Determination of hydroquinone in pure form and pharmaceutical preparations using Batch and FIA-Merging Zone techniques with spectrophotometric detection

Bushra B.Al-Mosulli[1], Aseel S.Al-Jomaili[2] and Hamssa S.Al-Janabi[1*]
[1] Department of Chemistry, College of science , University of Baghdad
[2] Department of Pharmacy, Al-Rasheed University College. Baghdad- Iraq

Abstract

In this study, a new, rapid and sensitive batch and flow injection-merging zones spectrophotometric methods for the determination of hydroquinone in a pure material and in pharmaceutical preparation were proposed. These methods were based on the oxidative-coupling reaction of HQ with 2,4-dinitrophenylhydazine (DNPH) in the presence of sodium periodate and sodium hydroxide to form a dark brown water slouble dye that is stable and has maximum absorption at 530 nm, graphs of absorbance versus concentration show that Beer's low is obeyed over the concentration rang of 1-40 and 3-300 µg.ml^{-1} of hydroquinone, with detection limits of 0.162 and 0.510 µg.ml^{-1} of hydroquinone for batch and FIA methods, respectively. The optimized FIA system is able to determine hydroquinone with a through put of 83 h^{-1}. All different chemical and physical experimental parameters affecting on the development and stability of the colored product were carefully studied and the proposed methods were successfully applied for the determination of hydroquinone in pharmaceutical preparations.

Keywords: Hydroquinone,oxidative-coupling, DNPH, FIA, Spectrophotometric determination.

التقدير الاني للهايدروكوينون بصيغته النقية والمستحضرات الصيدلانية باستخدام طريقتي الوجبة والحقن الجرياني–اندماج المناطق والتحسس بالطريقة الطيفية

بشرى بشير الموصلي[1]،اسيل صلاح الجميلي[2] ،همسة شاكرالجنابي*[1]
[1] قسم الكيمياء،كلية العلوم،جامعة بغداد،العراق
[2] قسم الصيدلة،كلية الرشيد الجامعة،العراق

الخلاصة

تتضمن الدراسة تطوير طريقة طيفية جديدة وسريعة للتقدير الكمي لمقادير ضئيلة من الهايدوكوينون في المحاليل المائية والمستحضرات الصيدلانية باستخدام طريقتي الدفعة التقليدية وتقنية الحقن الجرياني-اندماج المناطق. تعتمد الطريقتين على تفاعل الازدواج التأكسدي للهايدوكوينون مع كاشف 2و4- داي نايتروفنيل هايدرازين المؤكسد في الوسط قاعدي حيث تتكون صبغة آزو بني غامقة مستقرة وذائبة في الماء اعطت اعلى امتصاص عند طول موجي 530 نانومتر. تشير منحنيات الامتصاص مقابل التركيز انها مطاعة لقانون بير ضمن مدى التراكيز 1-40 ، 3-300 مايكروغرام.مل$^{-1}$ من الهايدروكوينون وبحدود كشف 0.162 ، 0.52 مايكروغرام.مل$^{-1}$ من الهايدوكوينون لطريقتي الدفعة والحقن الجرياني على التوالي وبمعدل نمذجة 83 أنموذج في الساعة بطريقة الحقن الجرياني، تم دراسة الظروف المثلى للتفاعل وجميع المتغيرات الفيزيائية والكيميائية المؤثرة على استقرارية الصبغة الناتجة بدقة وضبط عاليين، طبقت الطريقتين بنجاح في المستحضرات الصيدلانية الحاوية على الهايدوكوينون.

*Email: hamssa_62@yahoo.com

Introduction

In routine analysis flow injection systems are interesting because of their multiple advantages, that include the fast response, low-cost instrumentation, automated, reproducible and accurate results in addition to the large number of samples which can be analysed per a unit of time [1] the combination of this technique with the spectrophotometric detection significantly increases the advantages offered by these systems which are at the same time highly selective and sensitive, therefore low detection limits can be achieved [2]. Hydroquinone is an important phenolic compound used in a wide variety of biological [3] and industrial processes [4]. It is used principally as inhibitor in polymer industries to stop polymerization of acrylic acid, methyl methacrylate during storage and shipping processes. Hydroquinone is used as an intermediate in the manufacturing of antioxidants for rubber, dyestuffs and food products. The major use of hydroquinone is as a reducing agent in photographic developing solution [5-7]. There are varieties of methods a vailable in the literature for the determination of hydroquinone using titrimetry,voltammetric [8,9], colorimetry [10], thin layer and high performance liquid chromatography with different detectors [11,12], capillary electrochromatography [13,14], gas chromatography- mass spectrometry [15,16], spectrofluorimetry [17], voltammetry [18,19], spectrophotometric procedures [20-23], flow injection analysis with spectrophotometric detection [24,25], FIA and amperometric,fluorescence detection [26,27] , FIA-chemiluminescence [28-30].

