Development of FIA system for the spectrophotometric determination of hydroquinone in pure material and pharmaceutical formulations

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ABSTRACT

This article deals with the development of flow injection system for the spectrophotometric determination of Hydroquinone drugs. The analytical system is based on oxidation reaction using KMnO₄ as oxidizing agent for conversion of HQ to P-benzoquinone (BQ) in an alkaline medium, the absorbance is measured at 610nm. All different chemical and physical experimental parameters affecting on the development and stability of the colored product were carefully studied. The parameters optimized by using 20 µg ml⁻¹ standard solution of HQ, Beer’s law is obeyed over the concentration range of 1-26 and 3-125µg.ml⁻¹ of Hydroquinone with detection limits of 0.0125 and 0.25 µg.ml⁻¹ of Hydroquinone for spectrophotometric and FIA-Merging zone, respectively. The color product formed by passing of a 0.1 M sodium hydroxide as carrier at flow rate of 2.12ml.min⁻¹ is merged with a volume of 227.65 µL of sample and the volume of 227.65 µL of oxidant agent to conclude a final methodology for the determination of HQ accommodating 227.65µL as injection sample. Under these Conditions, the FIA system allowed to analyze was about 55 samples per hour. The method was successfully applied to the determination of HQ in Pharmaceutical formulations without any interference from common excipients used as additives. The results agree favorably with standard method.

Key words: Hydroquinone, Flow injection, oxidization reaction, spectrophotometric determination, KMnO₄.

INTRODUCTION

Hydroquinone (HQ) is used as a developer in black and white photography [1]. An antioxidant for fats and oils a polymerization inhibitor, a stabilizer in paints, varnishes, motorfuels, oils, an intermediate for rubber processing chemicals in the production of mono and dialkyl ethers and as de-pigmenting agent [2]. Besides its positive and beneficial utilization it bears some harmful and toxic aspects as well, which may produce serious health complications due to its release especially in water and air from mentioned and other sources. The possible health problems include irritation of skin, eyes, nose and throat, dizziness, headache, unconsciousness, tinnitus, breathing difficulties and others [3]. It has also been reported as a nephrocarcinogenic reagent [4]. It is extensively used in skin – toning preparations or skin lightening cosmetics and suggested to be effective at 1.5 -2.0%[1,5-8].

It is recommended that HQ must be used under prescription because its long –term contact in concentrations of greater than 5% can produce various side effects[6]. These side effects may be acute or chronic. Acute side effects are allergic and irritant contact dermatitis, post-inflammatory hyperpigmentation and nail discoloration. High concentrations of HQ (above 5-6%) have been implicated in persistent hypopigmentation or depigmentation, a condition known as leukoderma. Exogenous chronosis is a major chronic side effect of HQ. This condition is characterized by reticulated, ripple-like, sooty pigmentation on the forehead, cheeks and other areas of HQ application [7]. Despite its numerous useful applications, HQ has been reported as mutagenic in animals[8] and a possible nephrocarcinogen[4]. According to other report [9] mononuclear compounds such as benzene metabolites, caffeic acid and o-toluidine should express their carcinogenicity through oxidative DNA damage.
Several techniques have been reported in the literature for estimation of HQ in cosmetics such as voltammetry[10,11] high performance liquid chromatography (HPLC) [8,12-14] in different types of samples, titrimetry [15,16], Capillary electrochromatography (CEC)[17], Fluorimetry [18], GC/MS [19], electrochemical methods [20-22], chemiluminescence [1,23-26], Colorimetric analysis of small amount of HQ in styrene has also been reported [27, 28], flow injection analysis [29,31]. Spectrophotometric determination of HQ has been cited else where[32-36]. Due to easy instrumentation, low equipments, running cost, better repeatability, it’s a large through put per hour, board linear dynamic range and easy sample injection and preparation, the flow injection analysis technique such as FIA, rFIA, stop-flow or merging zone have clear advantages over the other techniques for the qualitative determination of environmentally toxic organic compounds in complicated matrices [37] and these techniques have some limitations such as use of expensive and or toxic ligands for complex formation and hence its expensive use for HQ complex determination in aqueous samples [32,34,38].

In contrast, our newly developed method is very fast, simple, economical and the main aim optimization is to find the experimental conditions which give the best response. Moreover, it has lower detection limits, better sensitivity and better application range for dilute aqueous samples where matrix effect minimizes the interfering effect of ions or reagents.

