FI - Spectrophotometric Determination of PropranololHydrochloric in pharmaceutical preparations

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Abstract:

A batch and flow injection spectrophotometric method is described for the determination of propranololhydrochloride 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 ofsodium 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 – 48.00µg/ml),with a limit of detection(signal / noise =3) of 0.145µg/mland 0.640µg/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

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الخلاصة

تم تطوير طريقتين تقليدية وحقن جرياني طيفية مضبوطة وحساسة لتقدير البروبانول . هايدروكلورايد في المحاليل المائية و المستحضرات الصيدلانية . تعتمد الطريقة المقترحة على ازوتة الكاشف العضوي 4-أمينو -6-كلوروبنزين -3,1-داي سلفون امايد ومن ثم الازدواج مع البروبانول . هايدروكلورايد وبوجود هيدروكسيد الصوديوم ليكون صبغة برتقالية ذائبة لها امتصاص اعظم عند 490 نانوميتر . الظروف الفضلى للتفاعل وبعض المؤثرات التحليلية تم تحديدها . كانت حدود الخطية بين10.00 – 0.25 و – 1.20 48.00 مايكروغرام . مل$^{-1}$ وحد كشف مقداره 0.145 و0.640 مايكروغرام . مل$^{-1}$ مع معامل ارتباط مقداره 0.9998و0.9997لكل من الطريقة التقليدية وطريقة الحقن الجرياني على التوالي . كذلك تم دراسة العوامل الفيزيائية و الكيميائية التي تؤثر على حساسية الطريقتين وتم تطبيق الطريقتين بنجاح في تقدير البروبانول . هايدروكلورايد في مستحضراته الصيدلانية مع انحراف قياسي نسبي افضل من 0.79 عندما (n=10).

Introduction:

Propranololhydrochloride (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,becuseits therapeutic index is very high[(1)].Many methods which is used for the determination of propranolol hydrochloride in pharmaceuticalpreparations most of them includetitrimetric[(2)],gravimetric[(3)],polarographic[(4)],spectrofluorometry[(5)],flow injection technique[(6,7)]and spectrophotometric method[(8-12)].FI spectrophotometric determination continue to be the most preferred method for analytical work because of its simplicity and reasonable sensitivity with significant economicaladvantages[(13)].The diazotization coupling reactions seem to be one of the most suitable spectrophotometric determination of drugs such as methyldopa[(14)],4-amino antipyrine[(15)],ethinylestradiol[(16)]and furosemide[(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 sodiumhydroxide.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 pulenence company/France. Tablets were provide from Act aviscompany 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 of 0.400gm 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 .

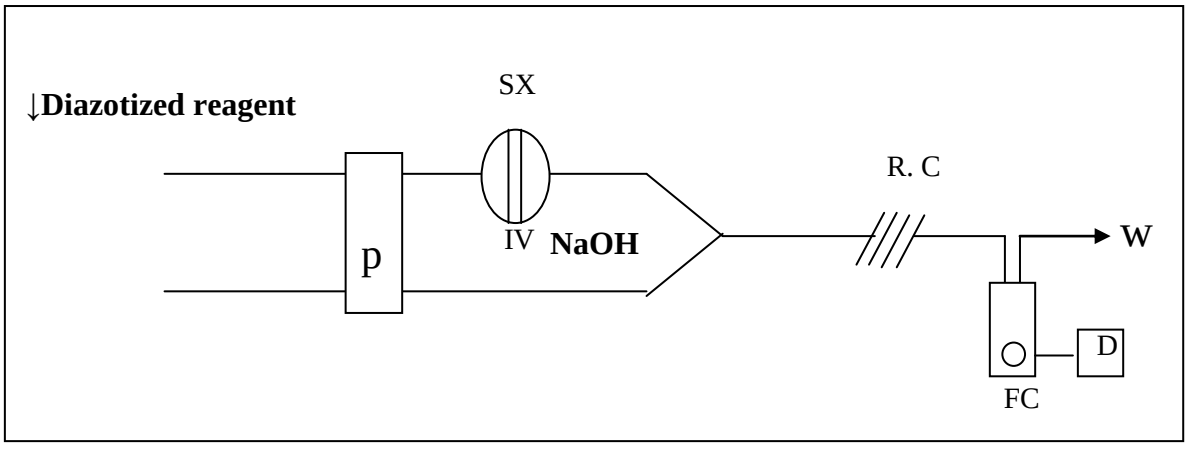
Pharmaceutical preparation:

Thecontents 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 solutionof propranolol was obtained. Thesesolution were diluted quantitatively to yield a concentrations in the range of calibration curve.