In the present paper, a method to determine hydroquinone in pure form and in pharmaceutical formulations by Batch and FIA-merging zone techniques with spectrophotometric detection was described, The manifold consisted in one channel and six-three ways valves. The proposed methods are based on oxidizing DNPH by sodium periodate to give a diazonium cation which reacts with drug in alkaline medium by electrophilic substitution at the phenolic ring to give a colored product. The proposed FIA method was designed in way that Hydroquinone, DNPH and NaOH were simply loaded in FIA system through the home made valves using a solution of $NaIO_4$ as a carrier .

Materials and apparatus

All spectral and absorbance measurements were carried out an a shimadzu UV-VIS 9200, Biotech Engineering management CO, LTD, (UK) digital double- beam recording spectrophotometry using 1 cm quartz cell. A quartz flow cell with 100 µL internal volume and 1cm bath length was used for the absorbance measurements. A one channel manifold was employed for the FIA spectrophotometric determination of hydroquinone. A peristaltic pump (YZ1515x BT one channel,china) was used to pump the reagents solutions. Injection valve (six- three ways homemade which including 3-loops made of Teflon) that loaded with drug (loop1),DNPH (loop2) and NaOH (loop3) based on merging–zone technique were employed using appropriate injection volumes of standard solutions and sample. Flexible vinyl tubes of 0.5mm internal diameter were used for the peristaltic pump ,while the reaction coil (RC) was made of glass with internal diameter of 2mm.The sodium periodate as carrier was combined with injected sample (hydroquinone, L_1) and they merged with the reagent 2,4-dinitrophenyl hydrazine, L_2) in basic solution (sodium hydroxide,L_3) then mixed in reaction coil (RC) with length of 50 cm, injection sample of 157 µL, flow rate of carrier of 18.4 $ml.min^{-1}$, the absorbance was measured at 530nm.

Chemical and reagents

All reagents were of analytical grade and distilled water was used throughout.

Hydroquinone stock solution (500 $\mu g.ml^{-1}$ = 0.00454M)

A 0.05g amount of pure HQ(SDI) was dissolved in sodium hydroxide (0.1M) then completed to 100ml in a volumetric flask with D.W.More dilute solutions were prepared by suitable dilution of the stock standard solution with distilled water.

Sodium hydroxide (0.5M) / A 2gm amount of NaOH (BDH) was dissolved in a 100ml volumetric flask and completed with distilled water.

2,4-dinitrophenylhydrazine (DNPH) ($5x10^{-3}$M)/ Prepared by dissolving 0.0991gm of pure DNPH(SDI) in 2ml of conc.sulpharic acid and completed the volume to a 100 ml in volumetric flask with distilled water.

Sodium periodate ($5*10^{-3}$M)/ A 0.1068gm amount of $NaIO_4$ (Merck) was dissolved in distilled water 100ml volumetric flask and completed to the mark with the same solvent. more dilute solutions were prepared by dilution of the stock solution with D.W.

Pharmaceutical formulations of hydroquinone/ Pharmaceutical formulations were obtained from commercial sources in some cosmetic creams.

1- Fediquin (Jorden), 4% **2-** Hydropaque (Syria), 4% **3-** Hydroquinone, (Syria), 2%

Procedure for the assay of pharmaceutical preparations(creams solutions)(100 µg.ml^{-1})

For the preparation of stock solution of lightening cream, 0.01 g of the each sample was taken in a pre-weighed beaker and 20 mL of methanol was added and throughly mixed using a glass rod , then completed to mark(100ml) with 0.1M NaOH .1ml of this solution was mixed with 1.5 ml of $5x10^{-3}$M DNPH and 1.5 ml of $5x10^{-3}$M $NaIO_4$ and 3ml of 0.5M NaOH in a 25 ml volumetric flask and this mixture was diluted with distilled water[18].

General procedure for calibration

A / Batch procedure

A 1.5 ml of $5x10^{-3}$M DNPH was transferred into a series of 25 ml volumetric flasks, to this solution was added equilmolar of sodium periodate solution ($5x10^{-3}$M)in basic medium with 3ml of 0.5 M sodium hydroxide solution. The solutions were shaken thoroughly. Then, an a aliquot volumes of 1ml of standard solution 500µg.ml^{-1} (0.00454M) of hydroquinone was transferred into the series of 25 ml volumetric flasks,then were diluted to the mark with distilled water,mixed well. After 10min., the absorbance of the colored product was measured at 530 nm against the corresponding reagent blank.