EXPERIMENTAL SACTION

Apparatus
All spectral and absorbance measurements were carried out by using a shimadzu UV-Visible,1200 digital double beam recording spectrophotometer(Japan), with 1cm quartz cells Biotech. Engineering Management CO.LTD.UV-9200(UK). A quartz flow cell with 100µL internal volume and 1 cm bath length (U.S.A) was used for the absorbance measurements. A one channel manifold (Figure10) was employed for the FIA spectrophotometric determination of Hydroquinone. A peristaltic pump (YZ1515X,China) was used to transport the reagents solutions. Injection value (6-three ways, Merging zone version, Home made) was employed to provide appropriate injection volumes of standard solutions and samples. Tubes:A- sample and reagent loops, made of teflon (0.5 mm internal diameter) B-Flexible vinyl tubing of 2mm with internal diameter tubing . Reaction coil was made of glass (I.D:1.5 mm ,length 100 cm, home made). Sodium hydroxide (figure10) as carrier was combined with injected sample (Hydroquinone) as Loop1 and reagent (KMnO4) as Loop2, then they merged with carrier (NaOH), mixed in reaction coil (RC) with length of 100 cm, injection sample loop (227.65 µL) and reagent volume (227.65µL), flow rate of 2.12 ml/min, the absorbance was measured at 610nm and at room temperature.

Reagents
Working Hydroquinone standard material was provided from state company for Drug Industries and Medical appliance (SDI) Sammara-Iraq of (99%purity) and stander solution of 500 µg.ml⁻¹ was freshly prepared by dissolving 0.0125 gm of hydroquinone in 5 ml sodium hydroxide 2M and then diluted with distilled water to the mark with 100 ml volumetric flask. Potassium permanganate of (99% purity) was obtained from Merck(Germany) a stander solution of 0.02 µg.ml⁻¹ was freshly prepared by dissolving 0.158 gm of KMnO4 in 50 ml distilled water. Sodium hydroxide (98% purity) from (RDL). Solution of 2M was prepared by dissolving 8gm in 100 ml distilled water,200 µg.ml⁻¹ of varies interferences by dissolving 0.125 gm in 250 ml distilled water. Dosage forms were obtained from commercial sources.

Procedure of Pure drug
An aliquot of sample containing 20 µg.ml⁻¹ of HQ was transferred into a series of 25 ml standard flask to cover the range of 1-26 µg.ml⁻¹. Avolume of 1.5ml of 0.02M KMnO4 solution and 2ml of 2M sodium hydroxide solution were added. The contents of flasks were diluted to the mark with distilled water, mixed well and left for 30 min. The absorbance was measured at 610nm (at room temp.). The color of the oxidant formed is stable for more than 4hr. For optimization of conditions and in all subsequence experiments, a solution of 500 µg was used and the final volume was 25 ml (i.e.20 µg/ml).

Analysis of Commercial dosage forms
For the preparation of stock solution of lightening cream, 0.25 gm of the each sample was taken in a pre-weighed beaker and 20 ml of methanol was added and thoroughly mixed using a glass rod , then completed to the mark with 0.1M NaOH.1ml of this solution was mixed with 1.5 ml of 0.02 M KMnO4 in a 25 ml volumetric flask, this mixture was diluted with D.W. The Sample thus prepared was transferred to the quartz cell and absorbance recorded in the same way as mentioned above, Cream samples were also prepared according to the method reported earlier [5] and the result were compared with those of the newly developed method.
RESULTS AND DISCUSSION

Hydroquinone (HQ) is slowly oxidized to BQ via a semiquinone (SQ) in alkaline medium [9] yielding highly soluble coloured which can be utilized as a suitable assay procedures for hydroquinone. The green colored product have maximum absorption at 610 nm. The blank at these wave length shows zero absorbance (Figure 1).

![Absorption spectra of HQ treated as described under procedure and measured against a reagent blank and a reagent blank measured against distilled water](image)

This conversion of HQ into BQ was taken as the basis for ultra-trace determination of HQ in aqueous solutions where the formation of greenish solution is responsible for absorption due to catalytic oxidation of the HQ in to BQ by KMnO₄. The conversion of HQ into BQ brings structural changes from benzenoid to quinoid ring according to chromophore theory [39]. A possible proposed mechanism for conversion of HQ into BQ via SQ can be worked out for Mn (VII) of KMnO₄ as shown in figure (2).

