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

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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-dinitrophenylhydrazine (DNPH) in the presence of sodium periodate and sodium hydroxide to form a dark brown water soluble dye that is stable and has maximum absorption at 530 nm, graphs of absorbance versus concentration show that Beer's law 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 طريقة الطيفية

والحقن الجرياني-اندماج المناطق والتحكم بالطريقة الطيفية

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الخلاصة

تتضمن الدراسة تطوير طريقة طيفية جديدة وسريعة للتقدير الكمي لمقدار ضئيلة من الهايدروكوينون في المحاليل المائية والمستحضرات الصيدلانية باستخدام طريقة الدفعة الطيفية وتقنية الحقن الجرياني. انطلاقاً من العناصر المختومتين على تفاعل الازدواج التأكسدي للهايدروكوينون مع كاشف 2,4-دانيترÓ-هيدزاين المؤكسد في الوسط قاعدي حيث تتكون صبغة آزوزية مستقرة وذائبة في الماء، اكتسبنا على انطلاق انتصاع عند طول موجي 530 نانومتر. تشير منحنى امتصاصات مقابل التركيز انها مطابقة لقانون بير ضمن مدى التركيز 1-40 ، 3-300 مايكروغرام.مل⁻¹ من الهايدروكوينون وبحدوث كشف 0.162 ، 0.52 مايكروغرام.مل⁻¹ من الهايدروكوينون لطريقة الدفعة الحقن الجرياني على التوالي وبحدوث نجاح 83 نموذج في السعة بتطبيق الطريقة الحقن الجرياني. تم دراسة المركبات المتماثلة من الهايدروكوينون على الاستقرارية الصبغية النتائج بدقة وضبط عاليين، نجحت الطريقتين بنجاح في المستحضرات الصيدلانية الحاوية على الهايدروكوينون.

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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 NaIO₄ 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, L₁) and they merged with the reagent 2,4-dinitrophenylhydrazine, L₂) in basic solution (sodium hydroxide, L₃) 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 µg.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 (5x10^{-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 througly 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 equimolar 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 \( \lambda_{\text{max}} = 530 \) nm, as shown in figure-1.

![Figure 1](image-url)

**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 $5 \times 10^{-3}$M 2,4-dinitrophenyl hydrazine and 0.3-2 ml sodium periodate ($5 \times 10^{-3}$M) on color development were used. The results obtained showed that 1.5ml of DNPH ($5 \times 10^{-3}$M) and 1.5ml of NaIO$_4$ ($5 \times 10^{-3}$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-dinitrophenylhydrazine) 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 ($4.5 \times 10^{-3}$M) added to affixed volume (1ml) of $4.5 \times 10^{-3}$ M hydroquinone, under optimum condition mentioned in the analytical procedure. The results obtained in figure 2 shows 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](image)

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

![Scheme 1- Reaction path.](image)
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, \( K = (1-\alpha)/\alpha^2 \) C; \( \alpha = A_m - A_s / A_m \), \( C = 1.816 \times 10^{-4} \) M (HQ) of the product in water under the described experimental of conditions was 3.244 \( \times 10^5 \) 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:-

**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\(^{-3} \) M NaN\(_3\) gave the highest absorbance and chosen for further experiments, as shown in figure 4.

![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.](image)

![Figure 4- Effect of the conc. of sodium periodate in (M)](image)
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](image)

**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](image)

**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](image)

**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 affects on the sensitivity of the colored reaction product and was investigated in the range of (50-200) cm. The results obtained showed that a coil 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 (Sb) and intercept (Sa) 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FIA procedure</th>
<th>Batch procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient, r²</td>
<td>0.9991</td>
<td>0.9997</td>
</tr>
<tr>
<td>Dynamic range (µg.ml⁻¹)</td>
<td>3-300</td>
<td>1-40</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y=0.0047x+0.1319</td>
<td>Y=0.0148x+0.0064</td>
</tr>
<tr>
<td>Reproducibility (%)</td>
<td>0.969</td>
<td>0.752</td>
</tr>
<tr>
<td>Linearity percentage, r²%</td>
<td>99.91</td>
<td>99.97</td>
</tr>
<tr>
<td>Slope, b (ml.µg⁻¹)</td>
<td>0.0047</td>
<td>0.0148</td>
</tr>
<tr>
<td>Intercept, a</td>
<td>0.1319</td>
<td>0.0064</td>
</tr>
<tr>
<td>Confidence limit of slope (b)</td>
<td>0.0047±0.987x10⁻⁵</td>
<td>0.0148±0.866x10⁻⁴</td>
</tr>
<tr>
<td>Confidence limit of intercept a</td>
<td>0.1319±0.00013</td>
<td>0.0064±0.00195</td>
</tr>
<tr>
<td>Standard deviation of residuals, S_y</td>
<td>0.0200</td>
<td>0.0036</td>
</tr>
<tr>
<td>Standard deviation of slope, S_b</td>
<td>6.1x10⁻³</td>
<td>0.866x10⁻⁴</td>
</tr>
<tr>
<td>Standard deviation of intercept, S_a</td>
<td>0.0093</td>
<td>0.0019</td>
</tr>
<tr>
<td>Limit of detection (LOD), µg.ml⁻¹</td>
<td>0.638</td>
<td>0.932</td>
</tr>
<tr>
<td>Molar absorptivity, (€) (L.mole⁻¹.cm⁻¹)</td>
<td>0.0517x10³</td>
<td>0.162x10⁴</td>
</tr>
<tr>
<td>Sandells sensitivity (µg.cm⁻²)</td>
<td>0.212</td>
<td>0.067</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>2.127</td>
<td>3.108</td>
</tr>
<tr>
<td>Sample throughput (hr⁻¹)</td>
<td>83</td>
<td>8</td>
</tr>
</tbody>
</table>

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 (n₁ + n₂-2=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.

<table>
<thead>
<tr>
<th>Pharmaceutical formulations</th>
<th>FIA</th>
<th>Batch</th>
<th>Official method recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rec*(%)</td>
<td>RSD* (%)</td>
<td>Present conc. (µg.ml⁻¹)</td>
</tr>
<tr>
<td>(1) Fediqum (Jorden)</td>
<td>50</td>
<td>99.980</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100.007</td>
<td>0.174</td>
</tr>
<tr>
<td>(2) Hydropaque (Syria)</td>
<td>50</td>
<td>99.980</td>
<td>0.410</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100.007</td>
<td>0.200</td>
</tr>
</tbody>
</table>

*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


