International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Normal Flow and Stopped Flow Injection Spectrophotometric Determination of Quercetin Dihydrate Dietary Supplements

Sadeem Subhi Abed[1], Hinda Ali Mahmood[2]

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

Abstract: ***Simple and sensitive normal flow injection(nFI) and stopped-FI spectrophotometric methods for the determination of quercetin dehydrate (QRC) in pure form were proposed, The methods were based on diazotization and coupling reaction between QRC and diazotized metoclopramide in alkaline medium to form orange–water soluble dye. The dye is stable and has a maximum absorbance at 438nm. A calibration graph shows that a Beers law is obeyed over the concentration range of 1-60µg.ml^{-1} and 0.8-50µg.ml^{-1} for (QRC) with a detection limit is 0.4166 µg.ml^{-1}, 0.1338 for nFI and stopped-FI methods, respectively. The reproducibility percentage (RSD% is 1.528 and 1.695 for both nFI and stopped-FI methods, and recovery percentage Rec% is (101.527) for nFIA and (104.36) for stopped-FIA. In nFIA the chemical and physical parameters are studied. The proposed method was applied on dietary supplements such as (Mega quercetin, Quercetin dehydrate).***

Keywords: Flavonoids, Quercetin dihydrate, spectrophotometric method, normal- Flow injection, Stopped flow injection

1. Introduction

Flavonoids are a specific class of phenolic plant phytochemicals represented by over 5000 compounds, subdivided into 13 categories that include anthocyanidins, catechins, flavonols, and flavones[1].The flavonol quercetin has shown much promise as an antioxidant agent, imparting a protective effect in reducing the risk of developing cardiovascular disease[2-4]. and certain types of cancer[5,6]. Quercetin dihydrate(QRC) is a type of flavonoids. Flavonoide possess the beneficial effect like antioxidant, anti-inflammatory, anti-platelet aggregatory activities and restore endothelial function and prevent LDL oxidation .Quercetein is found in redwine, green tea, onions, berries, citrus fruit, apples, garlic [7]. Since QRC is one of the most common flavonols and one of the most powerful antioxidants, it was important to develop a simple, precise and accurate method for the determination of QRC in different samples. Several methods were proposed in the literature to determine QRC in the samples of apple and tomato juice and fruits, wines, teas, serums and pharmaceutical preparations. These include HPLC [8-10], HPTLC [11] LC-MS [12], spectroscopic methods [13, 14], adsorptive stripping voltammetry [15], electro-chemical analysis [16] and fluorimetric methods [17]. The majority of methods required some pre-treatment of real samples, such as solid phase extraction [18] and molecularly imprinted polymers **(**MIPs).[19]

This paper describes spectrophotometric methods for determination of QRC by the diazotization coupling reactions with diazotized metoclopramide in alkaline medium. Metoclopramide was found to be a useful coupling reagents for diazotization reaction, because they produced a stable and rapid coupling organic products furthermore, these reagent is easily obtainable, highly purified and are soluble in ethanol therefore the proposed methods are considered as a new method for determination of flavonoids in flow injection method and stopped flow injection. In addition these methods have been satisfactorily applied for the determination of quercetin dihydrate in pure and pharmaceutical preparations.

2. Experimental

2.1 Apparatus

- All spectral and absorbance measurements were carried out by using Shimadzu UV/visible-260-digital double beam recording spectrophotometer using1-cm quarts cell (figure2).
- A flow cell with 50micro liter internal volume and 1cm bath length was used for the absorbance measurements.
- A two–channel manifold was employed for the FI spectrophotometric determination of QRC.
- A peristaltic pump was used to transport the carries solutions.
- Injection valve was employed to appropriate injection volumes of standard solutions and samples.
- Flexible vinyl tubing of 0.5mm internal diameter was used for the peristaltic pump. The peristaltic pump is interconnecting with a programmed timer which allows the flow to be stopped at a specific time after each injection (when the reacting mixture is in the detector flow cell) and then restarted to push the zone out of the detector.
- Reaction coil (RC) was of teflon with internal diameter of 0.5 mm.

2.2. Reagents

1) Standard solution of QRC (M.wt=338.27g/mol) (CarlROTH), stock solution of 100 µg.mL^{-1} was prepared by dissolving 0.01g of the pure compound in 50 ml absolute ethanol and complete the volumetric flask by absolute ethanol.
2) Diazotized metoclopramide solution(0.01M) (General company for samarra drugs-Iraq): prepared daily by

dissolving 0.1772gm of metoclopramide in 3ml distilled water and 2.5ml hydrochloric acid (1M) (BDH) in 50 ml volumetric flask. Cool the mixture to 0-50 c for 5 min using an ice bath. After 5 min add sodium nitrate (Merck) and stir the mixture. After 5 min the volume is completed to the mark with distilled water.

