	0.6483	100.65	0.6250

Conclusions

Following a study of the injection analysis literature, it was observed that only some researchers were used this region-based chemical incorporation technique for thiol MES determination. So, a research plan for our work is suggested a spectrophotometric determination of thiol-sensitive in pure, sampled form doses, urine and serum using a new CFIA design. It has a larger calibration range and a higher sample rate. These procedures can be used to determine the quantity of MES in g.mL^{-1} without needing to a preceding divorce action, heating or preparation of the specimen, or solid phase extraction. The CFIA technique main advantage is their wide operating range, as well as their acceptable sensitivity and suitability for routine evaluation in pharmaceutics quality control laboratories. This is due to their expertness and their result in decrease reagents waste and toxicity of organic reagents [40,41] when comparison with batch methods and official U.S. Pharmacopoeia titration method.

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