B / FIA procedure

A hydroquinone solution in the range 3-300 µg.ml^{-1} was prepared from the standard working solution of 500 µg.ml^{-1}. The injection volumes of (196.25µL, 196.25µL and 196.25µL) of HQ (L_1), DNPH ($5x10^{-3}$ M,L_2) and NaOH (0.5 M,L_3) respectively, were injected into the carrier of sodium periodate ($5x10^{-4}$M) with flow rate of 18.4ml/min for a one channel, the resulting absorbance of the dark brown product was conducted. Optimization of conditions were carried out on 100 µg.ml^{-1} of HQ.

Results and discussion

Batch spectrophotometric detection

The factors affecting on the sensitivity and stability of the colored product resulting from reaction between oxidized DNPH with HQ in alkaline medium were carefully studied and optimized. A typical spectrum for the azo dye formed was measured versus reagent blank which has negligible absorbance at λ_{max} = 530 nm, as shown in figure-1.

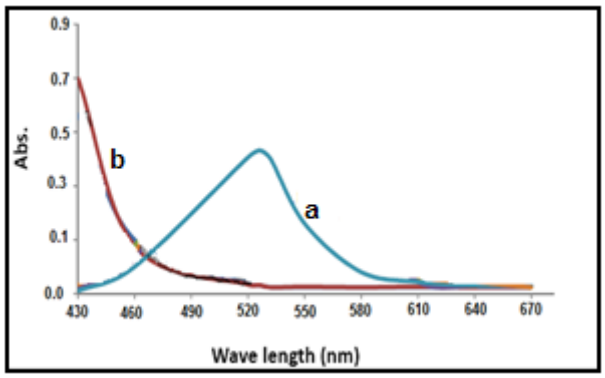


Figure 1- (a) Absorption spectra of the azo dye 20 µg.ml^{-1} of hydroquinone against reagent blank. (b) Blank against D.W.

The experimental conditions for the determination of hydroquinone were performed by varying one factors at a time and keeping the other parameters fixed and observing the effects of the product on the absorbance.

The effect of different volumes 0.3-2 ml of $5x10^{-3}$M 2,4-dinitrophenyl hydrazine and 0.3-2 ml sodium periodate ($5x10^{-3}$M) on color development were used.The results obtained showed that 1.5ml of DNPH ($5x10^{-3}$M) and 1.5ml of $NaIO_4$ ($5x10^{-3}$M) were efficient for color development .Absorbance of the dye formed increased and became more stable in alkaline , therefore, the effect of different alkaline solutions (0.5M) on colored product was studied such as potassium hydroxide, ammonium hydroxide, sodium hydroxide and sodium carbonate, maximum sensitivity and stability were obtained only when the reaction was performed in the presence of sodium hydroxide (0.5M), the effect of different volumes (0.1-7) ml of 0.5M of NaOH were studied . A volume of 3ml seems to be optimum for an intense colored product.

The experimental result also revealed that the color intensity reach maximum after oxidization of DNPH by $NaIO_4$ solution, then had been coupled with hydroquinone in an alkaline medium for 10 min and remained stable, therefore; a 10 min development time was suggested as the optimum reaction time,the complex remained stable for 150 min.

Acceleration of color intensity was applied by varying the addition sequence of the drug (HQ), the base (NaOH), the reagent (2,4-dinitrophenylhydrizine) and sodium periodate. A best absorption value was achieved by adopting the following sequence (Drug + DNPH + $NaIO_4$ + Base) and was used in all subsequent experiments.

The composition of the formed complex had been established using mole ratio method which was based on the measurement of series of solutions containing an increasing volumes (0.3-2) ml of oxidative formed reagent ($4.5x10^{-3}$M) added to affixed volume (1ml) of ($4.5x10^{-3}$ M) hydroquinone, under optimum condition mentioned in the analytical procedure. The results obtained in figure -2shows that a (1:1) azo dye was formed between hydroquinone (HQ) and diazonium cation reagent (DNPH) by electrophilic substitution at the phenolic ring to give a colored product.

