

Spectrophotometric Determination of Promethazine in Pharmaceutical Preparations

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الخلاصة

تم تطوير طريقتين تقليدية وحقن جرياني طيفية لتعيين دواء البروميثازين في المحاليل المائية والمستحضرات الصيدلانية تعتمد على تفاعل البروميثازين مع حامض السلفانيليك بوجود N-بروموسكسين أميد كعامل مؤكسد في الوسط المتعادل. تم قياس الناتج الأزرق-المخضر عند طول موجي 600 نانوميتر وتراوحت الخطية بين 2-100 و 2-30 مايكروغرام/ ملتر بروميثازين وكان حد الكشف مقداره 0.2 و 0.43 مايكروغرام/ ملتر بروميثازين بالطريقة التقليدية والحقن الجرياني على التوالي. تم دراسة تأثير العوامل الفيزيائية والكيميائية التي تؤثر على الطريقتين وتم تطبيق الطريقتين بنجاح في تعيين البروميثازين في المستحضرات الصيدلانية.

ABSTRACT

A batch and flow injection (FIA) spectrophotometric methods have been developed for the determination of promethazine in aqueous solution and in pharmaceutical preparations. The methods are based on the reaction of promethazine with sulphanilic acid in the presence of N-bromosuccinimide as oxidizing agent. The water soluble bluish-green dye produced is measured at λ_{max} 600 nm, linearity is observed from 2-30 and from 2-100 μgml^{-1} promethazine with detection limits of 0.20 and 0.43 $\mu\text{g ml}^{-1}$ promethazine by batch and FIA procedures, respectively. The effect of chemical and physical parameters has been carefully considered and the proposed procedures have successfully been applied to the determination of promethazine in pharmaceutical formulations.

INTRODUCTION

Promethazine [10,2-dimethylaminopropyl]phenothiazine] is a phenothiazine derivative and is extensively used as tranquilliser and antihistaminics in various dosage forms (1). Several, titrimetric(2), spectrophotometric(3), polarographic(4), gas chromatographic(5) and high-performance liquid chromatography(6) methods for the determination of promethazine have been described. The official methods(7) generally include non-aqueous titration for bulk drugs and an ultraviolet spectrophotometric method for dosage forms.

Oxidative coupling organic reactions seems to be one of the most popular spectrophotometric methods for the determination of several drugs such as sulphonamides (8) paracetamol(9), phenylphrine.HCl(10), methyl dopa(11) and folic acid (12).

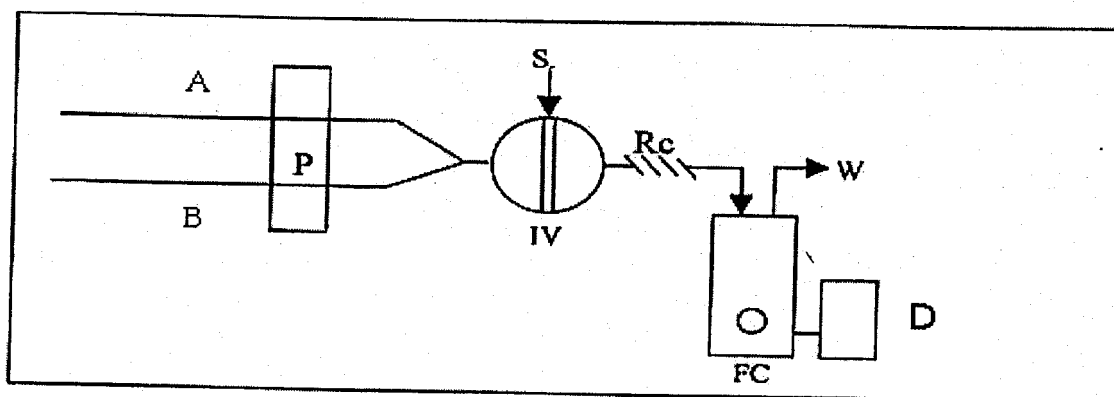
The objective of the investigation reported in this paper is to evaluate a spectrophotometric method for the determination of promethazine based on its reaction with sulphanilic acid in the presence of N-bromosuccinimide as oxidising agent. A stable water soluble bluish-green coloured product is formed which can be measured at 600nm. The reaction can be carried out either in batch or in FIA and the two approaches are compared. The method does not require temperature control or solvent extraction and can be applied successfully to pharmaceutical dosage forms containing promethazine .HCl (PMH).

