

FULL PAPER

Determination of diosmin and quercetindihydrate through charge transfer interaction with 4-methylamino phenol and normal flow injection system with spectrophotometric detection

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In this work, we used normal flow injection analysis for determining two drugs of Quercetin dehydrate symbolized as QE and Diosmin symbolized as DSN. The aim was to develop a simple, rapid, less expensive, linear, precise and accurate technique using p-Methylaminophenol Sulfate (MFS) in the presence of an oxidative agent of Sodium per iodate in aqueous medium. After the experimental conditions were passed, ideal conditions were adopted for this work, and the detection of the highest absorption of the two drugs was reached. Detected at the Maximum wavelength, we recorded them (QE= 641 nm and DSN = 665 nm). We studied each drug by flow injection technique chosen concentration rang from (2-160) mg .ml⁻¹ for QE and DSN with MFS reagent in presence of oxidative agent draw calibration graph which gave a linear relationship with correlation coefficients of 0.9994 and 0.9989, respectively. The sample response time for each drug was 20 seconds for QE and 19 seconds for DSN and the detection limit for the proposed approach was (1.4×10⁻⁵) for QE and DSN, depending on trace drug concentration. The proposed approach was used to study and determine the two suggested medications in injections and tablets with great effectiveness.

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KEYWORDS

Diosmin; quercetindihydrate; flavonoid; antioxidant; flow injection analysis.

Introduction

Several studies on the mutagenicity of quercetin dehydrate have been reported. Quercetindihydrate (QE) (Figure 1) is chemically known as 4 H-1 -Benzopyran-4-one-2(3,4dihydroxyphenyl)-3,5,7-trihydroxydihydrate, molecular formula of C₁₅H₁₀O₇ · 2H₂O, with formula Wt: 338.26, soluble in ethanol, and insoluble in water. QE is a natural pigment and one of the most abundant antioxidants in the diet, helping

body human combat free radical damage. QE belongs to a group of plant compounds called flavonoids [1,2]. Flavonoids have been reported to contain a large spectrum of pharmacological properties. The concentration of flavonoids is reported as QE in spectroscopic methods, which is used as a standard for calibration curves [3]. Various methods have been devised for the dedication of QE in different samples of planet like excessive-overall performance liquid chromatography, capillary electrophoresis,

UV spectrophotometry at 269nm [4], dispersive liquid-liquid microextraction and copied spectrophotometry. QE is determined via complexation reaction with numerous dissimilar inorganic reagents add to drug [5]. A sensitive approach has been devised based

on charge transfer of a compound generated between QE and (4-methylamino)-phenol sulfate (MFS) reagent in the presence of sodium periodate, which is easier and more suited than the reference spectrophotometric methods.

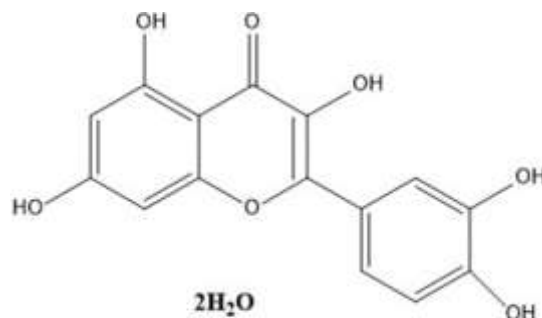


FIGURE 1 Chemical structure of QE drug

The second choice is Diosmin (DSN), which is a bioflavonoid, semi-synthetic drug, (3,5,7-trihydroxy-4-methoxyflavone-7-rutinoside). Diosmin Chemical formula $C_{28}H_{32}O_{15}$ is yellow-brown to brown powder Figure (2), with molecular mass: 608.54, insoluble in water, and soluble in DMSO and NaOH. Maximum absorption of DSN recorded in UV spectroscopy is at 268nm. Few analytical techniques have been proposed or

used for determination of the drug like voltammetry [6], HPLC [7,8], TLC [9,10] or FTIR [11,12]. Flow injection analysis and spectrophotometric methods are sensitive, suitable and comparatively inexpensive. For this project, spectrophotometric technique was used based on the response of drugs by (4-methylamino)-phenol sulfate (MFS) reagent by charge transfer interaction.

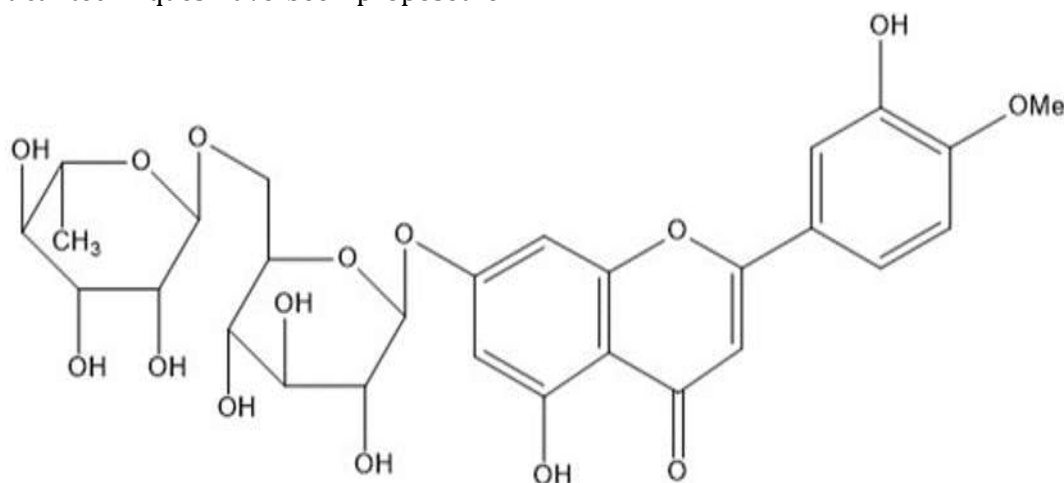


FIGURE 2 Chemical structure of DSN drug

The reagent (4-methylamino)-phenolsulfate (MFS) is the chemical name for the metol reagent. Chemical formula $C_7H_9NO_{12}H_2SO_4$ is the standard formula. Molecular mass of 172.19 g/mol as sulphate purity,

composition, and substance codes for compound sulphate are commonly used. Its function and applications are also commonly utilized. Figure 3 shows the chemical structure [13]:

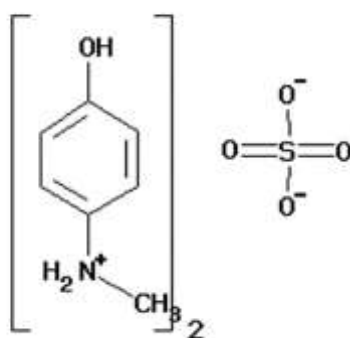


FIGURE 3 Structure of 4-methylamino-phenol sulphate (metol reagent)

Charge-transfer interaction of pharmaceuticals forms with (4-methylamino)-phenol sulfate (MFS) reagent is an important in biochemical and bio electrochemical energy transfer process [14]. Formation of stable charge-transfer (CT) compounds that results from the reaction between acceptors and drugs or biological compound [15]. The new charge-transfer interaction of drugs with (4-methylamino)-phenol sulfate (MFS) reagent has been structurally characterized via normal flow injection analysis. In this study, two pure standard solutions of pharmaceuticals forms (QE and DSN) were used to delve into interaction by charge transfer with p-Methylaminophenol Sulfate (MFS) in the presence of oxidative agent of Sodium per iodate in aqueous medium.

Experimental work

Chemicals and apparatus

Apparatus

Shimadzu spectrophotometer UV/VIS 260 digital double beam (Japan), with the use of 1cm path length and 50 μ L volume flow cell was applied. A peristaltic pump and an injection valve were prepared form Germany, Knauer. Flexible vinyl and Teflon tubing (0.5 mm) were used for various projects.

(4-methylamino)-phenol sulfate (MFS): or metol reagent solution (0.005 M)

The stock solution was prepared by mixing 0.8612 g of MFS reagent with M.Wt:172.19 mol^{-1} in 10 mL ethanol with 90 mL distilled water and volume was completed to mark in volumetric flask.

Sodium per iodate NaIO_4 (0.02 M): Oxidizing agent was used weighing about 0.428g of NaIO_4 (M. wt. 213.29 g mol^{-1} , purity 99%) dissolved in 5 mL distilled H_2O and the volumetric flask was completed to 100 mL.

Stock solution QE: (200 $\mu\text{g mL}^{-1}$) was organized by dissolving 0.02g of QE in 10mL of Ethanol and volume was completed with distilled water in 100 mL volumetric flask, stored at 8°C, and used within 1 week.

A stock standard DSN: (200 $\mu\text{g mL}^{-1}$) was prepared by dissolving 0.02 g DSN in 10 mL of 0.5 M (5%) sodium hydroxide. The solution was diluted with doubly-distilled H_2O . The stock solution was stored for 2 weeks in dark place [16].

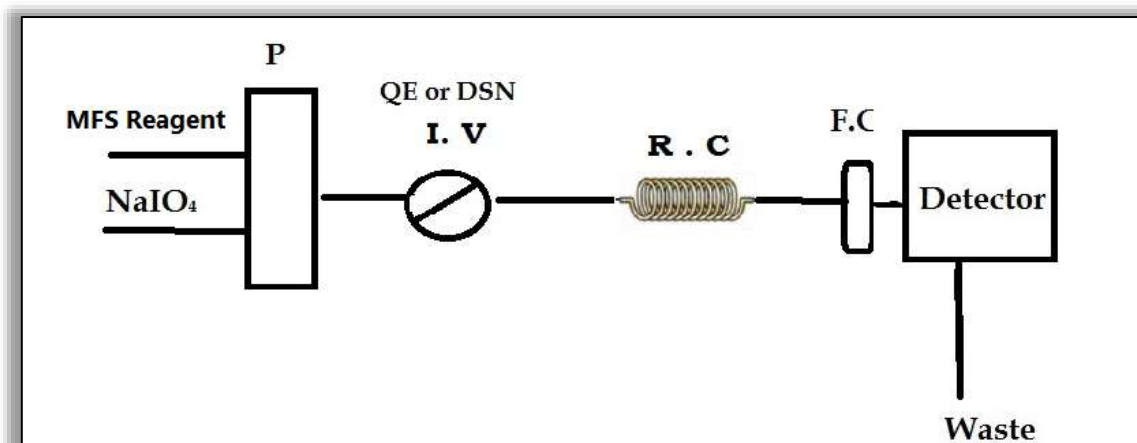
Sodium Hydroxide (0.5 M): It was prepared via dissolving 2g of sodium hydroxide in distilled water and then completed the flask to the mark with the same solvent to 100 mL.

General normal FIA procedures

Two channel manifolds were used for the spectrophotometric willpower of two drugs, solution of (4-methylamino)-phenol sulfate (MFS) reagent in 0.005 M for (2 – 160) $\mu\text{g mL}^{-1}$

¹QE and DSN drug prepared from stock solution. QE 200 μL portion was injected in the stream of 0.02 M NaIO_4 and was then joint by a stream of 0.005 M MFS reagent with a total movement rate of 2 mL/min and mixing the solutions in 150 cm reaction coil. The colored product absorbance measured at 641

nm for QE drug 200 μL portion was injected into the stream of the of 0.02 M NaIO_4 and 0.005 M MFS solution with a total flow rate of 2 mL/min. The reagent was mixed in 150 cm reaction coil. The colored product absorbance was measured at 665nm. Then, FIA manifolds were used as shown in Scheme 1.



SCHEME 1 Schematic manifold diagram for method (I.V, Injection valve; RC, reaction coil; P, Peristaltic pump; Detector; F.C, Flow Cell; waste; MET, metal solution and NaIO_4

Results and discussion

Absorption spectra

Determining QE and DSN drugs have been reported by charge transfer interaction (scheme 2) with the reagent solution (0.005 M) (4-methylamino)-phenol sulphate (MFS) to give a colored products at 641 nm for QE and 665 nm for DSN in the presence of (0.02 M) an Sodium per iodate(NaIO_4) oxidizing agent.

Interaction mechanism and pathway

Charge transfer interaction, an electronic transition among orbitals which can be centered on exclusive atoms, is called rate switch transition and absorption band is typically very sturdy. These transitions involve electron transfer from one part of a compound to another one. The electronic absorption spectra of all compounds in two solvents of ethanol and water were studied. The conc. of 0.0006 M to QE and 0.00033M to

DSN were prepared to interact with MFS in the presence of oxidative agent sodium periodate and UVV scan ranged from 200-800 nm. Figures (5 [A and B]) exhibit mainly two bands with $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions due to allowed transition of the electrons between energy levels. The both drugs QE and DSN are shown two types of transition at the wavelength (λ_{max} of 641 nm for QE and λ_{max} 665 nm for DSN). Absorptions fall inside the observed place; they frequently produce rich colors. The interaction given in Scheme 1 was assumed. The preoccupation spectrum of the blank is assumed in Figure 4 but colored product for drugs (QE and DSN) is assumed in Figure 5. Under the interaction circumstances, the color product might be stared as a kind of charge switch interaction. The rate transfer might be supposed to be captivating location related to electron transmission after the upper most busy (π) molecular detour of phenolic multiple (tablets) to the empty bottom molecular orbital (π^*) of four-methyl amino phenol.

