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Determination of Quercetin in Some Natural Products Using Reversed FIA-CL Method

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ABSTRACT

This paper reports a new and simple flow injection analysis with chemiluminescence (CL) system for determination of quercetin through the reverse flow injection technique. The method was based on the inhibition of the CL of H₂O₂–luminal–permanganate with quercetin. The reverse flow was used to avoid continuous monitors of CL which leads to unstable baseline. Various parameters associated with this flow system were studied and essential optimizations were carried out. Two calibration graphs were constructed for determination of quercetin in the range (6.0–12 µg/ml) with correlation coefficient (0.9994) for low concentration level and (20–190 µg/ml) with correlation coefficient (0.9962) for high concentration and a sampling frequency 80 samples/h. Possible interferences were studied and the results showed that the interferences caused less than 5% error. The method was applied successfully for the determination of quercetin in various natural products.

Keywords: Flow injection analysis, Chemiluminescence, Quercetin, Flavonoids, Natural products.

INTRODUCTION

Among all the flavonoids, which display a significant array of pharmacological activity, quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one) is most commonly presented in food such as onions, tea, apples and red wine ^[1]. Quercetin is important dietary constituent because it is most widespread and consequently, the most studied flavonoid. A lot of articles are dealing with beneficial pharmacological properties of quercetin in the field of allergy, vascular, inflammation, virology and carcinogenesis ^[2, 3].

Various methods have been described for the determination of quercetin mostly from plant materials: HPLC ^[4, 5], capillary electrophoresis ^[6] and derivative spectrophotometry ^[7]. Quercetin was also determined spectrophotometrically via coloring complexing reaction with many different inorganic reagents added ^[8, 9].

Progress in FIA-CL analysis has received much attention in various fields for its high sensitivity, rapidity, and simplicity. Polyphenols have been determined by their inhibition of CL

from the luminol- Ferric cyanide with detection limits in the range of ($6.8 \times 10^{-7} - 2.0 \times 10^{-8}$) M^[10]. Chlorophenols have been determined by enhance CL reaction of luminol, catalyzed by hose radish peroxide with detection limits varied from 0.01-10 μmol ^[11].

In this paper a new method for determination of quercetin in some natural products (cherry, onion, and tea) were developed by reversed FIA-CL method. The method was based on the inhibition of CL emission in the $\text{KMnO}_4/\text{luminol}/\text{H}_2\text{O}_2$ system by addition of quercetin.

MATERIALS AND METHODS

Reagents:

Hydrogen peroxide

A 0.1 M hydrogen peroxide was prepared daily by diluting 6.017 ml of H_2O_2 (GCC) (50%, sp.gr. 1.13) in a 1.0 l volumetric flask with distilled water. It was protected from light.

Sodium carbonate solution

A 0.1 M sodium carbonate solution was prepared by dissolving 10.599g of Na_2CO_3 (Fluka) in a little amount of distilled water, transferred to 1.0 l volumetric flask.

Luminol solution

A 1.0×10^{-2} M luminal solution was prepared by dissolving 1.771g of the solid (Surechem - LTD) in a little of 0.1 M sodium carbonate solution and the volume was completed to 1.0 l in a volumetric flask with the same solution.

Potassium permanganate solution

A 0.2 M KMnO_4 solution was prepared by dissolving 31.608 g of potassium permanganate in a little of water. The solution was boiled for 15 minutes then cooled and the volume was completed to 1.0 l in a volumetric flask. This solution was standardized against 0.1 M standard sodium oxalate solution^[12].

Apparatus:

Fig.(1) shows the schematic diagram of the FIA-CL system. A multi channel peristaltic pump (DESAGA Heidelberg), with 6 channels and variable speed. The silicon rubber pump tubes with (0.5 mm i.d) were used.

The rotary valve (Rheodyne U.S.A.) with variable sample volumes was used to inject the sample into flowing carrier streams. The flow cell was made by winding the length of glass tubing (0.8 mm i.d) to form coil of 100 μL volume. The spectrophotometer (Type CECIL CE303) was used as a detector. The chemiluminescent out-put was recorded by means of x-t recorder (Type PM 825A PHILIPS – one line recorder).