Apparatus:

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

In FIA,aflow 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 FI spectrophotometric determination ofpropranolol 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.



Fig(1):Manifold employed for FI-Spectrophotometric determination of propranolol . Where IV: Injection valve, RC: Reaction coil, SX: Sample, P: Peristaltic, D: Detector,FC:Flow cell,W: Waste.

Procedure for the batch method:

Into a series of volumetric flasks of 25.00 ml ,transfer1.00 mlof 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 10.00min.,in a water bath at 25.00 C° .The absorbance's were measured at 490 nm against blank.

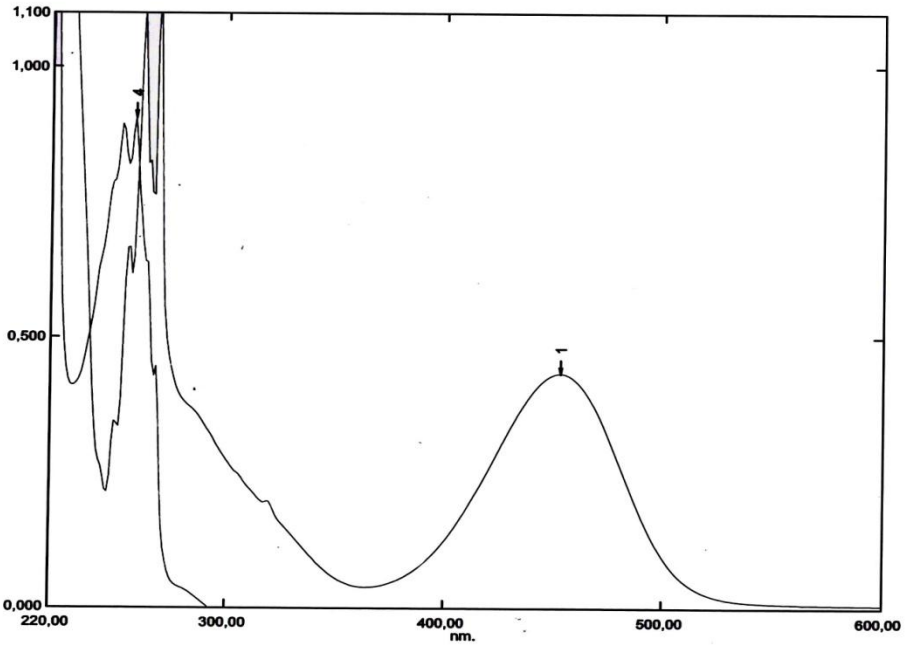
Procedure for the FIA method

100.00µl 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:

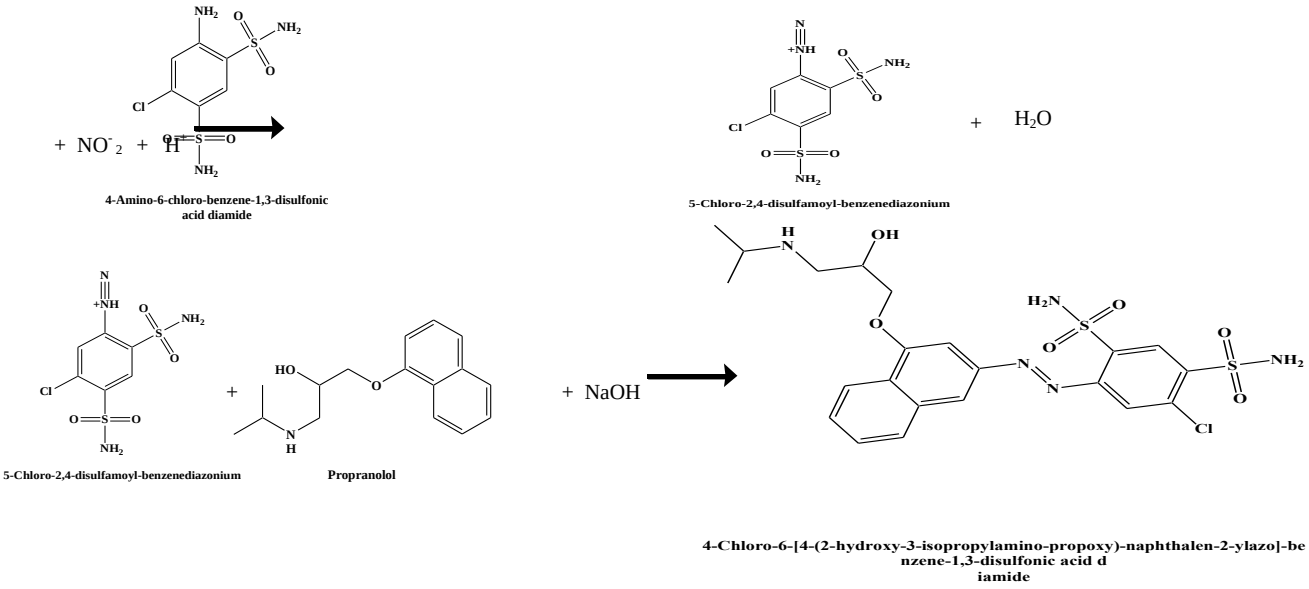
Propranolol drug reacted with diazotized of 4-amino-6-Chloro benzene-1,3-disulfonamide in the presence of sodium hydroxide, an intense Browncolor forms immediately and become stable after 10.00 min .The color product can be measured at 490 nm.Fig.2 showedthe spectrum directlyrelated with the concentration 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.