Charge-transfer transitions are diagnosed by their excessive intensity and the sensitivity in

their dynamisms to solve polarity (Figures 6, 7 and 8).

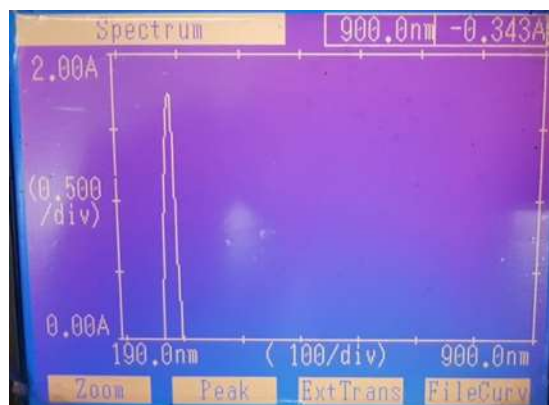


FIGURE 4 Spectrum of blank complexes

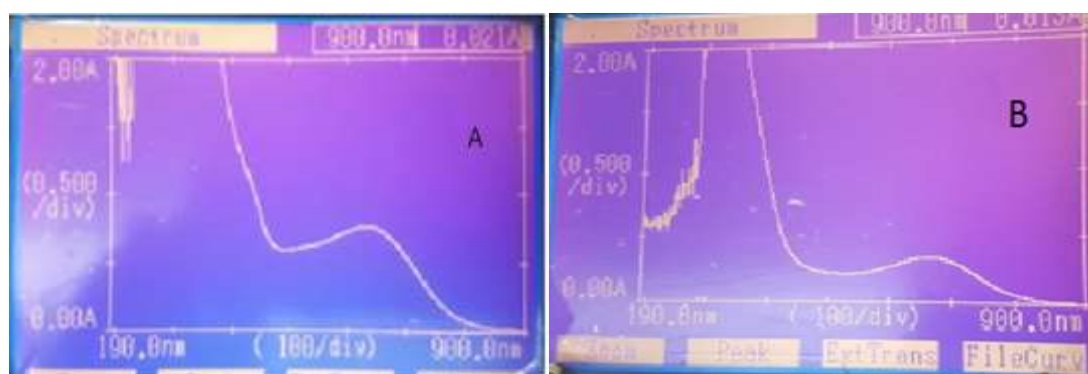


FIGURE 5 Spectrum (A) of QE product after charge transfer interaction: Spectrum (B) of DSN product after charge transfer interaction: presence of reagent solution (0.005 M) and Sodium periodate solution (0.02 M) oxidizing agent