General procedure:

The schematic diagram in Fig.(1) shows the FIA-CL system used. The procedure includes mixing of two main streams. In the first stream a 100 μl of KMnO_4 is injected into the carrier stream quercetin, while in the second stream luminol solution was merged with hydrogen peroxide stream before reaching the flow cell. The emission light produced in the flow cell

was detected and the peak height of the signal recorded as a CL signal (mV). Therefore, the concentration of quercetin was quantified.

RESULT AND DISCUSSION

Optimization of manifold designs

Three designs were tested for the determination of quercetin (20 and 30 µg/ml) as the carrier stream for each design; all designs were applied to avoid a continuous CL reaction of $\text{KMnO}_4/\text{luminol}/\text{H}_2\text{O}_2$. Manifold of Fig.(1) was found to give higher emission signal; therefore, this design was selected for further studies.

Optimization of experimental parameters

The physical and chemical parameters that participate in the reaction have been studied to obtain maximum sensitivity of the CL intensity. The parameters include sample volume, length of the mixing coil, flow rate and concentrations of reactants. Therefore, these optimizations were started using the following experimental physical and chemical variables for the determination of quercetin (30 µg/ml); 100µL injected volume, 60 cm lengths of the mixing coils (a and b), 3.0 ml/min flow rate, 5.0×10^{-3} M luminol, 1.0×10^{-3} M KMnO_4 , and 1.0×10^{-4} M H_2O_2 .

Physical optimization:

Effect of injected reagent volume

Effect of injected reagent volume (loop) on the CL intensity in the range of 40-150 µl was studied, while keeping all other variables constant. This study was applied for 30 µg/ml quercetin. The results obtained in Fig.(2) showed that there was an increase in the CL intensity up to 90µl, above that the CL intensity decreases. Therefore, injected reagent volume of 90µl was chosen as optimum for the present method.

Effect of mixing coils length

The length of two mixing coils in Fig.(1- a, and b) were also optimized with keeping all other variables constant. Different lengths of mixing coil from 0-100 cm for each coil were studied. A 30 cm length in each coil (a, and b) gave the best results for the determination of quercetin as shown in Fig.(3).

Effect of flow rate

All other optimized conditions were kept constant; the effect of flow rate on the CL intensity was studied in the range of 0.5-4.5 ml/min. The CL intensity increased with increasing flow rate up to 3.5 ml/min, above that the CL intensity decreased as shown in Fig. (4). The present CL reaction is very fast and the excited product at the entrance of the CL reaction flow cell needs rapid transport to the reaction coil of the cell for maximum light output to be monitored, while at the flow rate higher than 3.5 ml/min the reactants leaving the flow cell and CL performed outside the detector optical path. Therefore, 3.5 ml/min was selected as the best flow rate.

Chemical optimization:

Effect of different solutions

Quercetin was neither readily soluble in water nor in an acidic medium, while it was soluble in basic medium. Therefore, it was necessary to investigate effects of different bases on the CL intensity with keeping other experimental conditions at their optimized value. Three different bases (NaOH , KOH , and Na_2CO_3) with constant concentrations as the solvents for

quercetin were tested. The results in Table (1) show that the maximum CL intensity was obtained with Na₂CO₃ as a suitable base for dissolving quercetin.

Effect of luminol concentration

The influence of the luminol concentration on the CL intensity for 30 µg/ml of quercetin was studied in the range 1×10^{-4} – 1×10^{-2} M after keeping all parameters at their optimum values. The results in Fig.(5) showed that the maximum increase in the CL intensity was obtained as the concentration of luminol increased up to 1×10^{-3} M; above this concentration the CL intensity decreased due to the self quenching of luminol molecules. Therefore, 1×10^{-3} M luminol was selected for further studies.

Effect of KMnO₄ concentration

Keeping all other chemical and physical parameters at their optimized value, the effect of potassium permanganate concentration was studied in the range of 1×10^{-5} – 1×10^{-2} M on the CL intensity of 30 µg/ml quercetin. The results in Fig.(6) showed that 5×10^{-3} M KMnO₄ gives the largest CL intensity. The decrease in the signals at higher permanganate concentration is certainly due to the excess of permanganate, which overcasts the emitted light. Therefore, 5×10^{-3} M KMnO₄ was used as optimum concentration.

Effect of H₂O₂ concentration

Effect of the hydrogen peroxide concentration on the CL intensity for 30 µg/ml quercetin was investigated in the range of 1×10^{-4} – 5×10^{-3} M. Fig.(7) shows that the maximum increase in the CL intensity was obtained as the concentration of H₂O₂ increased up to 1×10^{-3} M. Therefore, 1×10^{-3} M of the H₂O₂ concentration was selected as the optimum.