3) Sodium hydroxide (BDH) : (0.1M): prepared by dissolving 1gm of the base in 250ml of distilled water.
4) Mega quercetin (Solary dietary supplements (USA)(1200mg/capsole) : Stock solution of 100 µg.ml $^{-1}$ was prepared by dissolving of 0.01g of the pure compound in 50 ml absolute ethanol (1.2g in each capsole) and complete the volume to the mark by absolute ethanol .
5) Quercetin dihydrate) Bulk dietary supplements (USA) (100g (3.5302) : Stock solution of 100 µg.mL^{-1} was prepared by dissolving 0.01g of the pure compound in 50 ml absolute ethanol and complete the volumetric flask by absolute ethanol .

2.3. General FIA procedure and stopped flow injection method

The manifold used for the determination of QRC was designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of the diazotized metoclopramide with QRC in sodium hydroxide medium. Maximum absorbance intensity was obtained when the sample (QRC 30µg.ml^{-1}) was injected into a stream of diazotized metoclopramide (0.005M) and then mixed with sodium hydroxide (0.1M) and the maximum spectrum value was measured at 438nm as given in (Figure 1), (Figure 2). The influence of different chemical and physical FIA parameters on the absorbance of the colored product was optimized as follows: QRC solution in the range of 0.7-60µg.ml^{-1}was prepared from the standard solution(100µg.mL^{-1}). A 102µL of 30 µg.ml^{-1} QRC was injected into the stream of diazotized metoclopramide (0.001M) then the mixture combined with (0.1M) NaOH at T-link with total flow rate 2 ml /min for the two channels.

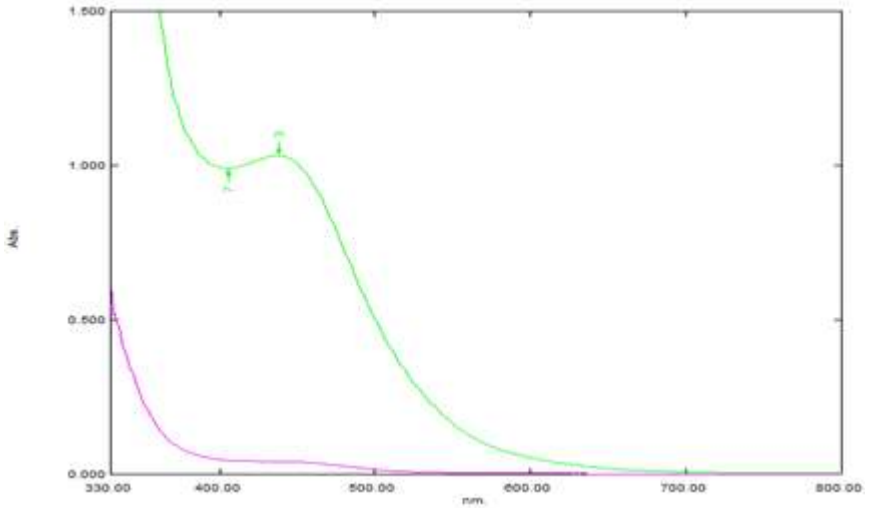

Figure 1: Absorption spectra of (30µg.ml^{-1}) QRC treated as described under procedure and measured against reagent blank ((0.01m)diazotized metoclopramide and (0.1M)sodium hydroxide) and the reagent blank measured against distilled Water

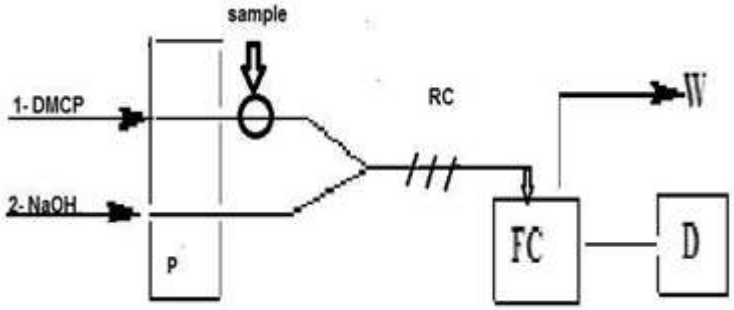

Figure 2: A schematic diagram of FIA manifold Where: (1) and (2), solutions of diazotized metoclopramide and sodium hydroxide respectively; P =peristaltic pump; Sample= injection sample QRC; ; RC= reaction coil; Fc= flow cell; D= detector; W= waste

Stopped –FI procedure can increase the sensitivity by increasing the reaction time in a stopping period, there by promoting more product. There is a stopping of the flow to hold an injected zone of sample in a mixing coil for promoting the reaction to take place without dispersion of the resulting product. In kinetic methods, the flow is stopped into the detector flow cell to monitor the evolution of the reaction [20].