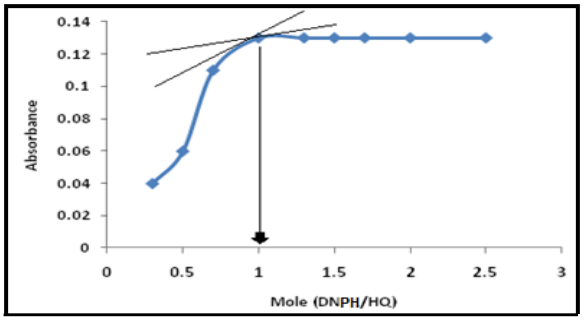
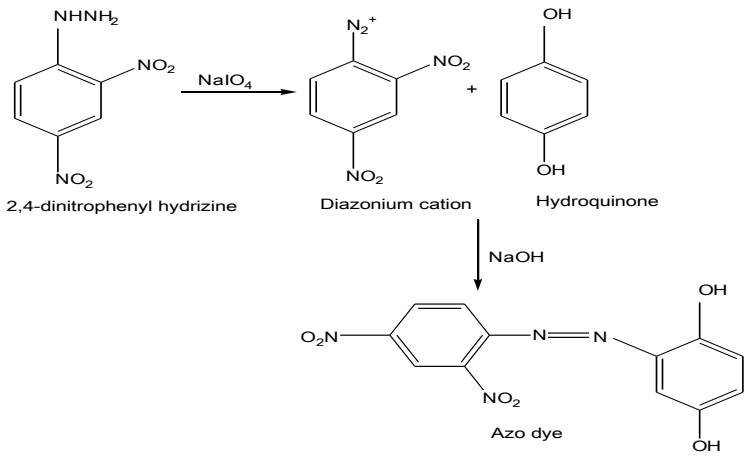


Figure 2- Mole ratio plot

The probable reaction path might be written as following (scheme-1).



Scheme 1- Reaction path.

The product formed was soluble in water.The apparent stability constant was calculated [31] by comparing the absorbance of a solution containing stoichiometric amount of hydroquinone 0.0045 M,(A_s)with a solution containing a five-fold excess of diazonium cation, (A_m) and according to analytical procedure. The average of stability constant, K [$K= (1-\alpha)/\alpha^2 C$; $\alpha=A_m-A_s/ A_m$, $C=1.816x10^{-4}M$ (HQ)] of the product in water under the described experimental of conditions was $3.244x10^5$ $L.mole^{-1}$

The regression equation obtained and the analytical features of the procedure are summarized in (table-1).It also summarized the main performance of the flow injection procedure developed for hydroquinone determination in order to make an effective comparison between the two approaches.

FIA-Spectrophotometric determination

The batch method for the determination of hydroquinone was adopted as a basis to develop a FIA procedure. The manifold used for the determination of hydroquinone was designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of oxidative formed of DNPH with hydroquinone in sodium hydroxide medium. Maximum absorbance intensity was obtained when the sample (100 $\mu g.ml^{-1}$ of HQ), the reagent ($5x10^{-3}M$ of DNPH) and the base (0.5M of NaOH) were injected into a carrier of sodium periodate ($5x10^{-4}M$) with flow rate of $18.4ml.min^{-1}$, as shown in figure -3.

The influence of different chemical and physical FIA parameters on the absorbance of the colored product was optimized as follow:-

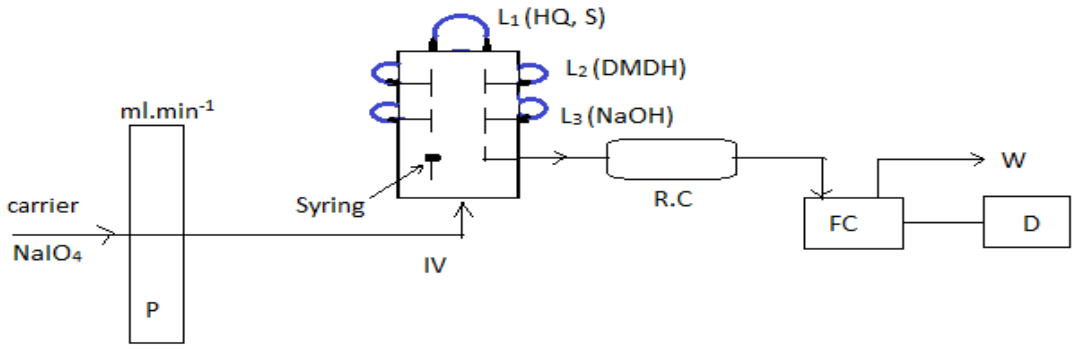


Figure 3- A schematic diagram of FIA manifold where, S; injection sample hydroquinone, P; peristaltic pump R.C; reaction coil, FC; flow cell, D; detector, IV; injection value, W; waste.

Optimization of chemical parameters

The effect of various concentrations of sodium periodate ($1x10^{-4}$-$5x10^{-3}M$) using for oxidizing DNPH was studied.It was found that $5x10^{-4}M$ $NaIO_4$ gave the highest absorbance and chosen for further experiments, as shown in figure -4.