![Proposed mechanism for conversion of HQ into SQ and BQ](image)

According to this mechanism, the spectrum of HQ also disappears and the spectrum of BQ dominates. This proves the involvement of Mn (VII) of permanganate for oxidation of HQ into BQ with subsequent reduction of Mn (VII) to lower state. The reduction of Mn (VII) into lower state is also evident from the lowering and the disappearance of Mn (VII) spectrum which is present at lower concentration of analyte in a range of 400-750 nm. The role of O₂ in second step is not essential but can assist Mn (VII) for its catalytic action.

![Change of absorbance with wavelength](image)
Optimization of parameters
The effect of various variables on the color development was studied to establish the optimum conditions for the accurate analysis of HQ.

Optimization of analytical wavelength
In the absence of interferences, the wavelength chosen for a quantitative determination in the wavelength 610 nm of maximum absorbance. Sometimes the adding coloring reagent may absorb very close to the same region were the determining substance does. Figure(3) describes the absorbance of 20 μg.ml⁻¹ HQ solution as BQ at different wavelength after 30 min with 0.02 M KMnO₄ solution.

Optimization of mixing amount of KMnO₄ solution
The effect of adding various amount of KMnO₄ solution on absorbance of 0.00454 M HQ solution is given in Figure(4), the highest absorbance value was observed at 0.02 M of KMnO₄ after 30 min mixing of the HQ in alkaline medium (NaOH) and KMnO₄ then measured at 610 nm.

Effect of order of adding reagent
Order of adding reagent plays very important role in accuracy of results and peak enhancement. In the present study it was observed that the addition of 1.5 ml of 0.02 M KMnO₄ to 1 ml of 500 μgml⁻¹ HQ, then added 2 ml of 2 M NaOH and dilution with water up to 25 ml resulted in a lower absorbance value. The greatest absorbance value was observed when we first took 1 ml of 500 μgml⁻¹ HQ, then added 2 ml NaOH solution (2M) and added 1.5 ml of 0.02 M KMnO₄ through mixing and diluting this in 25 ml volumetric flask to the mark with distilled water.

Optimization of time for development of stable color
In UV-Vis spectrophotometry the main problem is the instability of the absorbance value. The effect of time on absorbance presented in Figure(5).

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It is evident from fig(5) that the solution is quite stable within the range studied. However, 30-35 min. after mixing was chosen as optimum time for further study so that on could prepare, mix and process the solution according to his analytical skill. The stable color development in lesser time is due to tremendous oxidizing power of KMnO₄ for conversion of HQ into BQ.
Effect of temperature
The reaction between HQ and KMnO$_4$ in the presence of alkaline solution (NaOH) was found to be instantaneous. However, the reaction is complete within 15 min. at room temperature (25°C), but 35 min was sufficient to get maximum intensity and stability color after the addition of oxidizing agent and distilled water to final solution. The effect of temp. in range of 5-50°C were studied and the result obtained in figure(6) shows that the greatest absorbance at room temperature.

![Fig.6](image_url)

**Fig.6:** Effect of temperature on absorbance

Effect of sodium hydroxide concentration
It was found that the presence of a base led to increase the intensity of the coloured product, so 0.16M of NaOH was selected which was found that the best volume equal to 2ml of this base give high sensitivity which selected in subsequent, as shown in figure(7).

![Figure7](image_url)

**Figure(7):** Effect of NaOH concentration on absorbance

Interference study
The effect of some foreign compounds, which often found in pharmaceutical products, were studied by adding different amounts organic compounds to 1ml of 500 µg.ml of HQ. The color was developed following the recommended procedure described earlier. It was observed that organic molecules were not interfering except glucose with the determination at levels found in dosage forms, As shown in table(1).

<table>
<thead>
<tr>
<th>Interference(200) µg. ml$^{-1}$</th>
<th>Lactose</th>
<th>Maltose</th>
<th>Glucose</th>
<th>Sodium citrate</th>
<th>EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc.of HQ found</td>
<td>-11.092</td>
<td>-10.586</td>
<td>-18.54</td>
<td>-1.16</td>
<td>2.431</td>
</tr>
<tr>
<td>%</td>
<td>-11.833</td>
<td>-11.358</td>
<td>-18.945</td>
<td>-2.135</td>
<td>1.378</td>
</tr>
<tr>
<td>Rec.%</td>
<td>88.146</td>
<td>88.642</td>
<td>81.055</td>
<td>97.865</td>
<td>101.378</td>
</tr>
</tbody>
</table>