The stopped FI method can increase sensitivity of the measurement by increasing the residence (reaction) time, the elapsed time after sample and reagent are mixed together prior to detection of the reaction product. By stopping the flow the residence time can be prolonged without increasing the length of the reaction coil, thus avoiding an increase of dispersion [21, 22]

Travelling time " period from the injection to the stopping time" depend on the physical parameters in which increase with increase reaction coil and sample manifolds parameters, the travelling time after each injection was studies and it was found that the sample zone reached the flow cell 30 sec after each injection, and the time intervals between stopping and restarting the pump flow, in the range(10-100sec) was also studies, it found after 50 sec (Figure 3) the stopping time this time was selected as the optimum interval time.

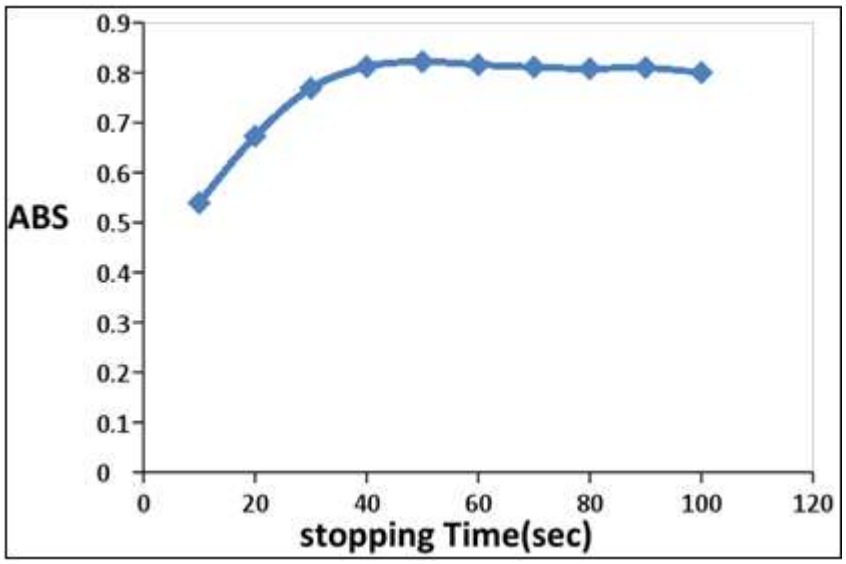

Figure 3: Effect of stopping time (sec)

3. Results and Discussion

The factors affecting on the sensitivity and stability of the colored diazotization coupling reaction between diazotized metoclopramide and QRC in an alkaline medium were carefully studied. . The influence of different chemical and physical FIA parameters on the absorbance of the colored product was optimized as followed :

Optimization of chemical parameters:
The influence of different chemical FIA parameters on the absorbance of the colored product was optimized as follows: The effect of different concentration of Diazotized metoclopramide was investigated. A concentration of (0.005M) of metoclopramide gave the highest absorbance and was chosen for further experiment (Figure 4).

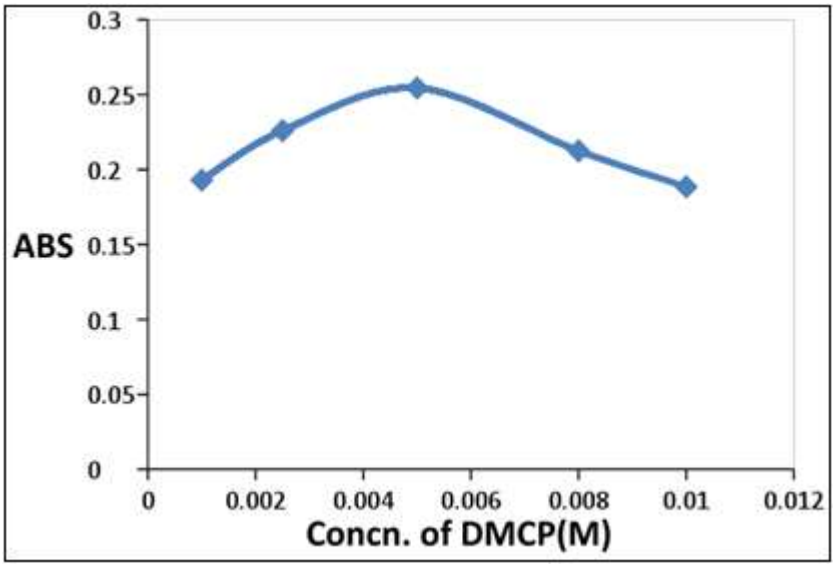

Figure 4: Effect of concentration of diazotized metoclopramide

It was observed that the reaction between diazotized metoclopramide and QRC depends on alkaline medium, The absorbance of the dye formed increased and became more stable in alkaline medium, therefore, the effect of different alkaline solutions(0.1M) on the colored product was studied such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, sodium acetate and sodium carbonate. As shown in figure (5a) maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of sodium hydroxide solution. The effect of different concentrations (0.01-0.3M)of NaOH(0.1 M) was studied. the effect of different concentrations of sodium hydroxide was studies and 0.1M was found to be the optimum (figure (5b).