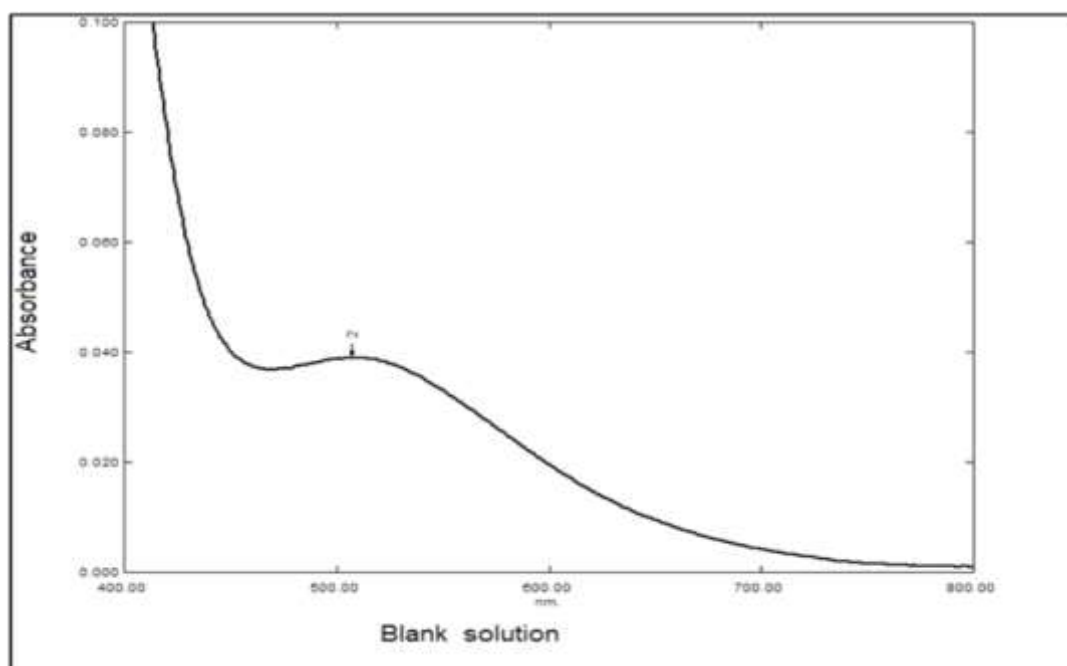


FIGURE 6 Spectrum for blank

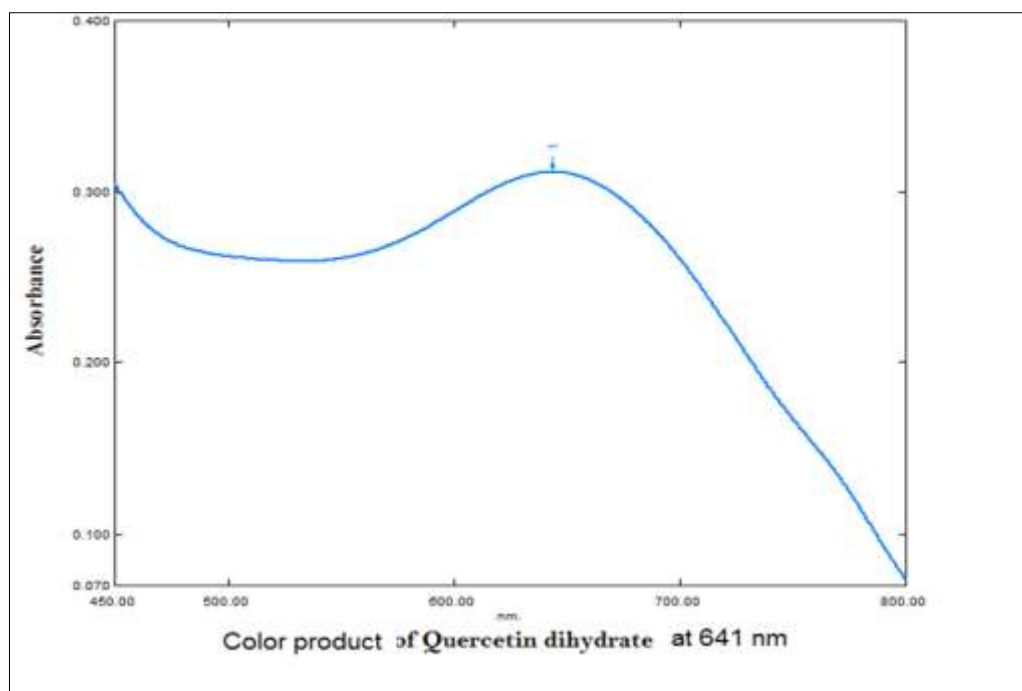


FIGURE 7 Spectrum of color product QE

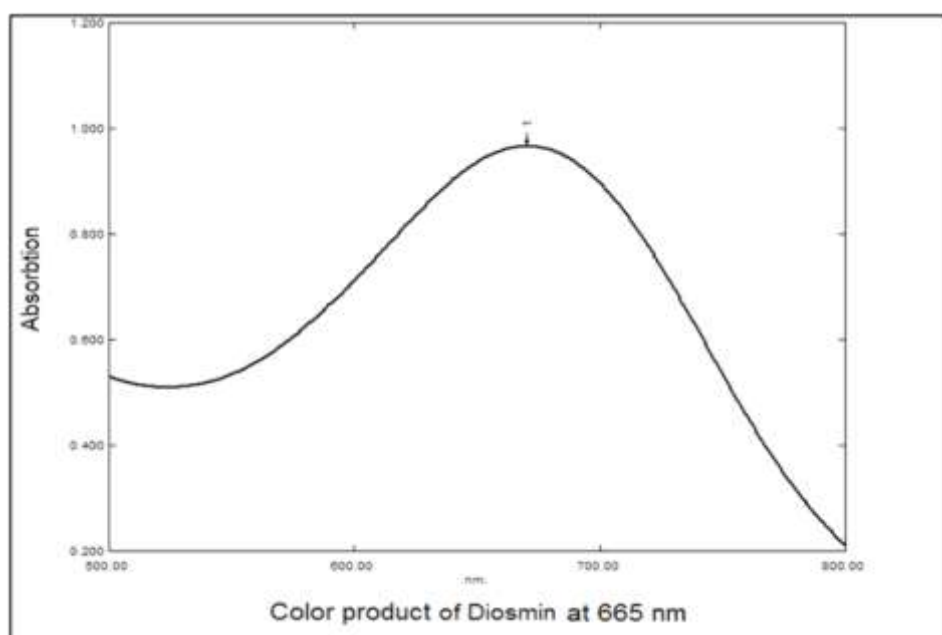


FIGURE 8 Spectrum of colour product of DSN

Optimization of chemical parameters

The chemical variables that impact in the presentation of the future method were specified. The optimum conditions method for all QE and DSN drugs were selected based on calibration curve, reproducibility, throughput and sensitivity.

Effect of (4-methylamino)-phenol sulfate (MFS) reagent concentration

The method for each drug (QE and DSN) included taking different concentrations of (4-methylamino)-phenol sulfate (MFS) reagent in the range of (1×10^{-3} to 10×10^{-3}) M examined with test approved in 10 mL bottle covering $50 \mu\text{g mL}^{-1}$ of (QE and DSN)

with 1 ml of NaIO_4 (0.02M) for measuring absorbance in normalflow injection method. It was found that the maximum absorbance intensity was obtained at 0.005 M to QE and

DSN. A decrease in absorbance occurred above these concentrations. Consequently, the concentration of 0.005 M was selected for further use as given in Figure 9.

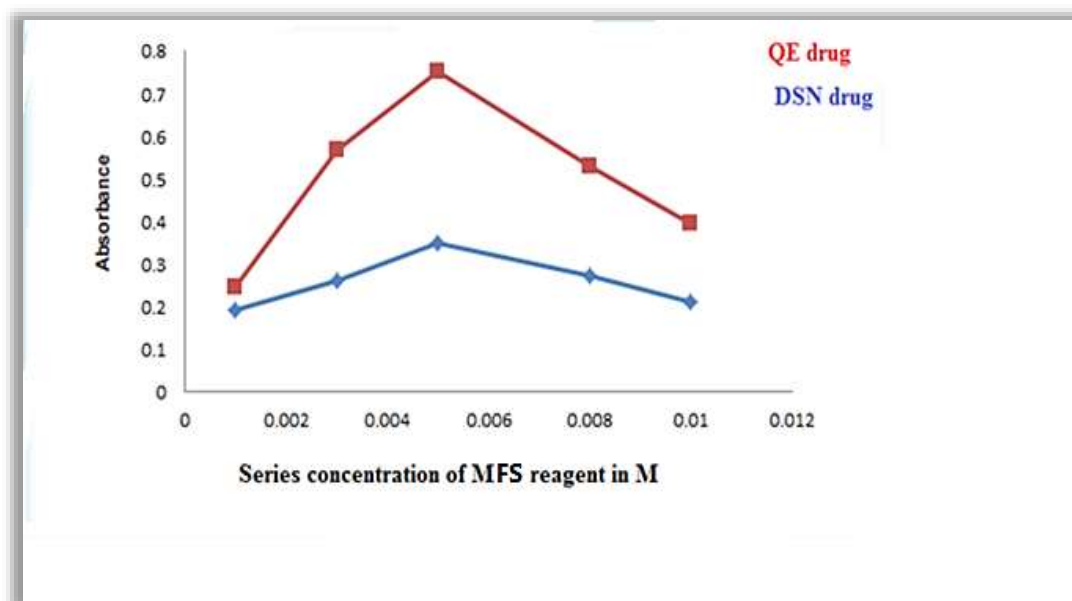


FIGURE 9 Series change concentration of MFS reagent with drugs

Effect of oxidative agents

As given in Figure 10, in this work we used various types of oxidative agents including NaIO_4 , $\text{K}_3[\text{Fe}(\text{CN})_6]$, $\text{K}_2\text{S}_2\text{O}_8$, FeCl_3 prepared concentration 0.02M from each agent for experimental conditions to obtain the best agent. Normal flow injection analysis

recorded the maximum absorbance of sodium periodate to oxidized (4-methylamino)-phenol sulfate (MFS) reagent to react with $50 \mu\text{g mL}^{-1}$ of each of the two drugs (Figure 11); the response concentration of oxidative agent of sodium periodate in maximum absorbance was explained at 0.02 M as the best one for QE and DSN.