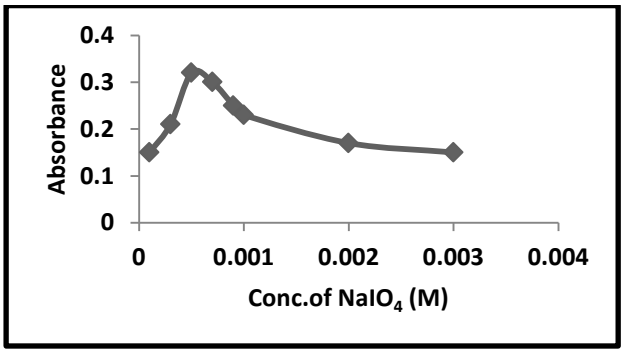


Figure 4- Effect of the conc. of sodium periodate in (**M)**

The effect of various concentrations of 2, 4-dinitrophenylhydrazine($3x10^{-3}$-$1x10^{-2}$M) was investigated. A concentration of ($5x10^{-3}$M) of DNPH, gave the highest absorbance and chosen for further experiments as shown in figure -5.

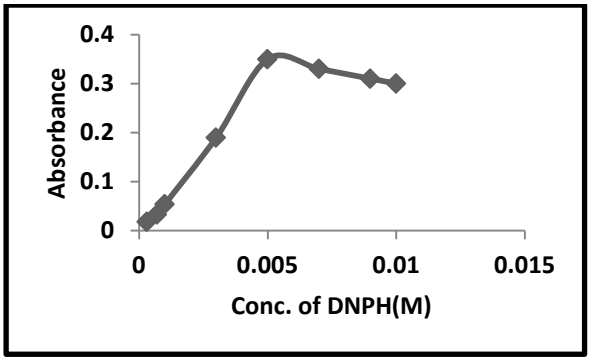


Figure 5- Effect of concentration of DNPH reagent in 0.5M of NaOH.

It was observed that the reaction between diazonium–DNPH and hydroquinone depends on the alkaline medium, therefore; the effect of different concentrations of sodium hydroxide(0.1-2M) was studied and 0.5M was found to be optimum as shown in figure-6.

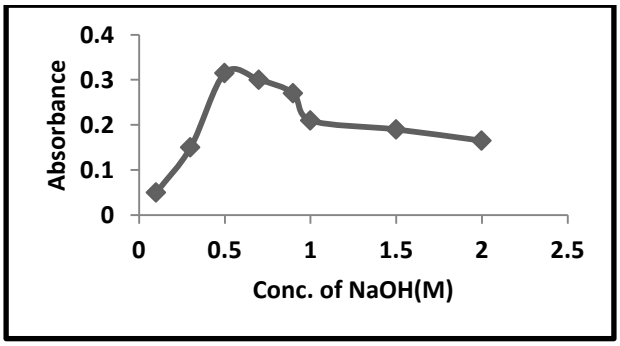


Figure 6- Effect of the concentration of NaOH (M)

Optimization of manifold parameters

The effect of total flow rate on the sensitivity of the colored reaction product was investigated in the range of 2-22 ml.min^{-1}. The results obtained showed that a total flow rate of 18.4ml.min^{-1} $NaIO_4$ as a carrier, gave the highest absorbance as shown in figure -7, and was used in all subsequent experiments .

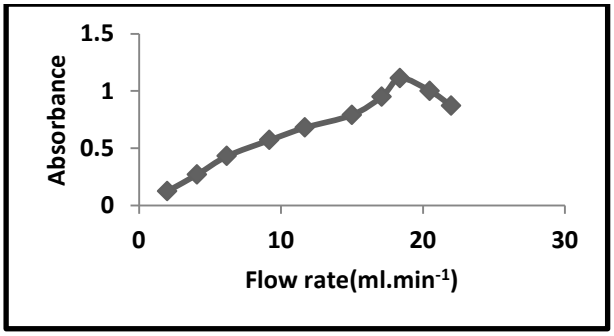
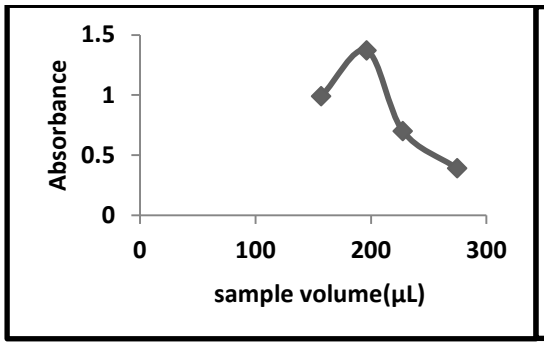


Figure 7- Effect of the total flow rate (ml.min^{-1})

The volume of the injected sample and the injected reagent were varied (157, 196.25, 227.65 and 274.75)µL and (157, 196.25, 227.65 and 274.75) µl, for sample and reagent volume, respectively using different lengths of sample and reagent loops.The results obtained showed that the injected

volumes of 196.25& 196.25 µL for sample and reagent respectively. gave the best absorbance, as shown in figures -8,9.