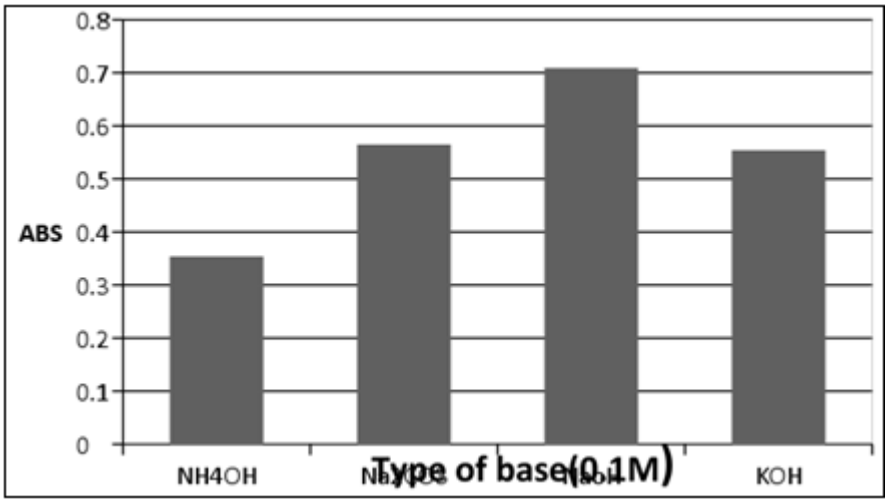

Figure (5a): Effect of type of base

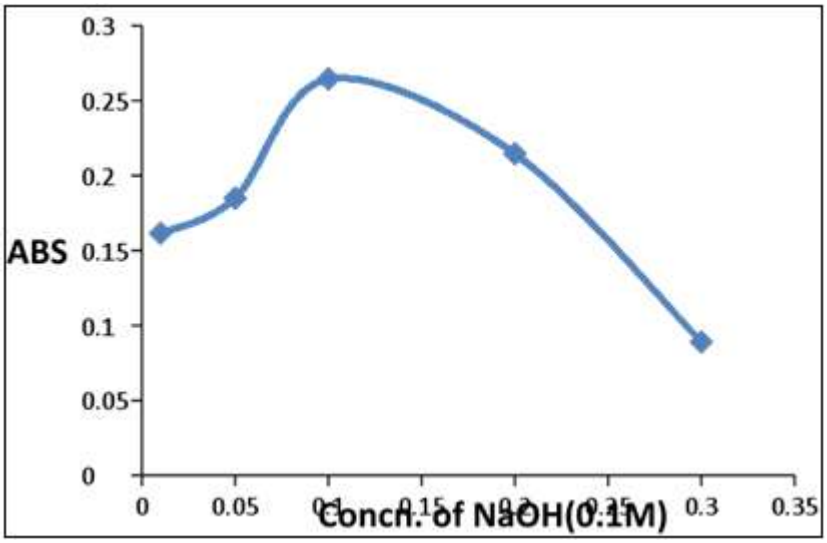

Figure (5b): Effect of concentration of NaOH

Optimization of physical parameter:

The effect of all physical parameters are studies in nFIA and stopped-FIA. The effect of total flow rate on the sensitivity of the colored reaction product was investigated in the range of (0.7-5 ml min^{-1}) for both method (nFIA, sFIA). The results obtained in figure (6) showed that a total flow rate 1.5ml min^{-1}, 2ml.min^{-1} gave the highest absorbance in the nFIA method , sFIA method respectively, and was used in this experiment.

The reaction coil Length is an essential parameters that effect on the sensitivity of the colored reaction product in nFIA and sFIA and was investigated in the range of 25-250cm. the results obtained in figure (7) showed that a coil length of 150cm, 75cm gave the highest absorbance in nFIA and sFIA, respectively and was used in the experiment.

The injection loop is the other parameters that effects on the sample throuput and was investigated in the range of (74-302 microliters). It was found that a loop of 102 microliters gave the highest absorbance for both methods (figure 8).