Recommended procedure

Table (2) illustrates summary of optimum physical and chemical conditions for the determination of quercetin using FIA-CL system.

Calibration graph:

The reversed FIA-CL system shown in Fig. (1) was performed under optimum conditions described in Table (2), the calibration graphs were constructed by plotting CL intensity (mV) versus quercetin concentration (µg/ml) as shown in Fig.(8) and (9) in two different low and high concentration requires respectively. The statistical results for calibration curve were summarized in Table (3).

The accuracy and precision of the proposed method was determined by five replicate determinations which made on the two different concentrations of pure quercetin solutions (30, and 110 µg/ml). These results were shown in Table (4).

Study of interferences:

To study the effect of interferences on the determination of quercetin using reversed FIA-CL method; different concentrations of several compounds were tested. The effects were calculated by comparing the CL intensity (mV) obtained with solutions containing 20 µg/ml of quercetin and different concentrations of interference compounds with 2-10 folds compared to concentration of the quercetin.

The results were summarized in Table (5) which shows the maximum tolerable concentrations of the various compounds.

Applications

The new method was applied for the determination of quercetin in three different natural products such as cherry, onion and black tea taken from Hawler markets.

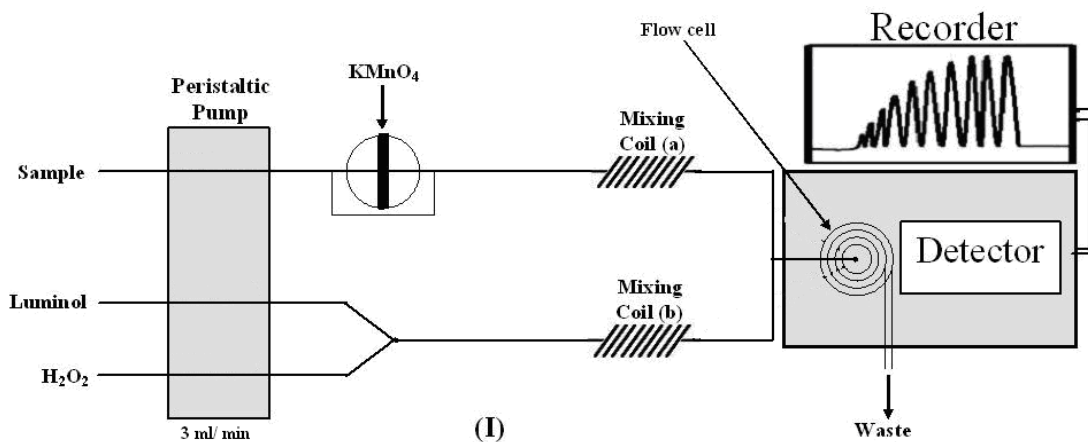


Fig. (1): Schematic diagram of the FIA-CL manifold used for the determination of quercetin.