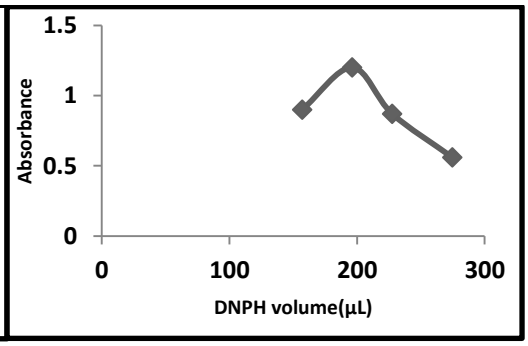


Figure 8- Effect of volume of sample loop(µL) **Figure 9**- Effect of volume of reagent loop(µL)

The coil length is an essential parameter that effects on the sensitivity of the colored reaction product and was investigated in the range of (50-200) cm.The results obtained showed that a coli length of 50 cm gave the highest absorbance as shown in figure -10 and was used in all subsequent experiments.

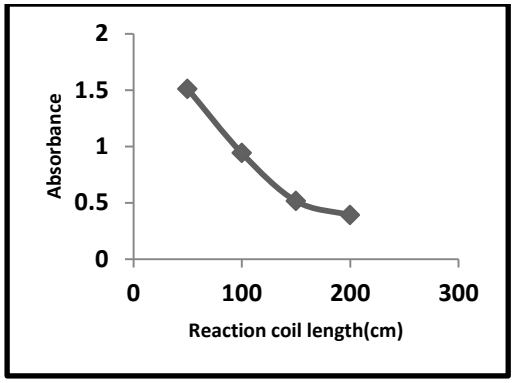


Figure 10- Effect of the length of the reaction coil (cm).

The injection time is also an important parameter that effect on the sample through put and was investigated by calculating the interval time between the sample injection and the appearance of the signal. The injection time of each sample was 43 sec., therefore; the sample through put was 83 samples/ h.

Analytical characteristics

The analytical characteristics such as linear range, detection limit, and relative standard deviation, recovery and correlation coefficient of each method were estimated under the optimized conditions, as shown in table -1. A standard calibration curve obtained for a series of hydroquinone standard solution and the main analytical figure of merits [32] of the developed procedure.Statistical evaluation of regression line gave the value of standard deviation for residuals (Sy/x), slop (S_b) and intercept (S_a) at 95% confidence limits for (n-2) freedom degrees are demonstrated in above table -1, these small points were referred to high repeatability and reproducibility of the developed FIA, the procedure with LOD and LOQ were calculated and shown in the same table.

Table 1- Analytical characteristics of the procedure developed for the determination of hydroquinone.

Parameters	FIA procedure	Batch procedure
Correlation coefficient,r^2	0.9991	0.9997
Dynamic rang (µg.ml^{-1})	3-300	1-40
Regression equation	Y=0.0047x+0.1319	Y=0.0148x+0.0064
Reproducibility (%)	0.969	0.752
Linearity percentage,r^2%	99.91	99.97
Slop, b (ml. µg^{-1})	0.0047	0.0148
Intercept, a	0.1319	0.0064
Confidence limit of slop(b)=b±tS_b, for n-2	0.0047± t 0.987x10^{-6}	0.0148± t 0.866x10^{-4}
Confidence limit of intercept a =a± tS_a, for n-2	0.1319± t 0.00013	0.0064 ± t 0.00195
Standard deviation of the residuals, Sy/x	0.0200	0.0036
Standard deviation of the slop, S_b	6.1x10^{-5}	0.866x10^{-4}
Standard deviation of intercept, Sa	0.0093	0.0019
Limit of detection (LOD), µg.ml^{-1}	0.638	0.932
Molar absorptivity, (€) (L.mole^{-1}.cm^{-1})	0.0517x10^3	0.162x10^4
Sandells sensitivity (µg.cm $^{-2}$)	0.212	0.067
Limit of quantification	2.127	3.108
Sample through put (hr^{-1})	83	8

Pharmaceutical applications

In order to demonstrate the applicability of the proposed methods were applied for the analysis of hydroquinone in pharmaceutical formulations. Table -2 summarizes the results obtained for these preparations; there is no interference from the excipients in accordance with those obtained by official method [33]. Finally, the results obtained by the proposed methods were compared with results obtained from standard method by applying the F-test and the t-test at 95% confidence limits. The calculated valued for F-test were (3.963) and (4.003), and t-test values were (2.239) and (0.789) for the batch and FIA methods, respectively, did not exceed the critical values of F-test = 19.009 and t-test=2.770 (n_1 + n_2-2=4). It reveals that there is no significant difference in precision and accuracy between the proposed methods and official method for the determination of hydroquinone in pharmaceutical formulations, as shown in table- 2.