**Table (1):** Interference effect of various organic molecules during HQ determination

Calibration plot
Employing the conditions described in the procedure, a linear calibration curve for hydroquinone is obtained in figure(8), which shows that Beer’s law is obeyed over the concentration range (1-26) µg.ml$^{-1}$ with correlation coefficient of 0.9997 with detection limit of 0.0125 µg.ml$^{-1}$.
Fig (8): The calibration curve of hydroquinone using spectrophotometric method

**Structure of the colored protect**
The stochiometry of the oxidization reaction between hydroquinone with KMnO₄ was investigated using job’s method. The result obtained in figure (9) shows that 1:1 drug to reagent was formed at 610 nm in presence of sodium hydroxide solution [40,41]

Fig (9): Continuous variation plot of the reaction between HQ and potassium permanganate (0.02M)

**Precision and Accuracy**
To evaluate the accuracy and precision of the methods, pure drug analyzed, each determination being repeated six times at three different concentration [42].

<table>
<thead>
<tr>
<th>HQ taken</th>
<th>HQ Found</th>
<th>%Rec</th>
<th>%E</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2.935</td>
<td>99.97</td>
<td>-0.0216</td>
<td>0.637</td>
</tr>
<tr>
<td>10</td>
<td>10.15</td>
<td>101.5</td>
<td>+1.5</td>
<td>1.269</td>
</tr>
<tr>
<td>14</td>
<td>14.18</td>
<td>101.285</td>
<td>+1.285</td>
<td>1.47</td>
</tr>
</tbody>
</table>

*Average of six determinations.

The result shown in table (2) indicate that satisfactory precision and accuracy could be attained with the proposed method. The %E and RSD % values were less than 1.5 % which indicate the high accuracy.

**Table (3): Application of the proposed method and pharmaceutical preparations for determination of hydorquinone drug**

<table>
<thead>
<tr>
<th>HQ Samples</th>
<th>HQ(µg.ml⁻¹)</th>
<th>Rec%</th>
<th>Average recovery%</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fediquin</td>
<td>5 4.863</td>
<td>99.97</td>
<td>99.993</td>
<td>0.314</td>
</tr>
<tr>
<td></td>
<td>10 10.163</td>
<td>100.016</td>
<td></td>
<td>0.316</td>
</tr>
<tr>
<td>Hydropaque</td>
<td>5 4.776</td>
<td>99.95</td>
<td>99.984</td>
<td>0.527</td>
</tr>
<tr>
<td></td>
<td>10 10.193</td>
<td>100.019</td>
<td></td>
<td>0.315</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>5 4.846</td>
<td>99.969</td>
<td>99.973</td>
<td>0.315</td>
</tr>
<tr>
<td></td>
<td>10 9.776</td>
<td>99.977</td>
<td></td>
<td>0.426</td>
</tr>
</tbody>
</table>


**Analytical Application**
Proposed method have been used Fediquin(40%), Hydropaque (40%), Hydroquinone (2%) drugs containing hydroquinone and it gave good accuracy and precision as shown in table (3), the proposed method compared with
standard method [41], since T-test and F-test shows that there was no significant differences the proposed method and standard method, the results obtained were tabulated in table (4).

Table (4): Comparison of hydroquinone determined in pharmaceutical preparation by the proposed method with standard method

<table>
<thead>
<tr>
<th>HQ Sample</th>
<th>Rec % Proposed method</th>
<th>Rec % Standard method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fediquin</td>
<td>99.903</td>
<td>99.75</td>
</tr>
<tr>
<td>Hydropaque</td>
<td>99.984</td>
<td>99.48</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>99.973</td>
<td>99.67</td>
</tr>
</tbody>
</table>

The proposed method was compared with other spectroscopic method in literature for the oxidation reaction of hydroquinone, as shown in table (5).

Table (5): Comparison of hydroquinone determination in the proposed method and other literature methods

<table>
<thead>
<tr>
<th>Reagent</th>
<th>λ max. nm.</th>
<th>Limit of detection</th>
<th>Linear range µg.ml⁻¹</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine B (RhB)</td>
<td>557</td>
<td>0.16µg.ml⁻¹</td>
<td>0.36-3.96</td>
<td>42</td>
</tr>
<tr>
<td>Ammoniummeta-vanadate</td>
<td>245.5</td>
<td>7µg.ml⁻¹</td>
<td>0.025-205</td>
<td>43</td>
</tr>
<tr>
<td>Methanol</td>
<td>293</td>
<td></td>
<td>0-1.3</td>
<td>44</td>
</tr>
<tr>
<td>Sulfuric acid</td>
<td>225</td>
<td></td>
<td>10-26</td>
<td>45</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>610</td>
<td>0.0125µg.ml⁻¹</td>
<td>1-26</td>
<td>Proposed method</td>
</tr>
</tbody>
</table>

Chemical and flow optimization
The flow injection manifold depicted in figure (10) were investigated in the relation to chemical and flow variable in order to obtain optimum conditions for system. They were optimized by making all variables constant and varying one each at a time.

