**FI - Spectrophotometric Determination of Propranolol Hydrochloride in pharmaceutical preparations**

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**Abstract:**

A batch and flow injection spectrophotometric method is described for the determination of propranolol hydrochloride in pure and pharmaceutical formulations. The proposed method based on the diazotization of 4-amino-6-chlorobenzene-1,3-di sulfonamide and followed by coupling with propranolol hydrochloride in the presence of sodium hydroxide to form orange soluble dye that has a maximum absorption at 490 nm. The optimum reaction conditions and other analytical parameter are evaluated. A graph of absorbance versus concentration shows that Beer's law is obeyed over the concentration range of (0.25 – 10.00µg/ml) and from (1.20 – 48.00 µg/ml), with a limit of detection (signal/noise = 3) of 0.145 µg/ml and 0.640 µg/ml. The correlation coefficient was 0.9997 and 0.9998 by batch and FI procedure respectively. The method was applied successfully for the determination of propranolol hydrochloride in pharmaceutical preparations. The relative standard deviation was better than 0.79 % (n=10).

**التقدير الطيفي بالحقن الجرياني لعقار البروبانول هايدروكلورايد في المستحضرات الصيدلانية منير عبد العالي عباس الدعمي فرع العلوم الطبية الاساسية / كلية طب الأسنان / جامعة كربلاء**

**الخلاصة**

تم تطوير طريقتين تقليدية وحقن جرياني طيفية مضبوطة وحساسة لتقدير البروبانول، هايدروكلورايد في المحاليل المائية والمستحضرات الصيدلانية. تتم استعمال الطريقة المقترحة على أروثة الكشفي العضوي 4 - أمينو - 6-كلوروبنيزين - 3,1-دي سلفون أميد ومن ثم الاتزامع مع البروبانول، هايدروكلورايد ويوجد هيدروكسيد الصوديوم ليكون صبغة برتقالية ذاتية لها امتصاصات أعظم عند 490 نانومتر. الظروف الفضلى للفعاء وبعض المؤثرات التحليلية تم تحديدها. كانت حدود الخطية بين 0.25 – 10.00 و 0.25 – 1.20 مل ميكروغرام. مل 1/3 كشف مقداره 0.145 و 0.640 ميكروغرام. مل 1/4 مع معدل ارتباط مقداره 0.9997 لكل من الطريقة التقليدية وطريقة الحقن الجرياني على التوالي. كذلك تم دراسة العوامل الفيزيائية والكيميائية التي تؤثر على حساسية الطريقتين وتم تطبيق الطريقتين بنجاح في تقدير البروبانول، هايدروكلورايد في مستحضراته الصيدلانية مع انحراف قياسي نسبي أفضل من 0.79 عندما (n=10).
Introduction:

Propranolol hydrochloride (1-isopropylamino-3-(1-naphthyloxy)-2 propranolol, the prototype of a pure beta-adrenergic blocking compound without intrinsic activity, represents an outstanding advance in the treatment of certain cardiovascular disorders and hypertension. It is one of the very good drugs of choice for sustained action dosage form, because its therapeutic index is very high\(^\text{1}\). Many methods which are used for the determination of propranolol hydrochloride in pharmaceutical preparations most of them includetitrimetric\(^\text{2}\), gravimetric\(^\text{3}\), polarographic\(^\text{4}\), spectrofluorometry\(^\text{5}\), flow injection technique\(^\text{6,7}\) and spectrophotometric method\(^\text{8-12}\). FI spectrophotometric determination continue to be the most preferred method for analytical work because of its simplicity and reasonable sensitivity with significant economical advantages\(^\text{13}\). The diazotization coupling reactions seem to be one of the most suitable spectrophotometric determination of drugs such as methyl dopa\(^\text{14}\), 4-amino antipyrine\(^\text{15}\), ethinylestradiol\(^\text{16}\), and furosemide\(^\text{17}\). The present investigated method describes asimple, accurate and sensitive method for the determination of propranolol hydrochloride in both pure and dosage forms. The proposed method is based on the diazotization of 4-amino-6-chlorobenzen-1,3-disulfonamide followed by coupling with propranolol hydrochloride in the presence of sodium hydroxide. The reaction can carried out in batch and in FIA and in this paper the two approaches are compared. The reaction products have been spectrophotometrically measured at 490 nm.

Experimental: All chemical Used of analytical grade reagent unless otherwise stated.

The propranolol hydrochloride was obtained from Rhone pululence company/France. Tablets were provide from Act avis company England).

Propranolol hydrochloride stock solution(1000 µg/ml):

0.100gm of propranolol hydrochloride was dissolved in 100.00 ml of distilled water in a volumetric flask of 100.00 ml.