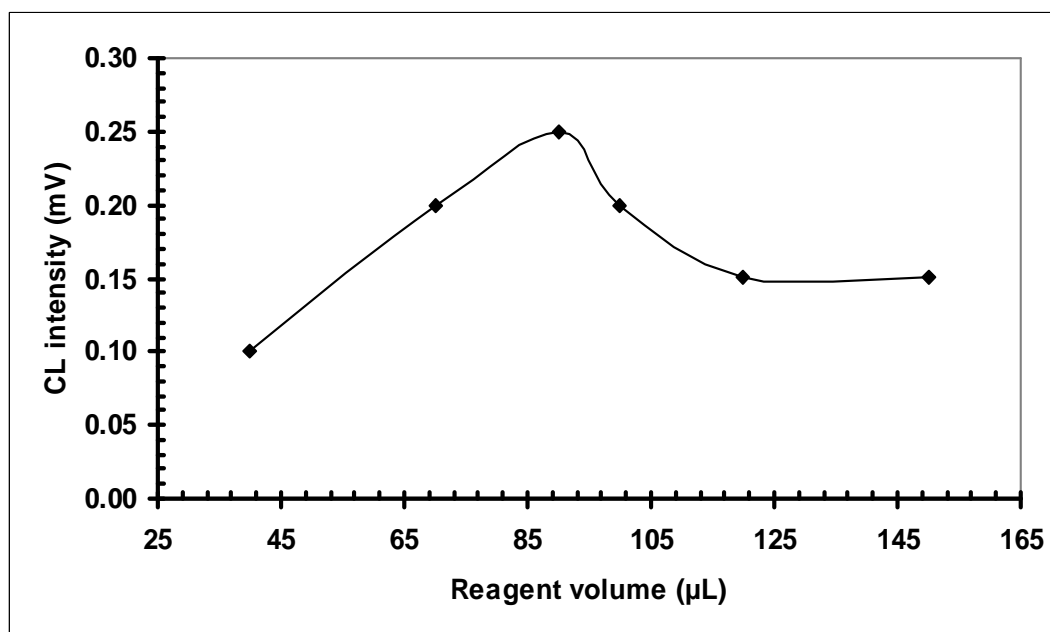


Fig. (2): Effect of injected reagent (KMnO₄) volume on the CL intensity

The samples were air dried and ground with a mortar. A 10 g of the dried sample powder was extracted with 80% methanol using solid-liquid extraction. After evaporation of 50% of the solution by rotary evaporator; ethylacetate was used for the extraction of aglycon flavonoids. The extracts were concentrated to dryness at room temperature. Quercetin was separated from other polyphenols by using preparative thin-layer chromatography (PTLC). Therefore, the obtained quercetin from all samples was dissolved in 0.01 M Na₂CO₃ and the volume completed by the same solution to 25 ml volumetric flask.

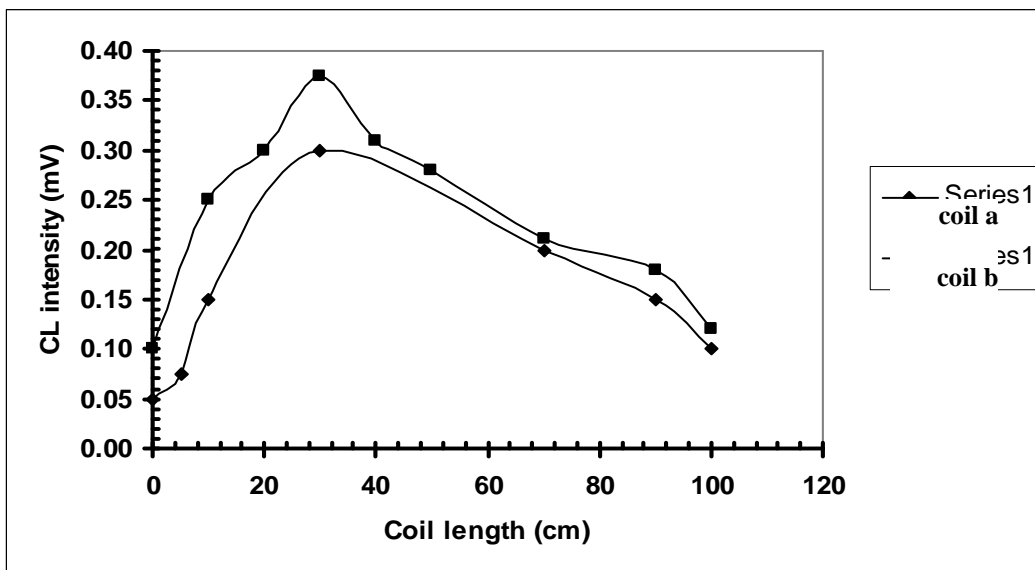


Fig. (3): Effect of mixing coils length on the CL intensity of 30 $\mu\text{g/ml}$ quercetin.