Figure(10): Flowgram of the whole manifold with double loop - 6- three way valve (IV; injection valve, RC; reaction coil, D; detector, W; waste)

Choice of base
The oxidative reaction of HQ with potassium permanganate can be conducted in basic medium. Therefore, bases (NaOH, KOH, NH₃OH, Na₂CO₃) were used at various concentration, sodium hydroxide was chosen as a favorable base for oxidation of drug using KMnO₄ as oxidizing agent. The use of a 0.1M of sodium hydroxide as a carrier stream at 2.12ml. min⁻¹, 227.65 of 2.5x10⁻⁵M KMnO₄ and 227.65µL of 50 µg.ml⁻¹ of HQ.

Effect of sodium hydroxide concentration
Various concentration (0.05-0.7M) of sodium hydroxide solution were used to obtain the optimum concentration of sodium hydroxide that can be used as carrier stream at 2.12 ml.min⁻¹ using 227.65 µl of 50µg.L⁻¹ of HQ 227.65 µL of 2.5x10⁻⁵mol.L⁻¹ of potassium permanganate, figure(11) shows the variation of absorbance with the molar concentration of sodium hydroxide which shows clearly that 0.1 M of sodium hydroxide is a propitious concentration.
Optimization of KMnO₄ concentration
The effect of adding various amount of KMnO₄ solution on absorbance of 50 µg ml⁻¹ HQ solution is given in figure(12). It is seen that the maximum absorbance of 2.5x10⁻² M occurs in the presence of various concentrations (1x10⁻³ - 4x10⁻² M) of KMnO₄, the concentration of 2.5x10⁻² M KMnO₄ solution showing an absorbance 1.189 was taken as optimum amount for further study because a calibration curve check of HQ in case of former solution did not result in better absorbance value at lower HQ concentration. According to literature [24], application of a higher KMnO₄ concentration would facilitate the determination of HQ in higher concentration range.

Physical parameters
Sodium hydroxide flow rate
The effect of sodium hydroxide flow rate was investigated in the range of (1.5-6.5) ml min⁻¹ to obtain the best absorbance where sodium hydroxide flow rate of 2.12 ml min⁻¹ gave the highest response as shown in figure(13).
Effect of sample volume
The sample volumes (157.196.25, 227.65, 274.75 µL) were evaluated using different length of sample loop and the results were plotted in figure (14). The results obtained showed that injected sample of 227.65 µL gave the best absorbance.

Effect of reagent volume
Different potassium permanganate volumes of (157, 196.25, 227.65, 274.75 µL) can be achieved by inserting different lengths of reagent loop. Figure (15) shows that 227.65 µL is the optimum volume.

Effect of temperature
The effect of temperature on the reaction of \([\text{HQ-MnO}_4\text{-OH}]\) system was studied. It was found that there was no effect of the temperature on the reaction for temp.range( 5-50°C).

Effect of mixing coil
Different delay reaction coil length (50, 100, 150, 200 cm) were used to measure the absorbance of colored product at 2.5x10^{-2}M potassium permanganate, 0.1 M base (NaOH) and 50 µg.ml^{-1} HQ. Figure(16) shows that 100cm gave the highest absorbance and was used in all subsequent experiments.
Calibration curve
Series of HQ solutions (3.5, 10, 15, 20, 30, 40, 50, 75, 100, 125) µg ml⁻¹ were prepared from stock solutions under the optimum conditions of reagent and manifold variables as indicated in table (6). The calibration curve is linear in the concentration range (3-125) µg.ml⁻¹ with a detection limit of 0.25 µg.ml⁻¹. Figure (17) shows the variation of absorbance of color product versus HQ concentration and table (7) shows the treatment of the results [46,47] in linear regression terms.