4-amino-6-chlorobenzen-1,3-disulfonamide solution(5.00×10^{-2}M):

1.430gm of 4-amino-6-chlorobenzen-1,3-disulfonamide dissolved in 100.00 ml of distilled water in a volumetric flask of 100.00 ml.

Sodium nitrite solution(0.500 w/v%):

Sodium nitrite solution was prepared by dissolving of 0.500gm in 100.00 ml of distilled water in a volumetric flask of 100.00 ml.

Sodium hydroxide solution (0.100 M):
Sodium hydroxide solution was prepared by dissolving 0.400 gm in 100.00 ml distilled water in a volumetric flask of 100.00 ml. And then standardization of this solution with standard solution of HCl.

**Pharmaceutical preparation:**

The contents of Ten tablets of propranolol, each containing 10.00 mg propranolol, were accurately weighed individually and finely powdered. Powdered sample containing 10.00 mg propranolol was weighed and dissolved in 25.00 ml ethanol and 2.50 ml of (1.00M) hydrochloric acid solution. The solution was then filtered and transferred into 100.00 ml volumetric flask. The solution was finally made up to the mark with water. A 100.00 µg/ml solution of propranolol was obtained. These solution were diluted quantitatively to yield a concentrations in the range of calibration curve.

**Apparatus:**

All spectral and absorbance measurements were carried out on a Shimadzu UV–visible 260 digital double beam recording spectrophotometer using 1 cm silica cell.

In FIA, a flow cell with 50.00 µl internal volume and 1.00 cm path length was used for the absorbance measurements. A two-channel manifold (Fig. 1) was employed for the FIA spectrophotometric determination of propranolol drug. (Rheodyne – USA) injection valve was employed to provide appropriate injection volumes of standard solutions and samples. Flexible vinyl tubing of 0.50 mm internal diameter used for peristaltic pump. Reaction coil (RC) was made from Teflon with internal diameter of 0.50 mm. Channel 1 was used to transport the diazotized of 4-amino-6-Chloro benzene-1,3-disulfonamide solution. Channel 2 was used to transport Sodium hydroxide solution. The sample was injected into the carrier solution of diazotized reagent, through the injection valve. Solution were propelled by peristaltic pump with individual flow rate of 0.60 ml min⁻¹, the absorbance measured at 490 nm.

![Diagram](image_url)

**Fig(1):** Manifold employed for FIA-Spectrophotometric determination of propranolol.

Procedure for the batch method:

Into a series of volumetric flasks of 25.00 ml, transfer 1.00 ml of 1.00M Hydrochloric acid followed by 0.50 ml of 5.00 × 10⁻² reagent and 6.00 ml of 0.50 w/v % sodium nitrite solution and cool in an ice – bath for 5 min., followed by addition of increasing volume of propranolol drug covered the calibration curve concentration and then addition of 12.50 ml of 0.10 M of sodium hydroxide solution, the solution were diluted to the mark with deionized water and the reaction mixtures were allowed to stand for 10.00 min., in a water bath at 25.00 °C. The absorbance's were measured at 490 nm against blank.

Procedure for the FIA method

100.00 µl sample is injected into a 0.60 ml.min⁻¹ stream of 2.50×10⁻² M diazotized reagent solution in 75.00 cm reaction coil, and the stream allow to merge with another stream of 7.50×10⁻² M Sodium hydroxide solution. The reaction is carried out by passing the mixture maintaining and the absorbance measured at 490 nm.

Results and discussion:

Propranolol drug reacted with diazotized of 4-amino-6-Chloro benzene-1,3-disulfonamide in the presence of sodium hydroxide, an intense Brown color forms immediately and become stable after 10.00 min. The color product can be measured at 490 nm. Fig.2 showed the spectrum directly related with the concentration of propranolol drug and can be used for their spectrophotometric determination. It was found that the sensitivity of the color product depends on the reaction conditions and were established for sodium nitrite (from 0.300 – 0.025 w/v%), 4-amino-6-Chloro benzene-1,3-disulfonamide (from 7.50×10⁻²–7.50×10⁻³ M) and sodium hydroxide (from 1.00×10⁻¹ – 5.00×10⁻³ M) by altering one variable at a time and studying the absorbance at 490 nm as a function of time. The obtained results show that 0.12 w/v% of sodium nitrite, 1.00×10⁻³ M of 4-amino-6-Chloro benzen-1,3-disulfonamide and 5.00×10⁻² M of sodium hydroxide are the concentration that can give a higher absorption intensity at 490 nm for 50.00 μg of propranolol in a final volume of 10.00 ml.