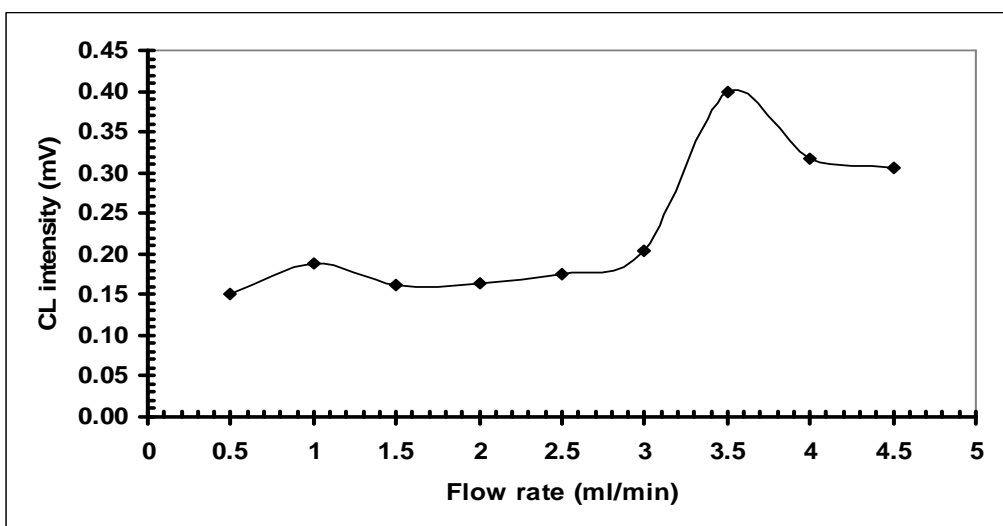


Fig. (4): Effect of flow rate on the CL intensity of 30 $\mu\text{g/ml}$ quercetin.

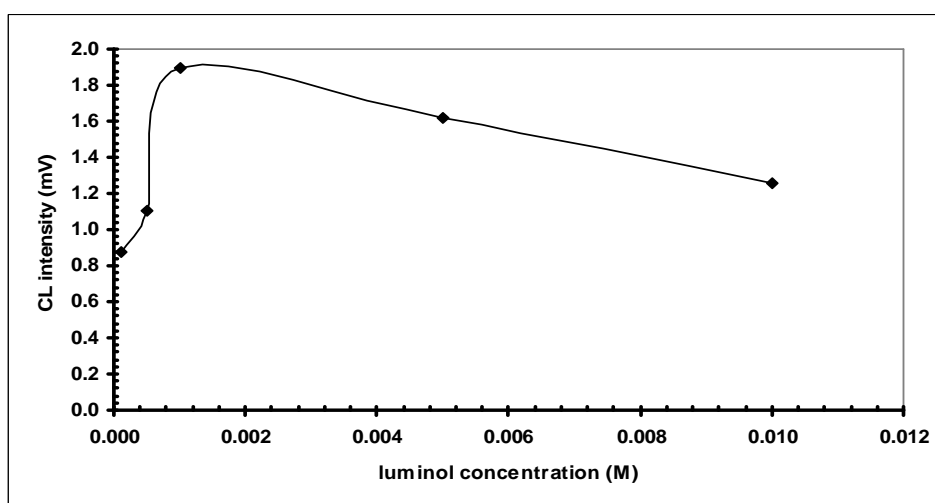


Fig. (5): Effect of luminol concentration on the CL intensity of 30 $\mu\text{g/ml}$ quercetin.

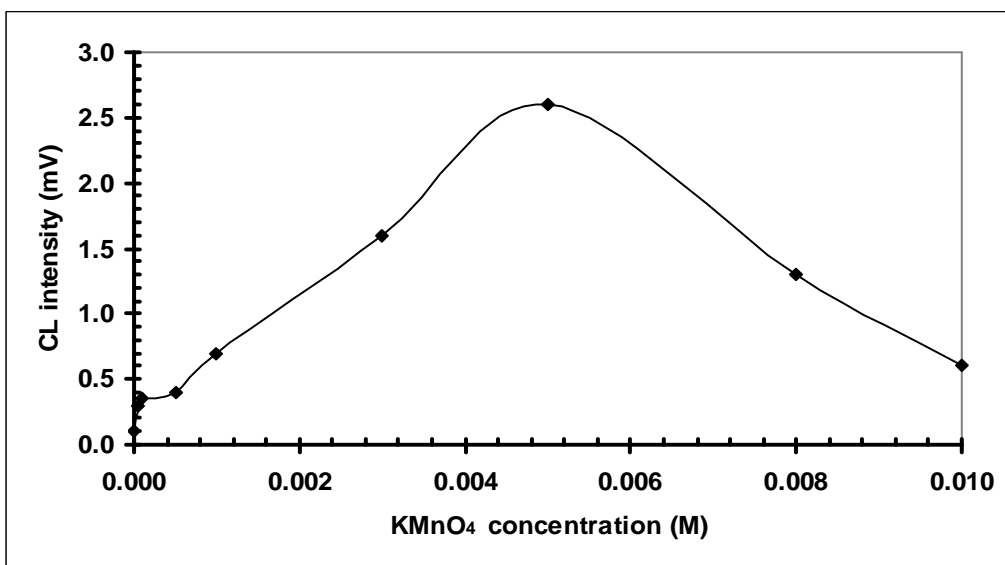


Fig. (6): Effect of KMnO₄ concentration on the CL intensity of 30 µg/ml quercetin.