EXPERIMENTAL

Apparatus:

All spectral and absorbance measurements were carried out on a Shimadzu uv-visible 210 and 260 digital double-beam recording spectrophotometer using 1-cm silica cells . In FIA, a flow cell with 50 μ l internal volume and 1-cm path length, was used for the absorbance measurements. A two-channel manifold (Fig.1) was employed for the FIA spectrophotometric determination of (PMH) drug. A peristaltic pump (Ismatec, Labor Technik – Analytik, CH – 8152, Glatbrugg – Zurrich- Switzerland) was used to transport the carrier solutions. Rheodyne (USA) injection valve was employed to provide appropriate injection volumes of standard solutions and samples. Flexible vinyl tubing of 0.5 mm internal diameter was used for the peristaltic pump. Reaction coil (RC) was of Teflon with internal diameter of 0.5 mm.

Channel A was used to transport sulphanilic acid solution and channel B to transport N-bromosuccinimide solution. The sample was injected into the stream of the mixture of sulphanilic acid with N-bromosuccinimide solution, through the injection valve. Solutions were propelled by peristaltic pump with individual flow rate of 1.5 ml min.⁻¹. The absorbance was measured at 600 nm.



Fig(1). Manifold employed for FIA-Spectrophotometric determination of (PMH) drugs with sulphanic acid and N-bromosuccinimide where: W.Waste, IV. Injection valve, Rc.Reaction Coil, S. Sample, P.Peristaltic pump, FC.Flow cell, D.Detector.

Reagents:

All chemicals were of analytical reagent grade unless other wise stated. promethazine.HCl standard material was provided from the state company for drug industries and medical appliances, Sammara-Iraq. Histazine tablets and syrup were obtained from the united pharmaceutical manufacturing company, Amman, Jordan.

Promethazine hydrochloride stock solution ($500 \mu\text{g ml}^{-1}$)

A 0.0500 g amount of promethazine.HCl was dissolved in distilled water; the solution was then made up to 100ml in a volumetric flask with distilled water. More dilute solutions were prepared by simple dilution with distilled water.

N-Bromosuccinimide solution (10^{-2} M)

Prepared by dissolving 0.1779 g of N-bromosuccinimide in distilled water and made up to 100 ml in a volumetric flask with distilled water. More dilute solutions were prepared by simple dilution with distilled water

Sulphanilic acid solution (10^{-2} M)

Prepared by dissolving 0.1732 g of sulphanilic acid in distilled water and made up to 100 ml in a volumetric flask with distilled water. More diluted solutions were prepared by simple dilution with distilled water.

Procedure of pharmaceutical preparations

Tablets

Each tablet containing 25 mg of promethazine.HCl. Weigh and finally powder 10 tablets. Extract and accurately weigh portion of the powder equivalent to a bout 0.0500 g of pure drug and dissolve in distilled water, shake and filter the solution into a 100-ml volumetric flask and wash the residue with distilled water and dilute to

volume with distilled water to obtain (500 ppm) solution of the drug. A concentration of 100 ppm of the drug was prepared by simple dilution of the above solution with distilled water.

Syrup: (1mg ml⁻¹)

Each 1 ml of syrup contains 1 mg of promethazine.HCl, Transfer 50 ml of the syrup solution to a 100-ml volumetric flask and dilute to 100 ml with distilled water to obtain (500 ppm) solution of promethazine.HCl. More dilute solutions were prepared by simple dilution with distilled water.

Procedure for the batch method

Into a series of 25ml calibrated flasks, transfer increasing volumes of (PMH) solution (100 µgml⁻¹). Add 3ml of 2×10^{-3} M of N-bromosuccinimide solution and shake well, followed by 2 ml of 4×10^{-3} M of sulphanilic acid solution. Dilute the solution to the mark with distilled water and allow the reaction mixture to stand for 30 min at room temperature. Measure the absorbance at 600nm against a reagent blank prepared in the same way but containing no promethazine.HCl.