The development of the color of product from a mixture containing 5.00 μg.ml⁻¹ propranolol in 0.12 w/v% sodium nitrite, 1.00×10⁻³ M 4-amino-6-Chloro benzene-1,3-disulfonamide and 5.00×10⁻² M sodium hydroxide gave evidence that the color develops during the first 10.00 min. and remains stable for more than 24.00 hr. The effect of temperature on the color intensity of the dye was studied. In practice, high absorbance was obtained when the color was developed at room temperature (25.00 ±2.00 °C) than when the calibrated flask were placed in an ice – bath at (0.00±2°C) or in a water bath at(45.00 ±2.00 °C).
Fig(2): Absorption spectrum of (5.00µg/ml) propranolol treated as described under procedure and measured against reagent blank.

The stoichiometry of the reaction was investigated using mole ratio method. The results obtained (Fig.3) show a 1:1 drug to reagent product was formed. The formation of the dye may be probably occur as follows:
The Regression equation obtained, from a series of propranolol standards and the analytical figures of merit of this procedure are summarized in Table 1 in which are also summarized the main performance of the flow procedure developed for propranolol determination in order to make an effective comparison between the two approaches.

Table 1 Analytical characteristics of the proposed methods for the determination of propranolol drug.

<table>
<thead>
<tr>
<th>parameter</th>
<th>Batch method</th>
<th>Flow injection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>490</td>
<td>490</td>
</tr>
<tr>
<td>Beer’s law limits ($\mu$g/ml)</td>
<td>0.25 – 10.00</td>
<td>1.20 – 48.00</td>
</tr>
<tr>
<td>Molar absorptivity (l.mol$^{-1}$.cm$^{-1}$)</td>
<td>$2.52 \times 10^4$</td>
<td>$0.51 \times 10^9$</td>
</tr>
<tr>
<td>Sandal sensitive ($\mu$g. cm$^{-2}$)</td>
<td>$7.82 \times 10^{-6}$</td>
<td>$3.86 \times 10^{-5}$</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$Y=0.096X+0.012$</td>
<td>$Y=0.018X+0.008$</td>
</tr>
<tr>
<td>Slope</td>
<td>0.096</td>
<td>0.018</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.012</td>
<td>0.008</td>
</tr>
<tr>
<td>RSD% for (5 $\mu$g/ml)</td>
<td>0.84</td>
<td>0.79</td>
</tr>
<tr>
<td>Recovery% for (5 $\mu$g/ml)</td>
<td>100.90</td>
<td>99.21</td>
</tr>
<tr>
<td>Sample Through-put (hr$^{-1}$)</td>
<td>30</td>
<td>120</td>
</tr>
</tbody>
</table>

**FI- Spectrophotometric determination:**

The batch method for determination of propranolol was adopted as a basis to develop FI procedure, using the manifold indicated in Fig.1. The absorbance intensity of the colored was studied the different FI parameters on the reaction between propranolol and diazotized of 4-amino-6-Chloro benzene-1,3-disulfonamide in the presence of sodium hydroxide such as Sodium nitrite concentration(from 0.300 – 0.025 w/v%), 4-amino-6-Chloro benzene-1,3-disulfonamide concentration(from 7.50×10$^{-2}$ – 7.50×10$^{-3}$ M), Sodium hydroxide concentration(from 1.00×10$^{-1}$ – 5.00×10$^{-3}$ M), flow rate of carrier solution (from 0.15 – 2.50 ml/min. in each channel), length of the reaction coil (from 25.00 – 250.00 cm) and the volume of sample loop(from 50.00–200.00 µl). The results
obtained showed that a concentration of 0.17 w/v%, 2.50×10⁻² M and 7.50×10⁻² M were optimum for Sodium nitrite, 4-amino-6-Chloro benzene-1,3-disulfonamide and sodium hydroxide respectively. A flow rate of 0.60 ml/min. in each channel, a reaction coil length of 75.00 cm and an injection sample volume of 100.00 µl were the best conditions which provided the highest absorbance at 490 nm with the lowest blank value.

A calibration curve obtained for a series of propranolol standards and the main analytical figures of merit of the developed procedure are indicated in Table 1.

The increase in the temperature of the reaction coil does not increase the absorbance and caused a degradation of the colored product and low sensitivity and stability of the reaction products.

Interference effect study:

In order to evaluated the possible analytical applications of the proposed method, the influence of frequently encountered excipients and additives were studied by analyzing sample solution containing 2.50 µg/ml of propranolol with 5.00 µg/ml amounts of possible interferents. The results obtained (Table 2) indicated that no serious interference occurred from the classical additives tested.

Table 2 Influence of excipients and additives as interfering species in the determination of propranolol drug.