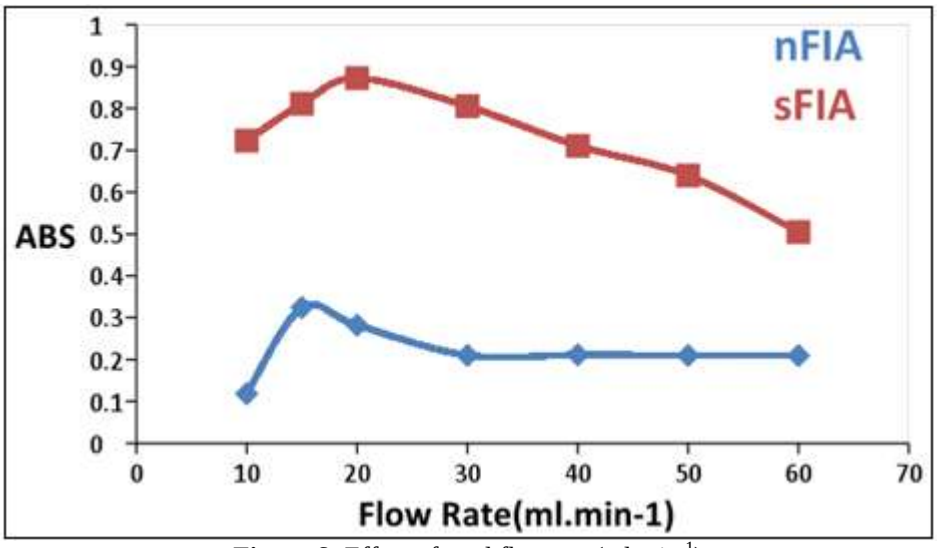

Figure 6: Effect of total flow rate(ml.min^{-1})

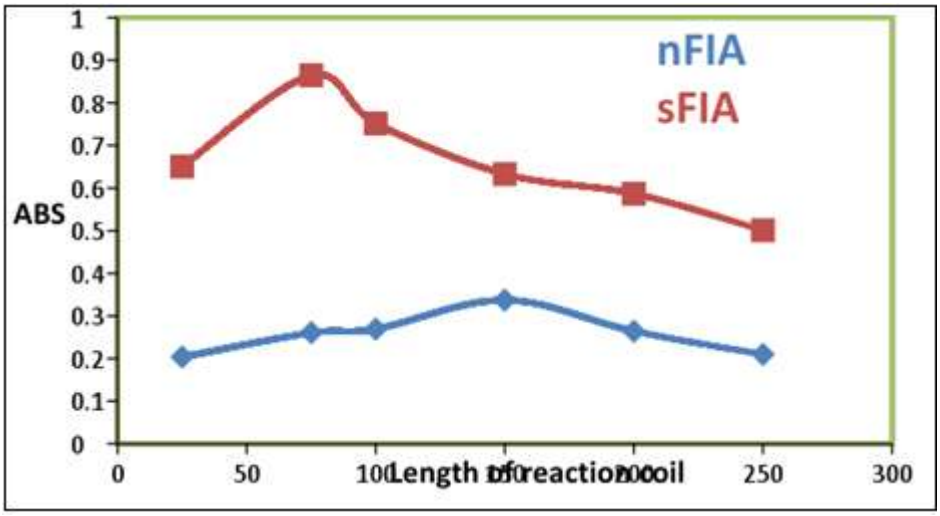

Figure 7: Effect of Reaction coil (cm)

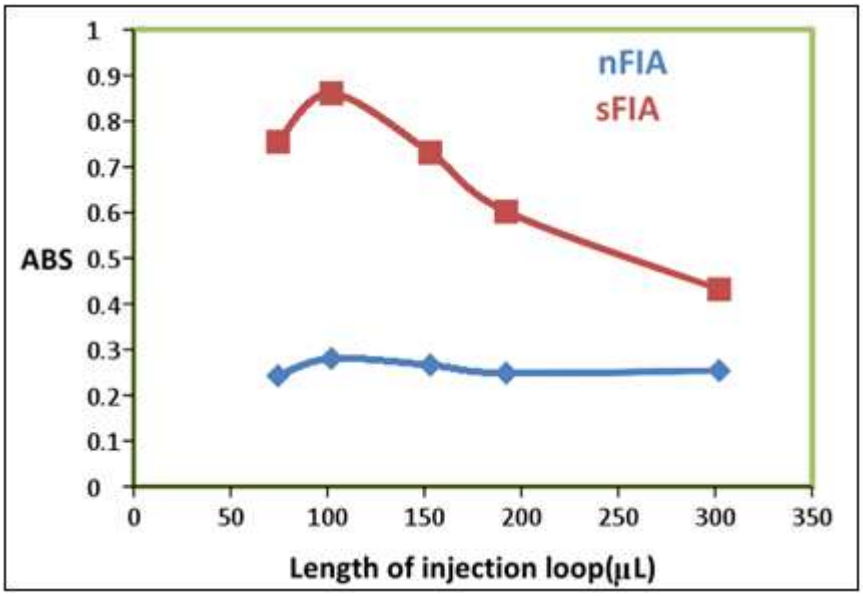

Figure 8: Effect of Length of injection loop(micro liter)