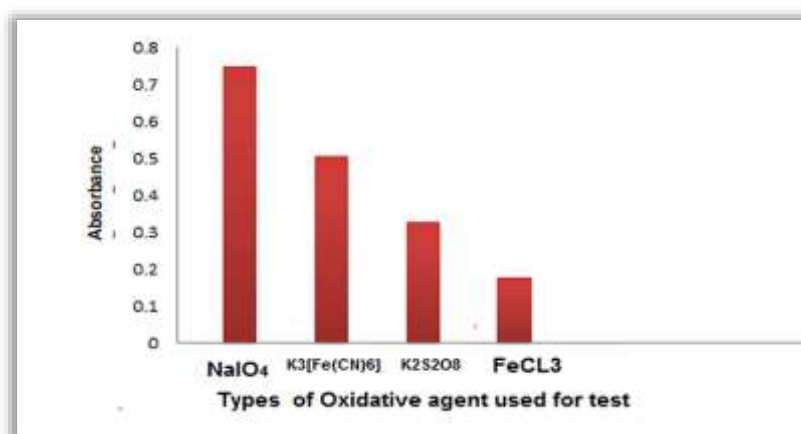


FIGURE 10 Type of oxidant agents used for testing each drug

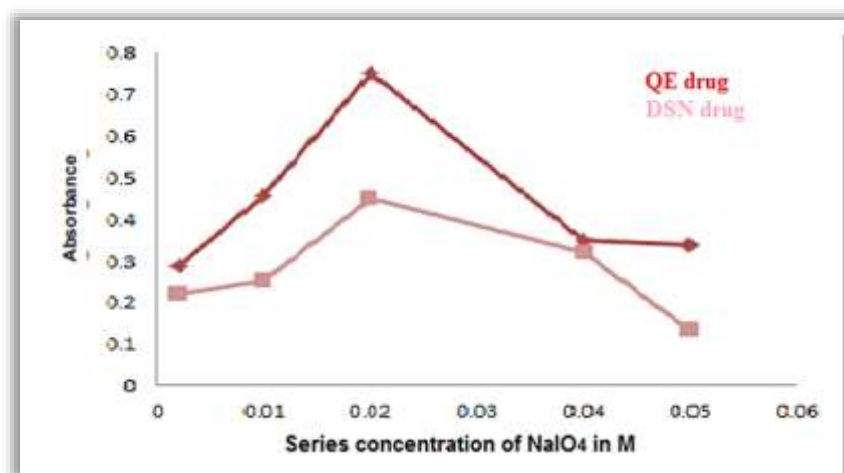


FIGURE 11 The effect of NaIO₄ concentration (M)

Optimum conditions in this work

Effect of physical parameters for experimental method (normal flow injection analysis) was measured for two drugs (QE and DSN). The optimization conditions were realized by altering one change condition and the others at steady state. Table 1 summarizes the optimization conditions for both analyte drugs. The result of entire movement rate on the response of the method was examined in the range of (0.8 to 2) mL min⁻¹ correspondingly (Figure 12). In each carrier solutions in normal flow injection analysis, the sign absorbance was sharp up to 2 mL min⁻¹ for drugs; therefore, the flow rates of 2 mL min⁻¹ were designated as optimum movement solutions for (QE and

DSN) correspondingly, which will be used in the next studies. The second effect is injection sample volume (Figure 14). The volume (60 to 200 μL) was injected into the carrier stream for each drug. It can be understood that 150 μL as an injected volume logged the best response for QE and 100 μL for DSN at higher absorbance. As third effect, length of reaction coil of mixing coil length on the reply changed into enhanced in range of [0 (without reaction coil) to 250] cm. Rendering to the consequences (Figure13), lengths of 75 cm changed into recorded as top-quality period that provided the most absorbance for the colored merchandise for every QE and DSN correspondingly and may be utilized in subsequent research.