Table 2- Application of the proposed methods for determination of hydroquinone in pharmaceutical formulations.

Pharmaceutical formulations	Proposed methods						Official method recovery
	FIA			Batch			
	Present conc. (µg.ml^{-1})	Rec*(%)	RSD* (%)	Present conc. (µg.ml^{-1})	Rec*(%)	RSD* (%)	
(1) Fediqum (Jorden)	50	99.980	0.156	5	99.970	0.314	101.17
	100	100.007	0.174	10	100.016	0.316	
(2) Hydropaque (Syria)	50	99.980	0.410	5	99.950	0.527	102.30
	100	100.007	0.200	10	100.019	0.315	

***average of six measurements.**

Conclusion

The proposed method was superior to other reported methods by showing a high repeatability in results and reproducibility in injection time.The application of oxidative coupling reaction of hydroquinone with DNPH in an alkaline medium to the spectrophotometric determination of drug in pharmaceutical preparations was described by batch and FIA- merging zone techniques. Although the batch system was the advantages of a higher sensitivity and lower limit of detection over the FIA system, the developed FIA has several advantages over the batch method, through the injection valve (six-three ways homemade) needs of low consumption of sample & reagents, simplicity, rapid, inexpensive since it requires simple instrumentation and large dynamic rang with high sample through put (83 sample h^{-1}) were important features of the FIA systems. In addition to competitive precision and good linearity, the proposed procedure show relevant selectivity allowing analysis of a wide concentration range of hydroquinone in dosage forms without separation steps with satisfactory results and providing suitable alternative to other many methods.

Reference

1. Rueda, M.E., Sarabia, L.A., Herrero, A. and Ortiz, M.C. **2003**. Optmization of a flow injection system with electrochemical detection using the desirability function , Application to the determination of hydroquinone. *Analytica chimica Acta*, 479 , pp: 173 – 184 .
2. Valcavcel, M. and Luque de Castro, M.D.**1987**. "*Flow Injection Analysis Principles and Applications"*, Ellis Horwood , Chichester, P: 198.
3. Chambers , J.Q. **1988** . *In the chemistry of Quinonoid compounds ; Patai , S. , and Rapport , Z.* , Eds ; willey & Sons ; New York . 11, P: 719 .
4. Nielson, A.H., Allard, A.S., Hnning, E.A. and Rememberger, M. **1991**. Toxical . *Environ* . *Chem* . , 30, P: 3.
5. Leto,W.,Yan, Z., Yongling, D. and Chunming ,W.**2012**.Simultaneous determination of catechol and hydroquinone based on poly (diallyldimethylammonium chloride) functionalized grapheme-modified glassy carbon electrode. *j. solid Electrode* , 16, pp:1323-1331.
6. Renata,K.M.,Prisscila,C. and Eder,T.G.**2006**, The use of a graphite-castor oil polyurethane composite electrode for the determination of hydroquinone in photographic developers, *Talanta* , 68,pp:708-712.
7. Budavari . S.**1989**.*The Merck Index. An Encyclopedia of Chemicals, Drugs and Biological*. 11th ed . Ed. Merck and Co. Inc. , Rahway , NJ.
8. *The United States Pharmacopoeia*.**1990**. The National Formulary 17, United States Pharmacopeial Convention Inc., Rockville, MD. PP: 665-666.
9. Haijun, D.,Jiaqi,Z. and Xiaodan, H.. **2011**."Avoltammetric sensor based on graphene-modified electrode for simultaneous determination of catechol and hydroquinone.*J.Electro.Anal.Chem.*650,PP: 209-213.
10. Whettem, S. M. A. **1979**.The determination of small amount of Hydroquinone in styrene, *Analyst* ,74, p:185.
11. Risner , C.H. **1993**. The quantification of HQ , catechol , phenol , 3-methy cateohol, scopoletin , m+p-cresol and O-cresol in indoor air samples by HPLC , *J.Chromatogr*. , 16(18), PP: 4117 – 4140.
12. Huang, J.X., Stuart, J.D., Melander, W.R. and Horvath, C. **1984**. High performance liquid Chroma. of substituted P-benzoquinones and P-hydroqninones Interplay of on-column redox reaction and chromatographic retention process. *J.Chromatogr*.,316, PP :151-161.
13. Desiderio , C. , Ossicini , L. , and Fanali , S. **2000**. Analysis of HQ and some of its ethers by using capillary electrochromatography. *J. Chromat*. A., 887,PP: 489 – 496.
14. Firth, J. and Rix, I. **1986**. Determination of hydroquinone in skin-toning creams using high-performance liquid chromatography. *Analyst*, 111,P: 129.
15. Perl, I.M., Tisza , S.K., Kurin Csorgei, E.K. and Szalai, I. **1995**. Quantitation of hydroquinone, 1,4-benzoquinone, and 1,4-cyclohexandione: one by one simultaneously. *J.High Resolute Chromatogr*. , 18(12) ,P: 749.
16. Pryor, W.A., Stone, K., Zong, L.K. and Bermudez, E. **1998**. Fractionation of aqueous cigarette tar extracts:fractions that contain the tar radical cause DNA damage. *Chem. Res. Toxical* , 11(5) ,P: 441.