- **Calibration Curve in normal flow injection and stopped flow injection**

After fixing the optimum condition of nFIA for the determination of QRC standard calibration graph were constructed (Figure 9). The analytical values of statistical treatments for the calibration curves are summarized in table(2)

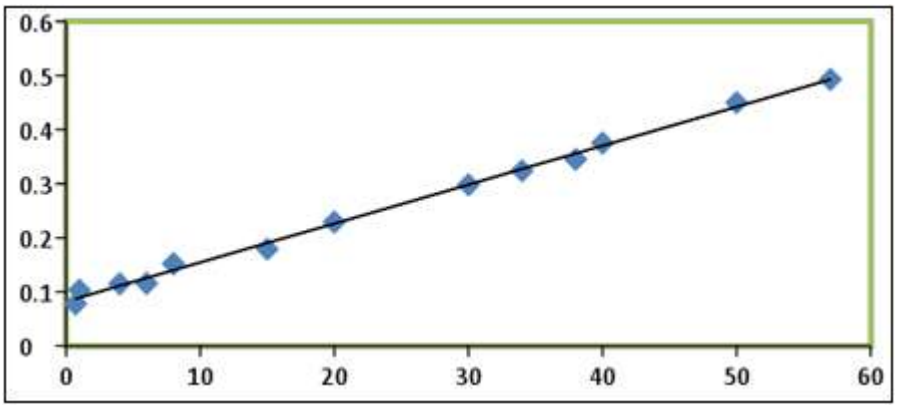

Figure 9: calibration curve for the determination of QRC in normal flow injection analysis

The calibration graph were constructed in sFIA (Figure 10) after study the physical parameters , and the statistical treatment are summarized in Table(2).

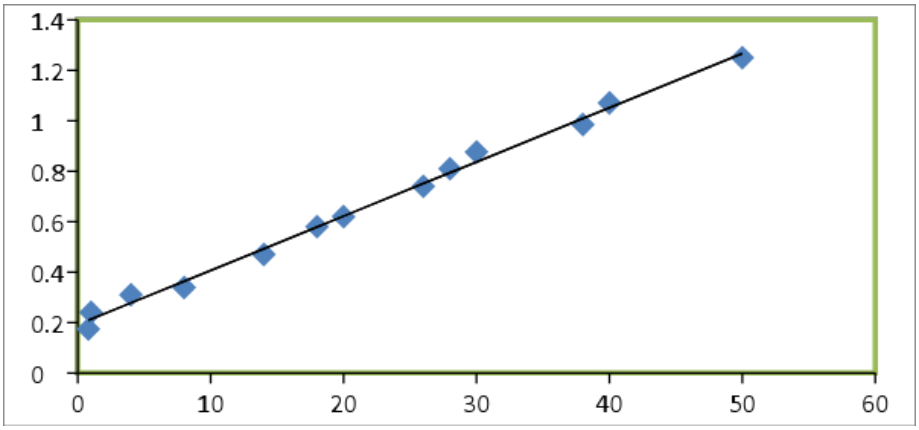

Figure 10: Calibration curve for the determination of QRC in stopped flow injection

Table 2: Analytical values of the calibration graphs for the determination of QRC

Parameters	nFIA procedure	sFIA procedure
Regression equation	Y= 0.0072x+0.0819	Y= 0.0215x+0.1918
Correlation coefficient	0.9967	0.9947
Reproducibility (%)(RSD%)	<1.528	<1.695
Recovery %	<101.5275	<104.36
Limit of detection (µg.mL^{1}); 3SDB/b	0.4166	0.1338
Limit of detection (µg.mL^{-1});Ŷ=YB+3SB	0.04338	
Linearity range	1-60 µg.ml^{-1}	0.8-50 µg.ml^{-1}
1.11	1.388	Limit of quantification
3.971x10^{-5}	1.988x10^{-5}	Standard deviation of the slope, Sb
6.319x10^{-3}	5.036x10^{-3}	Standard deviation of the intercept, Sa
0.4651	0.0984	Sandell"s sensitivity, S (µg cm^{-2}) per 0.001 absorbance unit, S = M / ε
35	70	Sample through-put (h^{-1})

Accuracy and precision

The accuracy and precision of the method, at three different concentrations, for five replicates of each concentration were tested. The results shown in Table 3 were studied depending upon the value percentage of (E %), (Rec. %)and (RSD %), were obtained. The relative standard deviation (RSD %) value in all cases indicating good repeatability of the suggested methods.