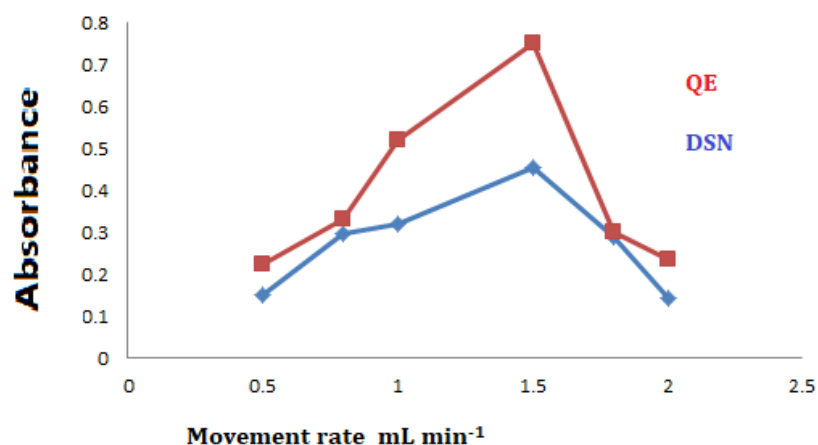
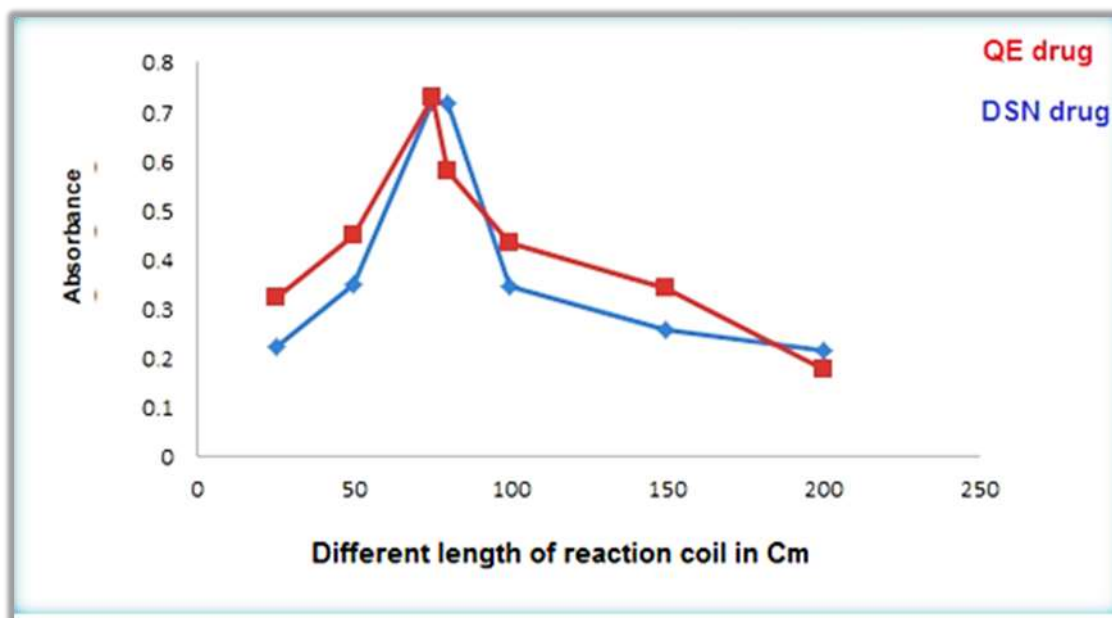
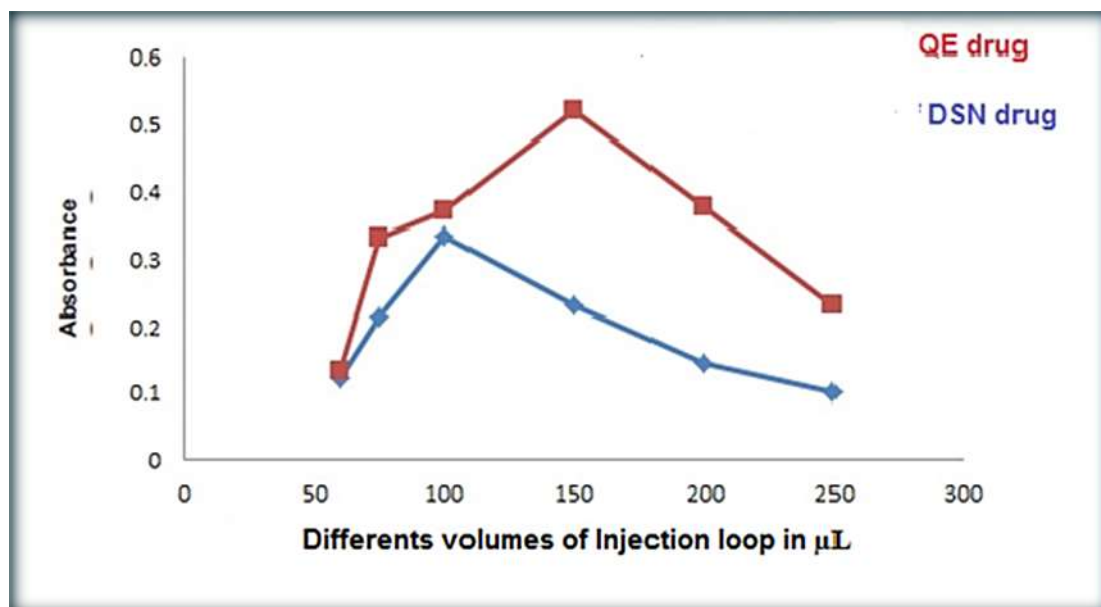


FIGURE 12 Movement rate on the response of the method give maximum absorbance for QE

TABLE 1 The experimental conditions for two pharmaceuticals forms (QE and DSN)

Parameter	QE	DSN
Total flow rate (mL.min ⁻¹)	2,5	2,5
Sample volume (μL)	150	100
Reaction coil (cm)	75	75
λ _{max} (nm)	641	665

**FIGURE 13** 75 cm length of reaction coil gives higher absorbance for QE**FIGURE 14** 150μL volume gives higher absorbance for QE, while 100 μL volume gives higher absorbance for DSN

Calibration graph

This specific wavelength corresponds to the maximal absorbance value in drugs from pure

standards. Thus, measuring absorbance at 641 nm provides a useful quantitative estimation of total QE content, while measuring absorbance at 665 nm provides a

useful quantitative estimation of total DSN content. Therapy of the colored products gives response absorbance and plotted against the attentiveness of pharmaceuticals forms contingent on the designated limits as mentioned in Table 1. Two series of drugs solutions were chosen in the range of (2-160)

$\mu\text{g mL}^{-1}$. To obtain linear relationship, we followed Lambert Beer law (Figure 15). Table 3 summarizes the other analytical statistical treatments calculated for the calibration curve. Dispersion of colored product was studied by using optimum conditions and the nFIA manifolds.

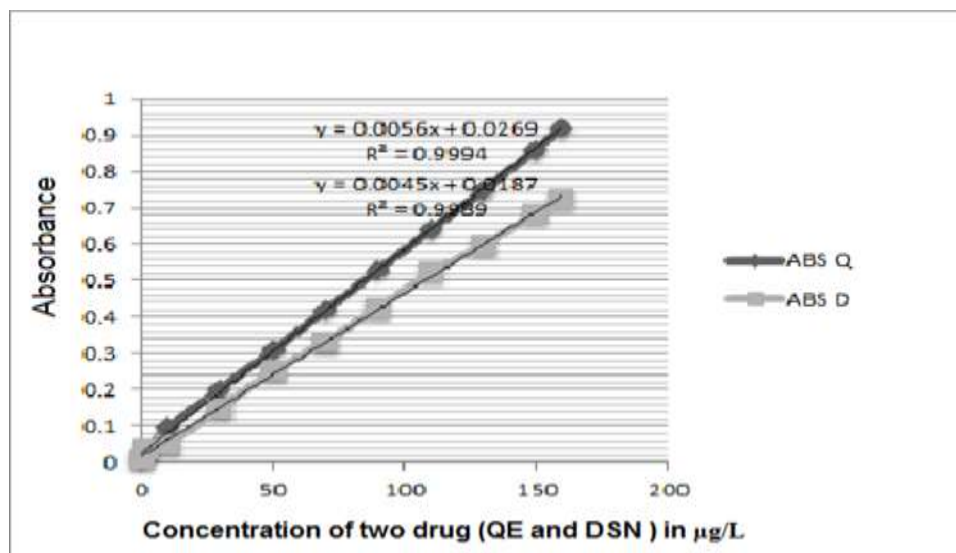


FIGURE 15 Calibration curve for estimation of QE and DSN

From the calibration curve, absorbance of pharmaceuticals forms against (0-160) ppm concentration and linear equation can be drawn. It is easy to apply the statistics

including the collection and interoperation of numerical data, and the estimation analytical values that are given in Table 2.