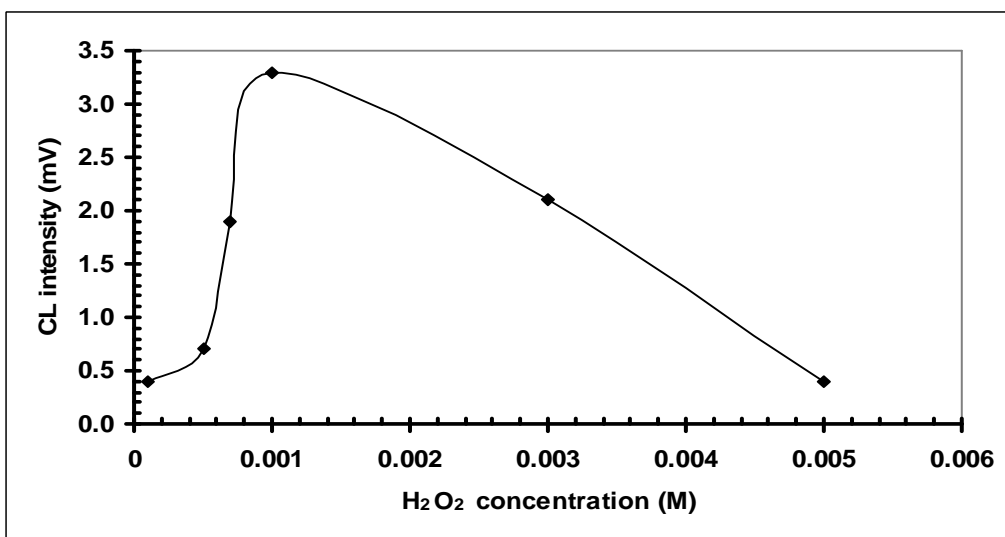


Fig. (7): Effect of H₂O₂ concentration on the CL intensity of 30 µg/ml quercetin.

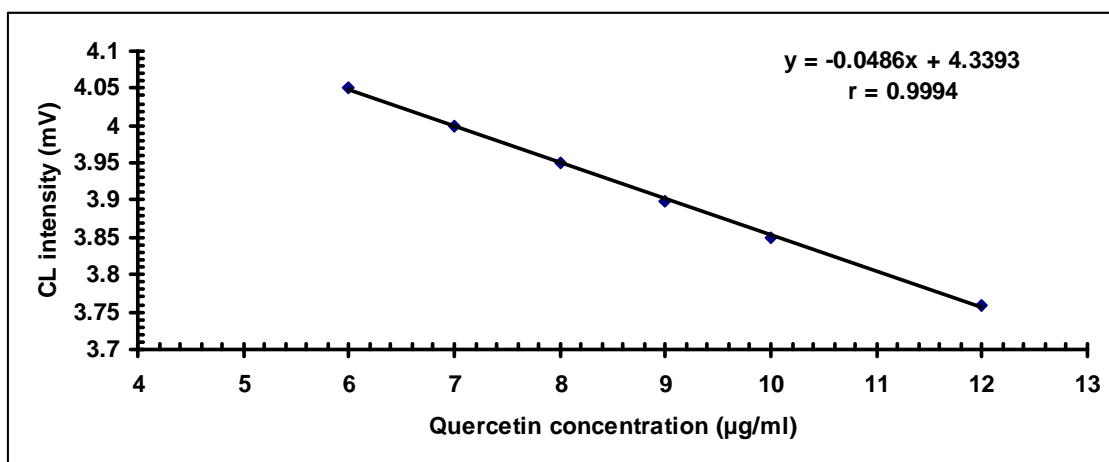


Fig. (8): Low concentration calibration graph for the determination of quercetin using reversed FIA-CL method.