<table>
<thead>
<tr>
<th>Additives or Excipients</th>
<th>Amount of additive / (2.50 µg/ml) of drug</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium stearate</td>
<td>5.00</td>
<td>101.20</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5.00</td>
<td>99.86</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.00</td>
<td>98.93</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.00</td>
<td>100.74</td>
</tr>
<tr>
<td>Starch</td>
<td>5.00</td>
<td>101.23</td>
</tr>
<tr>
<td>Citric acid</td>
<td>5.00</td>
<td>99.34</td>
</tr>
</tbody>
</table>

Average of five determination

Analytical application:

The developed method is very adequate for the determination of propranolol in aqueous solution and in pharmaceutical preparation at a concentration level of traces and without requiring any previous separation step nor a temperature or PH control. Moreover the proposed procedures are very economical when compared to other method such as those based on the use of HPLC. In comparison of the batch with FI procedure, the later is more convenient than the former method because of its speed (sample through – put of 120.00 injection/hr.) and wider linear range of the calibration curve (Table 1). The precision of the method was evaluated by analyzing pure sample of propranolol and a good recovery was obtained (Table 1). Finally the proposed method was applied successfully to the analysis of some tablets containing propranolol. The results in Table 3 are in accordance with those obtained by the official method.
Table 3 Determination of propranolol in pure dosage and in pharmaceutical preparation.

<table>
<thead>
<tr>
<th>Drug sample</th>
<th>Amount of drugs taken (µg/ml)</th>
<th>Batch method</th>
<th>Flow injection method</th>
<th>Official method(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery %</td>
<td>RSD %</td>
<td>Recovery %</td>
<td>RSD %</td>
</tr>
<tr>
<td>Pure Propranolol</td>
<td>10.00</td>
<td>100.40</td>
<td>0.44</td>
<td>99.64</td>
</tr>
<tr>
<td>Propranolol Tablet</td>
<td>10.00</td>
<td>99.22</td>
<td>0.48</td>
<td>100.60</td>
</tr>
<tr>
<td>Propranolol Tablet</td>
<td>5.00</td>
<td>100.90</td>
<td>0.84</td>
<td>99.21</td>
</tr>
</tbody>
</table>

Average of five determination

**Comparison with another methods:**

The proposed (Batch and Flow injection) method comparison with the reported Spectrophotometric methods for the determination of propranolol in pharmaceutical preparations (Table 4).

Table 4 Comparison of the proposed method with the reported methods for the determination of propranolol drug.

<table>
<thead>
<tr>
<th>Reagent used</th>
<th>λ_{max} (nm)</th>
<th>Beer’s law limits (µg/ml)</th>
<th>Molar absorptivity (L.mol^{-1}.cm^{-1})</th>
<th>Remark</th>
<th>Ref.no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supracen Violet 3B</td>
<td>575</td>
<td>1.20 – 12.50</td>
<td>1.225×10^4</td>
<td>Involved extraction</td>
<td>18</td>
</tr>
<tr>
<td>Alizarin Red-S</td>
<td>515</td>
<td>25.0 – 200.0</td>
<td>0.096×10^4</td>
<td>Involved extraction</td>
<td>19</td>
</tr>
<tr>
<td>Erthrosin-B</td>
<td>525</td>
<td>10.0 – 80.0</td>
<td>0.163×10^4</td>
<td>Involved extraction</td>
<td>19</td>
</tr>
<tr>
<td>Bromothymol Blue</td>
<td>414</td>
<td>3.00 – 25.00</td>
<td>----</td>
<td>Involved extraction</td>
<td>20</td>
</tr>
<tr>
<td>Ce(IV) in H_2SO_4 medium</td>
<td>478</td>
<td>150 – 350</td>
<td>----</td>
<td>Involved heating to 90 Cº for 25 min.</td>
<td>21</td>
</tr>
<tr>
<td>p-Nitroaniline,NaNO_2 and NaOH</td>
<td>490</td>
<td>5.00 – 50.00</td>
<td>----</td>
<td>Absorbance were recorded after 30 min.</td>
<td>22</td>
</tr>
<tr>
<td>Methylene Violet</td>
<td>378</td>
<td>2.00 – 25.00</td>
<td>----</td>
<td>Involved extraction</td>
<td>23</td>
</tr>
<tr>
<td>Proposed reagents in Batch method</td>
<td>490</td>
<td>0.25 – 10.00</td>
<td>2.52 × 10^4</td>
<td>Containing no extraction step nor a temperature or PH control</td>
<td>This work</td>
</tr>
<tr>
<td>Proposed reagents in Flow injection method</td>
<td>490</td>
<td>1.20 – 48.00</td>
<td>0.51× 10^4</td>
<td>Containing no extraction step nor a temperature or PH control Moreover its very economical, speed and wider linear range</td>
<td>This work</td>
</tr>
</tbody>
</table>
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