Table 3: Accuracy and precision of the proposed method

Proposed method	Con.of QRC (µg.ml^{-1})		E%	Rec%	RSD%
	present	found			
nFIA	20	20.29	+1.45	101.45	1.528
	30	29.916	-0.28	99.72	0.9556
	40	40.611	+1.527	101.527	0.7089
sFIA	18	18.10	+0.55	100.55	1.695
	26	25.49	-1.96	98.04	0.854
	40	40.89	+2.22	102.22	0.972

Analytical application

The proposed methods were applied successfully to the analysis of some pharmaceutical preparations containing QRC(the application is quercetin dehydrate, mega quercetin), and they gave a good reproducibility and recoveries as shown in Table 4. The results obtained by the proposed and reference methods (There are various methods available for estimation of QRC like U.V. HPTLC method reported . not a single U.V. method reported for estimation of quercetin in formulation ((procedure is a spectrophotometric method (preparation of standard stock solution by weighed an accurate amount 100mg of quercetin dihydrate was dissolved in 20ml methanol and diluted up to 100ml by distilled water to obtain 100mg/ml concentration of quercetin dihydrate , This solutions was subjected to scanning between (271nm-372nm)) . present work is done to develop a simple U.V. spectroscopy using normal flow injection method and stopped flow injection method . for capsule forms were compared statistically by means of the F-test and t-test and the proposed methods and the reference methods were found no significant differences in precision and accuracy between the proposed methods and the reference method (Table 5).

Table 4: Application of the proposed methods to the determination of QRC in capsule forms

Pharmaceutical preparation	Proposed method	Present concn (µg.ml^{-1})	Found conc (µg.ml^{-1})	E%	Rec%	RSD%
QRC 100g(3.5302)	nFIA	20	19.59	-2.05	97.95	1.9176
		30	30.70	+2.30	102.30	0.3764
		40	40.29	0.725	100.725	0.151
	sFIA	18	18.10	+0.55	100.55	0.073
		40	40.80	+2.00	102.00	0.1493
		26	25.54	-1.76	98.24	0.105
Mega quercetein (1200mg/capsole)	nFIA	20	20.29	+1.45	101.45	0.2962
		30	30.15	+0.50	100.50	0.4751
		40	40.36	+0.90	100.90	0.5492
	sFIA	18	18.10	+0.55	100.55	0.1286
		26	25.68	-1.23	98.77	0.1102
		40	40.92	+2.30	102.30	0.2282

Table 5: The comparison of the proposed method with standard method

Pharmaceutical preparation	Proposed methods						Standard method Rf.
	nFIA			sFIA			Rec%
	Rec%	T	F	Rec%	T	F	
QRC Pure	100.585	2.734	7.988	100.13	2.5561	2.415	99.699
QRC	100.125			100.12			99.853
Mega quercetin	100.975			100.53			100.0
	$S^1$1=0.1809, $S^2$2=0.02265 S= 0.319			$S^1$1=0.0547 $S^2$2=0.02265 S=0.9166			

***values at 95% confidence level, n1= n2 = 3, ttab =t0.05/2 ,n-1= 2.776 where t has**
ν = n1 + n2 - 2 degrees of freedom= 4, Ftab = 19.0 where F has ν1 = n1 - 1, ν2 = n2 - 1 degrees of freedom = 2.

The results obtained by the proposed and standard methods for dosage forms were compared statistically by means of the F-test and t-test at 95% confidence level [23] and were found the calculated t and F-values (Table 4) did not exceed the theoretical values, which indicates that there is no significant difference between either methods in terms of accuracy and precision F-test, which is defined as testing differences between standard deviations (S1, S2) of data sets, provides a simple method for comparing the precision of two sets of measurements using the following equation:

F=S1/S2 or F=S2/S1 (F<1)

Student's t-Test is an ideal way of comparing the mean one set of measurements with another. The value of t-test is chosen based on the desired confidence level. A 95% confidence level test is generally used.

4. Conclusion

The application of diazotization–coupling reaction of diazotized metoclopramide in sodium hydroxide medium to the spectrophotometric determinations of the quercetin dihydrate in pharmaceutical preparations was described by nFIA and sFIA systems. Although the nFIA system has the advantages of simplicity ,reproducibility , time saving, low reagent consumption need of small sample volume, large dynamic range and high sample throughput . This is important feature of the nFIA system.

The proposed methods offer can be applied to the analysis of a wide concentration range of QRC in real samples with

satisfactory results. The proposed methods are simple and inexpensive since it requires simple instrumentation.