TABLE 2 Analytical values of statistical treatments for the calibration graph

Parameter	Quercetindihydrate	Diosmin
Leaner equation	$0.0056x+0.0269$	$0.0045x+0.0187$
Coefficient correlation, r	0.9994	0.9989
Linearity percentage, r^2 %	99.94	99.89
Linearity ($\mu\text{g.mL}^{-1}$)	2-160	2-160
Slope, b ($\text{mL}.\mu\text{g}^{-1}$)	0.0056	0.0045
Intercept, a	0.0269	0.0187
Time response for drug	20 Sec	19 Sec
Dispersion	1.096	1.71
Standard deviation SD	0.331	0.264
Molar absorptivity ($\text{L mol}^{-1}\text{cm}^{-1}$)	2019.5	2895.8
LOD ($\mu\text{g.mL}^{-1}$)	1.4×10^{-5}	1.4×10^{-5}
LOQ ($\mu\text{g.mL}^{-1}$)	4.51×10^{-4}	4.5×10^{-5}
Sampling rate (per hour)	20	19

Accuracy and precision

As shown in Table 3, both drugs were assessed by injections of pure drug solution

at dissimilar attentions. The precision and accuracy of the results presented in Table 3 tell us that the closeness of repeated measurement from each other means a

measure of internal agreement among a set of replicate observations while accuracy shows numerical differences between an analytical

result and accepted value for the measure quantity.

TABLE 3 Accuracy and precision for both suggested drugs (QE and DSN)

Conc. of drugs	Present	Found	E %	Rec. %	RSD %
QE($\mu\text{g}/\text{mL}$)	30	30.38	1.27	101.26	0.508
	50	50.73	0.0146	100.015	0.322
	70	70.49	0.007	100.007	0.363
DSN ($\mu\text{g}/\text{mL}$)	30	31.03	3.433	103.433	1.343
	50	51.77	3.54	103.54	0.606
	70	70.73	1.043	101.043	0.594

Stoichiometric measurements

Job's method of continuous variation [17,18] was applied to determine the measure of the measurable association amid the crops and reactants of a assumed chemical interaction in footings of their comparative relations of mass or volume of a binding by keeping the molar concentrations of reactants constant of drugs solutions with $A\ 1.0 \times 10^{-4}\ \text{M}$ normal solution with drugs base. A sequence of solutions was ready in the entire volume of

drug and MFS substance was reserved at 10 mL for drugs and reagent, correspondingly. The absorbance was measured in normal flow injection with UV absorbance detector at the optimum wavelength for each drug color product. We explain determining drugs with reagent by the incessant differences method (Job's method). The results showed that 1:1 (drug: dye) shaped the electron charge transfer amid drugs and 4-methylaminophenol. The results obtained are represented in Figure 16.

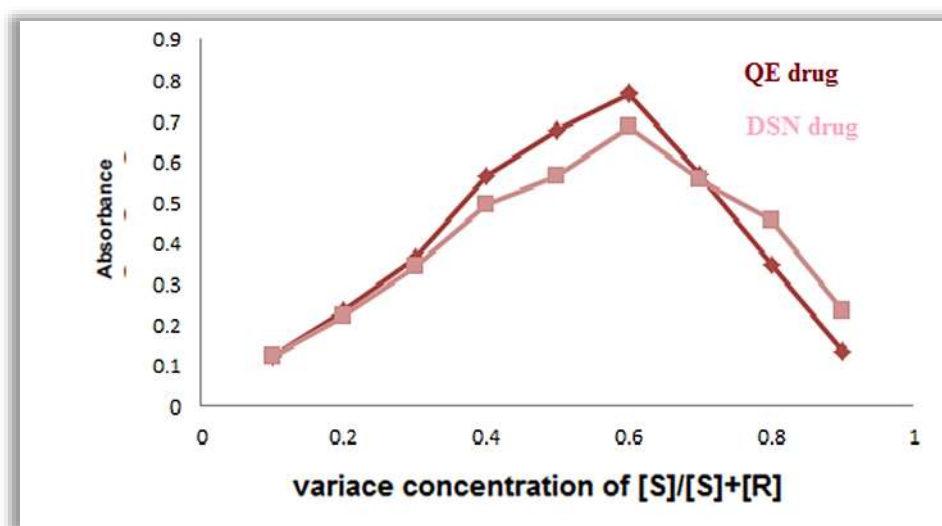
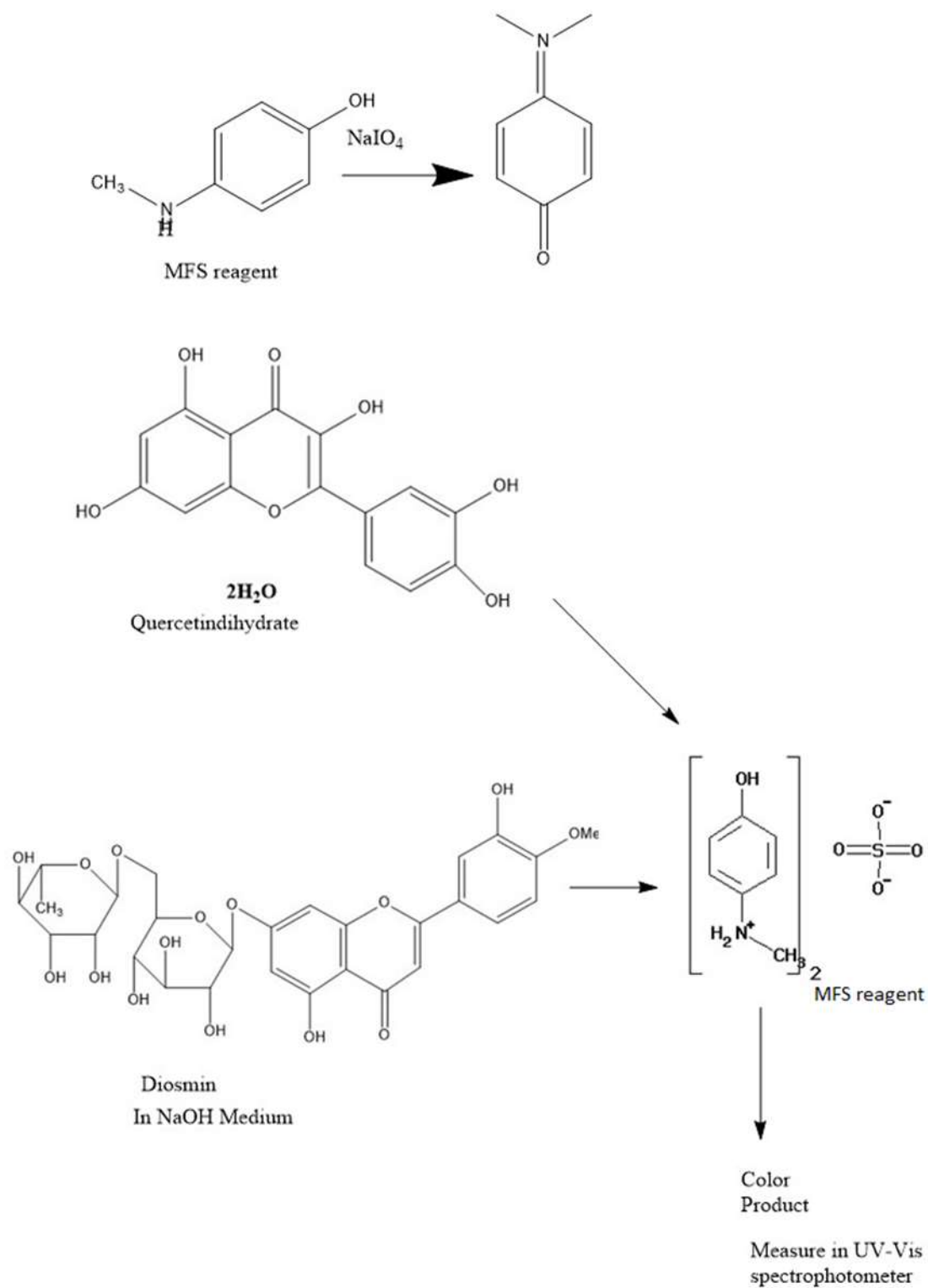