Table (6): The optimization of the chemical and FIA parameters using Merging Zones – Flow injection analysis for determination of HQ drug

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Optimum value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH Conc.</td>
<td>0.1 M</td>
</tr>
<tr>
<td>KMnO₄ Conc.</td>
<td>2.5x10⁻³ M</td>
</tr>
<tr>
<td>Flow of rate of NaOH</td>
<td>2.12 ml.min⁻¹</td>
</tr>
<tr>
<td>Sample volume (µL)</td>
<td>227.65 µL</td>
</tr>
<tr>
<td>Oxidant volume (µL)</td>
<td>227.65 µL</td>
</tr>
<tr>
<td>Reaction coil length (cm)</td>
<td>100 cm</td>
</tr>
</tbody>
</table>

Figure (17): The calibration curve of Hydroquinone at 0.1M NaOH as a carrier stream at flow rate 2.21 ml.min⁻¹

Table (7): The linear equation results for the color product by the injection of 227.65 µL of HQ sample through the adopted system [HQ – MnO₄⁻ – OH]

<table>
<thead>
<tr>
<th>Linear range Conc. (µg.ml⁻¹)</th>
<th>Slope (b) at confidence limit 95% for (n-2) (b=s±t)</th>
<th>Intercept (a) at confidence limit 95% for (n-2) (a=s±t)</th>
<th>t from table at confidence limit 95% for (n-2)</th>
<th>Calculate t⁰.₀.₉₉₉₂</th>
<th>Correlation coefficient (r)</th>
<th>Linearity R² %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-125</td>
<td>0.0096±0.00014</td>
<td>0.1299±0.00085</td>
<td>2.31</td>
<td>100.964</td>
<td>0.9995</td>
<td>0.9992</td>
</tr>
</tbody>
</table>

Repeatability
Repeatability was studied at determination of hydroquinone via measurements of oxidization of drug with potassium permanganate in basic medium to form color product (green) for FIA system [HQ-MnO₄⁻-OH]. Variable concentration of HQ 50,100 µg.ml⁻¹ were injected. Each concentration was injected successfully for five times. Figure (18) shows clearly that relative standard deviation is better than 1.5% in most cases can be obtained of a high repeatability by a short analysis time.

Figure (18): Repeatability for five successive measurement of hydroquinone for 50,100 µg.ml⁻¹ as injected sample
Determination of HQ in pharmaceutical formulations by proposed method and spectro method (40)

Table (8) shows the agreement between the results in both methods and the paired t – test illustrates that the flow injection analysis method has no significant difference when compared with the standard adopted method ,therefore it can be regarded as an alternative determination method a part from many advantages this method had.

Table (8) : Comparison between proposed method and spectro method for the determination of HQ in pharmaceutical formulations

<table>
<thead>
<tr>
<th>Sample</th>
<th>HQ found in samples (mg.ml$^{-1}$)</th>
<th>di μg.ml$^{-1}$</th>
<th>Xd μg.ml$^{-1}$</th>
<th>Sx</th>
<th>Paired t-test = Xd - X̅/Sx d</th>
<th>T form table at confidence limit 95% (n-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Merging Zone FIA</td>
<td>Spectro Method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fediquin</td>
<td>0.041</td>
<td>0.0398</td>
<td>0.0012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydropaque</td>
<td>0.042</td>
<td>0.0397</td>
<td>0.0023</td>
<td>0.00153</td>
<td>0.002</td>
<td>1.325</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.021</td>
<td>0.0199</td>
<td>0.0011</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interference:
The effect of some foreign compounds[40] , which often found in pharmaceutical products , were studied by adding different amount organic molecules to 5 ml of 500 µg.ml$^{-1}$ of hydroquinone . The color was developed following the recommended procedure described earlier. It was observed were not interfering with the determination of levels found dosage form.

CONCLUSION

The work described in this research comprises more than a complete new intuition for a compact home made new injection valve using Merging Zone technique .The simplicity , speed , reliability and the mode of working its well comparison with standard spectrophotometric method indicates that the method presented in this research work can be used as an alternative method for the spectrophotometric method.The work has been developed for the determination of HQ in pure material and pharmaceutical preparations in alkaline medium based on oxidization reaction using potassium permanganate as reagent for conversion of HQ to BQ [9] analysis of the former at the extent of formation of the later is very useful for analysis of HQ at ultra – trace level .It is a very good example of redox reaction and may be employed in situation involving useful oxidation products of such types .To our knowledge no what so ever this technique was used in any published work everywhere else using FIA- Merging Zone technique for determination of HQ. This method has clear edge over other methods employing expensive and or hazardous reagents ,the proposed method does not require temperature control or solvent extraction step. Moreover , the lower detection limitachieved with highly reproducible in results by this method which is not possible by other methods.

REFERENCES


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