References

[1] Croft, K.D. 1998. The chemistry and biological effects of flavonoids and phenolic acids. Ann. New York Acad. of Sci. 854:435–442.
[2] Hertog, M.G.L. and P.C.H. Hollman. 1996. Potential health effects of the dietary flavonol quercetin. Euro. J. Clin. Nutr. 50:63–71.
[3] Keli, S.O., M.G.L. Hertog, E.J.M. Feskens, and D. Kromhout. 1996. Dietary flavonoids, antioxidant vitamins, and incidence of stroke. Arch Intern. Med. 154:637–642.
[4] Knekt, P., R. Järvinen, A. Reunanen, and J. Maatela. 1996. Flavonoid intake and coronary mortality in Finland. BMJ. 312:478–481.
[5] Knekt, P., R. Järvinen, R. Seppänen, M. Heliövaara, L. Teppo, E. Pukkala, and A. Aromaa. 1997. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Amer. J. Epidem. 146:223–230.
[6] Leighton, T., C. Ginther, L. Fluss, W.K. Harter, J.Cansado, and V. Notario. 1992. Molecular characterization of quercetin and quercetin glycosides in Allium vegetables phenolic compounds in food and their effects on health II. ACS Symp. Ser. 507:220–238.
[7] H. Sandhar, B. Kumar, S.Prasher, *Int. pharmaceuticasciencia*,**2011**,(1) 25-41
[8] F. Wang, T. Yao, S. Zeng, Determination of quercetin and kaempferol in human urine after orally administrated tablet of ginkgo biloba extract by HPLC, *J. Pharm. Biomed. Anal.*, **33**, 317–321 (2003).
[9] K. Ishii, T. Furuta, Y. Kasuya, High-performance liquid chromatographic determination of quercetin in human plasma and urine utilizing solid-phase extraction and ultraviolet detection, *J. Chromatogr.*, B: *Anal. Technol. Biomed. Life Sci.*, **794**, 49–56 (2003).
[10] S. Wang S, D. Di, X. Liu, S. Jiang, Determination of luteolin and quercetin in the capsule of Lamiophlomis Rotata (Benth.) Kudo by HPLC coupled with weighted least squares linear regression. *J. Liq. Chromatog. Rel. Technol.*, **30**, 1991–1999 (2007).
[11] Y. Zheng, L. Ye, L. Yan, Y. Gao, The electrochemical behavior and determination of quercetin in choline chloride/urea deep eutectic solvent electrolyte based on abra-sively immobilized multi-wall carbon nanotubes modified electrode, *Int. J. Electrochem. Sci*., **9**, 238–248 (2014).
[12] L. Wang, M. E. Morris, Liquid chromatography-tandem mass spectroscopy assay for quercetin and conjugated quercetin metabolites in human plasma and urine, *J. Chromatogr*. B, **821**, 194–201 (2005).
[13] N. Pejić, V. Kuntić, Z. Vujić, S. Mićić, Direct spectrophotometric determination of quercetin in the presence of ascorbic acid, *Il Farmaco*, **59**, 21–24 (2004).
[14] V. Kuntić, N. Pejić, S. Mićić, V. Vukojević, Z. Vujić, D. Malešev, Determination of quercetin in pharmaceutical formations via its reaction with potassium titanyloxalate. Determination of the stability constants of the quercetin titanyloxalato complex, *J. Serb. Chem. Soc.*, **70**, 753–763 (2005).
[15] G. J. Volikakis, C. E. Efstathiou, Fast screening of total flavonols in wines, tea-infusions and tomato juice by flow injection/adsorptive stripping voltammetry, *Anal. Chim. Acta*, **551**, 124–131 **(**2005).
[16] S. U. Rakesh, P. R. Patil, V. R. Salunkhe, P. N. Dhabale, K. B. Burade, HPTLC method for quantitative determi-nation of quercetin in hydroalcoholic extract of dried flower of *Nymphaea stellata* Wild, *Int. J. ChemTech Res.*, **1**, 931–936 (2009).
[17] M. Shaghaghi, J. L. Manzoori, A. Jouyban. Determination of total phenols in tea infusions, tomato and apple juice by terbium sensitized fluorescence method as an alternative approach to the Folin–Ciocalteu spectrophotometric method, *Food Chem.*, **108**, 695–701 (2008).
[18] M. Oman, M. Škerget, Ţ. Knez, Application of supercritical fluid extraction for the separation of nutraceuticals and other phytochemicals from plant material, *Maced. J. Chem. Chem. Eng.*, **32**, 183–226 (2013).
[19] Al-Bayati ,Y. K. and Aljabari, F. I. **2016.**Synthesis of ibuprofen-molecularly imprinted polymers used as sensors to determine drug in pharmaceutical preparation. *Asian J. of Chemistry*, 28(6), pp:1376-1380.
[20] J. Ruzicka, and E. H. Hasan, "Flow Injection Analysis" , 3rd Ed., John Wiley and Sons, Inc., New York, 2005.
[21] J. Ruzicka, and E. H. Hasan, "Flow Injection Analysis" , 2rd Ed., John Wiley & Sons, New York, 1988, p156-166.
[22] K. Grudpan, P. Ampan, Y. Udnan, S. Jayasvati, S. Lapanan tnoppakhun, J. Jakmunee, G.D. Christian, J. Ruzicka, Talanta 58 (2002) 1319.
[23] Miller, J. N. and Miller, J. C. **2000**. *Statistics and Chemometrics for Analytical Chemistry*. Fourth Edition. Pearson Education Limited, London.