FIGURE 16 Job's method or continuous variation graph for the reaction of each (QE and DSN) with MFS reagent

In continuous variation method, volumes 0.5 – 4.5 mL of 0.005 M portions of MFS (VS) were diazotized using its equimolar and coupled according to analytical procedure with the corresponding complementary

volume of each drug to give a total volume of 5 mL for VS+ VR in 2 mL of 0.02M sodium periodate and diluted to 25 mL with distilled water.



SCHEME 2 Proposed mechanism of the reaction between drugs with MFS reagent

TABLE 4 Application of the suggested method on determination of QE and DSN in supplements

Supplements	Conc. µg /mL		E %	Rec. %	RSD %
	Present	Found			
Mega Quercetin (1200 mg)	30	30.38	1.25	101.25	0.507
	50	52.64	5.27	105.28	0.476
	70	72.70	3.85	103.85	0.230
Diosmin(capsules 750 mg)	30	30.36	1.19	101.19	2.248
	50	51.84	3.68	103.68	1.161
	70	70.28	0.413	100.41	0.472

*Average of five determination

According to the difficulties of using the standard method for determination of QE and DSN in its pharmaceutical preparations, so that a standard addition method has been used for its simplicity which proves that the proposed method was applied successfully for the determination of drugs method of

standard additions used to overcome the matrix effects, which cause the reduction or enhancement of analyte signal. Equal volumes of example solution are occupied and results planned as shown in Figure 17 below:

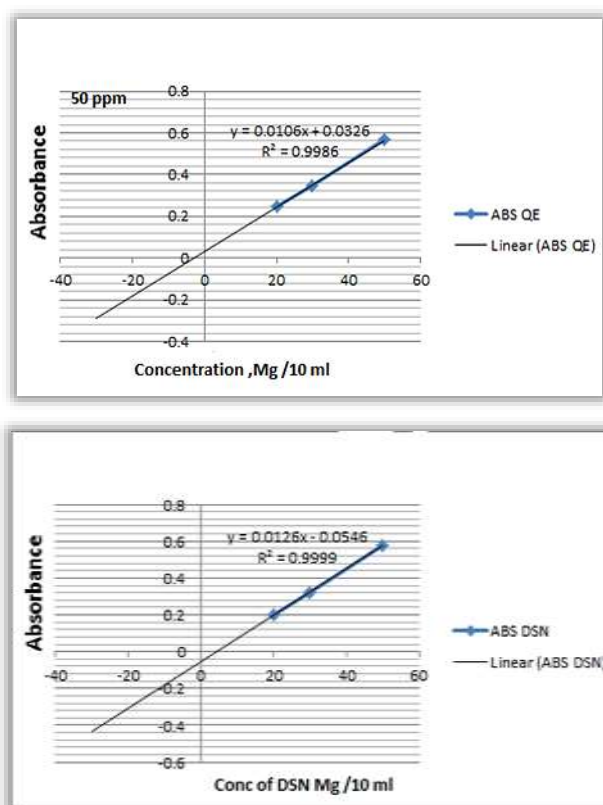
**FIGURE 17** Graphs of standard addition method of application (QE and DSN) in tablet

TABLE 5 Comparison between the present FIA methods and UV method

Pharmaceutical preparation	Suggested FIA methods			Standard method
	Rec %	t**	F**	Rec %
Pure QE	100.06			100.01
Pure DSN	102.67			100.04
Mega Quercetin 1200 mg	103.09	1.310	10.2243	100.13
Diosmin capsule 750 mg (Nutrition Greenlife,USA)	101.76			100.11

Conclusion

By using normal flow injection technique, the donor-acceptor compounds between MFS reagent and (QE and DSN) were investigated. This study reported a molar of 2:1 ratio. The UV-Vis spectra demonstrated the transition in the presence of oxidizing agent and MFS π acceptors as an analytical substance for the growth method for the accurate estimation of two drugs (QE and DSN) in pharmaceutical dosage forms. The applied nFIA gives very stiffed procedure as a cheap and simple instrument. The results obtained gave RSD % ≤ 5 . The proposed method used inexpensive reagent with excellent charge transfer transition. The mechanism was dependent on the interaction of drugs amino group (n-donor) with the oxidized MFS (π -acceptor) reagent to form custody transfer compound, which produced a colored product that was slow. In addition, the recommended methods were applied to the analysis of QE and DSN in dietary supplements and were compared statistically with an official UV method. The statistical analysis disclosed no noteworthy variance in correctness and exactness amid the current methods and the official method.

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