17. Pistonesi, M.F., Dinezia, M.S., Centurion, M.E., Lista,A.G., Fragoso,W.D.**2010**. Simultaneous determination of hydroquinone,resorcinol,phenol,m-cresol and p-cresol in untreated air samples using spectrofluorimetry and a custom multiple linear regression-successive projection algorithm.*Talanta*.83,PP:320-323.

18. Vieira, I.C. and Filho.**2000**. Biosensor based on Paraffin / graphite modified with sweet potato tissue for the determination of hydroquinone in cosmetic cream in organic. phase, *Talanta* , 52 ,PP: 681-689.

19. Zhao, D.M., Zhang, X.H., Feng, I.J. and Wang, S.F. **2009.**Simultaneous determination of hydroquinone and catechol at PASA/MWNTs composite film modified glassy carbon electrode , coll.surf.*B:Biointer ferences* 74 , PP:317-321.

20. Sirajuddin, Bhanger, M.I. , Niaz , A. , Shah , A. and Rauf , A. **2007**.Ultra _ trace level determination of hydroquinone in waste photographic solution by uv _ vis spectro photometry, *Talanta* , 72 , PP:546 – 553.

21. Afkhami , A. and Khatmi, H.A. **2001**. Indirect kinetic – spectrophotometric determination of resorcinol , catechol and hydroquinone. *J. Anal. Chem.* ,56,P:429.

22. Shahine, S. and Mahmoud, R.**1978**. Indirect spectrophotometric determination of some reducing agents, using phenolphthalin as reagent, *Microchim , Acta*. , 70 , PP: 431-434,

23. Gavcia,P.L.,Santoro,M.I.R.M.,Singh,A.K and Kedor-Hachmann, E.R.M.,**2007**. Determination of optimum wavelength and derivative order in spectrophotometry for quantitation of hydroquinone in creams. *J.Pharmac.Sci*, 43, PP: 397-404.

24. Rebeeiro, P.R.S.,N eto, J.A.G.,Pizza,L. and pizz, H.R. **2005**. Flow-injection spectrophotometric determination of methyldopa in pharmaceutical formulations. *Talanta*, 67, P:240.

25. Ruedo,M.E.,Sarabia,L.A.,Herrero,A. and Ortiz,M.C. **2003**.Optimization of a flow injection system with spectrophotometric detection of HQ in cosmetics. *Anal.chim.Acta*, 479,pp:173-184.

26. Zhou, J.X., Wang, E.K.**1992**. Liquid chromatography amperometric detection of catechol, resorcinol, and hydroquinone with a copper- based chemically modified electrode. *Electro analysis*, 4, P: 183.

27. Pistonesi,M.,Centurion,M.E.,Pereyra, M. and ,Lista,A.G. **2004**. Synchronous fluorescence for simultaneous determination of hydroquinone and resorcinol in air samples. .*Anal. Bioanal. Chem.* 378,PP:1648-1651.

28. Fan, S.L., Zhang, L.K. and Lin, J.M.**2006**. Post-column detection of benzenediols and 1,2,4-benzenetriol based on acidic potassium permanganate. chemiluminescence, *Talanta*, 68, PP:646-652.

29. Haghighi, B. and Dadoshvand, R.**2006**. Flow injection chemiluminesce analysis of phenolic compounds using the NCS-Luminol system. *Anal. Bioanal and chem*., 384, PP:1246-1253.

30. Corominas, B.G.T., Icardo, M.C., Zamor, L.L., Mateo, J.V.G. and Calatayud, J.M.**2004**. Atandem. Flow assembly for the chemiluminometric determination of hydroquinone. *Talanta*, 64, p:618-625.

31. Al-Abachi, M.Q. and Al-Ghabsha, T.S.**1983**. "*Fundamental of Analytical Chemistry*" press of Mosul University, Mosul, p. 346.

32. Miller, J.N. and Miller, J.C.**2000**. *Statistics and Chemometrics for Analytical Chemistry*, 4th ed., pearson education limited, London.

33. United State British Pharmacopoeia on CD-ROM. **2013**.Version 5, 3rd Ed.,Vol.1,Copyright by System Simulation Ltd, The Stationery Office Ltd.,London.