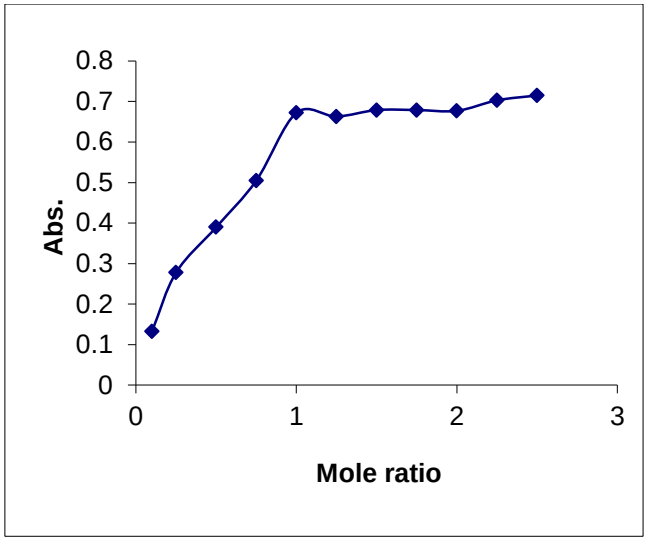
The development of the color of product from a mixture containing 5.00 $\mu g.ml^{-1}$ propranolol in 0.12 w/v% sodium nitrite,1.00×10^{-3} M 4-amino-6-Chloro benzene-1,3-disulfonamide and 5.00×10^{-2} M sodium hydroxide gave evidence that the color develops during the first 10.00 min. and remains stable 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±2C°) or in a water bath at(45.00 ±2.00 C^{o}).



Fig(2): Absorption spectrum of (5.00µg/ml) propranolol treated as described underprocedure 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:





Fig(3): Mole ratio of drug to reagent

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.

Table1 Analytical characteristics of the proposed methods for the determination of propranolol drug.

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

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(from7.50×10^{-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 75.00cm 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 5.00µg/mlamounts of possible interferents.The results obtained(Table.2) indicated that no serious interference occurred from the classical additives tested.

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

Additives or Excipients	Amount of additive / (2.50 µg/ml) of drug	Recovery %
Magnesium stearate	5.00	101.20
Sucrose	5.00	99.86
Lactose	5.00	98.93
Glucose	5.00	100.74
Starch	5.00	101.23
Citric acid	5.00	99.34

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 wasobtained(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.

Table3 Determination of propranolol in pure dosage and in pharmaceutical preparation.

Drug sample	Amount of drugs taken (µg/ml)	Batch method		Flow injection method		Official method(1)
		Recovery %	RSD %	Recovery %	RSD %	Recovery %
Pure Propranolol	10.00	100.40	0.44	99.64	0.38	101.30
Propranolol Tablet	10.00	99.22	0.48	100.60	0.46	
Propranolol Tablet	5.00	100.90	0.84	99.21	0.79	

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

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

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