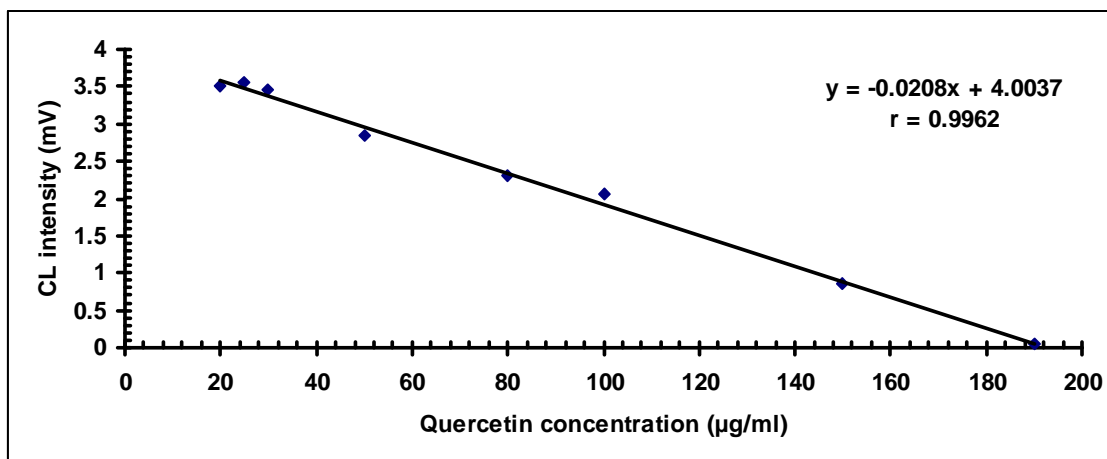


Fig. (9): High concentration calibration graph for the determination of quercetin using reversed FIA-CL method.

The resultant solutions were used to determine the concentration of quercetin in the samples. The results obtained with the present method accepted with those obtained by means of an HPLC technique, and shown in Table (6). The same procedure was carried out except that the final solution was completed with methanol for the determination of quercetin by HPLC method.

Table (1): Effect of different bases on the CL intensity

Concentration of quercetin (µg/ml)	CL intensity (mV)		
	0.01 M NaOH	0.01 M KOH	0.01 M Na ₂ CO ₃
20	0.65	0.472	1.98
30	0.41	0.228	1.83

Table (2): Summary of optimum conditions for quercetin determination.

Parameters	Optimum value	
Reagent volume	90 µl	
Mixing coils length	a	30 cm
	b	30 cm
Flow rate	3.5 ml/min	
luminol	1x10 ⁻³ M	
Potassium permanganate	5x10 ⁻³ M	
Hydrogen peroxide	1x10 ⁻³ M	

Table (3): Statistical data of the calibration graphs for the determination of quercetin using reversed FIA-CL method.

Analyte	Detection limit (µg/ml)	Calibration range	Linear range (µg/ml)	Correlation coefficient (r)
quercetin	6	Low concentration	6-12	0.9994
		High concentration	20-190	0.9962

Table (4): Accuracy and precision of the method.

Quercetin concentration (µg/ml)	Mean of CL intensity (mV)	% Error	S.D.	RSD
30	3.39167	0.82233	0.024	1.690
110	1.69322	0.32909	0.046	0.965

Table (5): Effect of interferences on the CL intensity of 20 µg/ml quercetin.

Interference	Concentration of interferences (µg/ml)	% Error
Glucose	200	0.22
Sucrose	200	0.76
Galactose	200	-1.28
Fructose	200	-2.12
p-Cresol	170	0.87
Ascorbic acid	155	-3.2
phenol	175	2.76
Catechol	160	-2.43
Tannin	75	4.11
Resorcinol	45	-1.98

Table (6): Determination of quercetin in natural products.

Sample	Quercetin found (µg) per 25 ml		% Error
	Proposed method	HPLC method	
Cherry	62.436	61.212	1.99
Onion	101.616	102.988	-1.332
Black tea	46.455	44.892	3.4816

CONCLUSION

A new reversed FIA-CL method was proposed for the determination of quercetin based on inhibition of CL of permanganate-luminol-hydrogen peroxide system. The proposed method is accurate and precise with a wide linear range; %RSD in this method was 0.965-1.690. The analytical frequency of this method was 80 samples/h and with good correlation coefficient (0.9994-0.9962) for 15 measurements of low and high concentration calibration respectively. This method can be applied to analysis of quercetin in different natural products. It is characterized by mixing the samples and reagents rapidly with a high degree of reproducibility.

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