Procedure for the FIA method

Samples containing different concentrations of (PMH) drug were prepared by simple dilution with distilled water of the stock solution (500 µg ml⁻¹). The FIA spectrophotometric measurements were carried out using the manifold shown in Fig.1, employing 0.5mM of sulphanilic acid and 0.5mM N-bromosuccinimide solutions with a flow rate of 1.5ml min⁻¹ in each channel. 50 µl of samples and standard solutions were injected and the absorbance of the resulting dye product was measured at 600 nm. Optimizations of conditions were carried out on 100 µg ml⁻¹ of (PMH) solution.

RESULTS AND DISCUSSION

For the optimization of conditions and in all subsequent experiments, a solution of 500 µgml⁻¹ promethazine.HCl was used and the final volume was 25ml.

Batch spectrophotometric determination

When a very dilute aqueous solution of (PMH) is mixed with sulphanilic acid reagent and oxidised with N-bromosuccinimide, an intense bluish-green colour forms after 5 min, which became stable after 30 min. The colour has a maximum absorption at λ_{\max} 600 nm. Fig (2) shows the spectra of the bluish-green colour formed (A) and of the reagent blank (B).

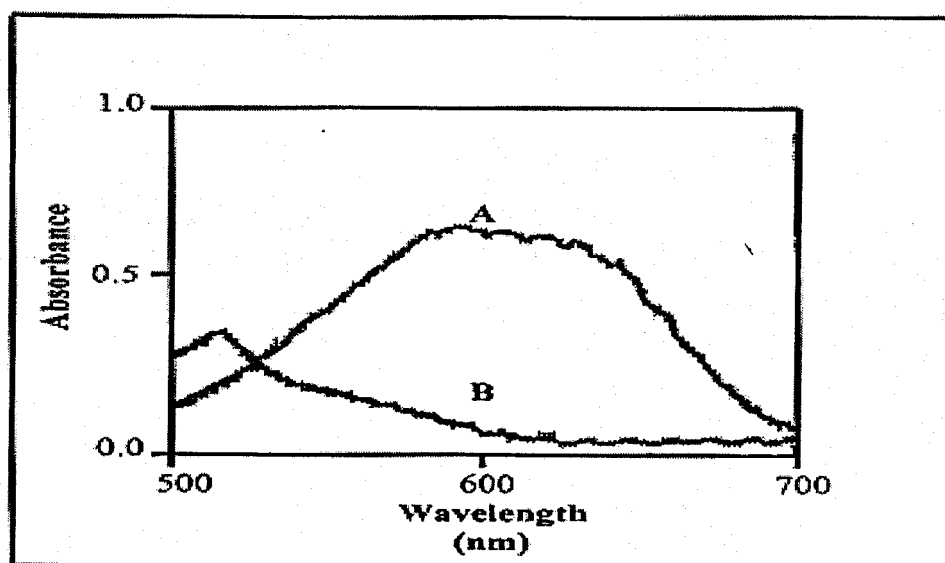


Fig.2 Absorption spectra of A (500 $\mu\text{g/ml}$) of promethazine.HCl treated as described under procedure and measured against reagent blank, and B the reagent blank measured against distilled water

The best experimental conditions for the determination of (PMH) were established for sulphanilic acid from (0.016 to 0.8 mM) and N-bromosuccinimide (from 0.008 to 0.4 mM) by altering one variable at a time and studying the absorbance at 600 nm as a function of time. The obtained results show that 0.32mM of sulphanilic acid and 0.24mM of N-bromosuccinimide are the concentrations that can give a higher absorption intensity at 600 nm for 500 μg of (PMH) in a final volume of 25 ml (i.e. 20 $\mu\text{g ml}^{-1}$).

The development of the colour of (PMH) from a mixture containing 20 $\mu\text{g ml}^{-1}$ in 0.32mM sulphanilic acid and 0.24mM N-bromosuccinimide gave evidence that the colour develops immediately and remains stable after 30 min. for more than 120 min.

The effect of temperature on the colour intensity of the dye was studied. In practice, high absorbance was obtained when the colour was developed at room temperature (25 $^{\circ}\text{C}$) than when the calibrated flasks were placed in an ice-bath at (0 $^{\circ}\text{C}$) or in a water bath at (60 $^{\circ}\text{C}$).

The stoichiometry of the reaction was investigated using molar ratio method (13). The results obtained (Fig.3) show a 1:1 drug to sulphanilic acid product. The formation of the dye may probably occur as follows (8):

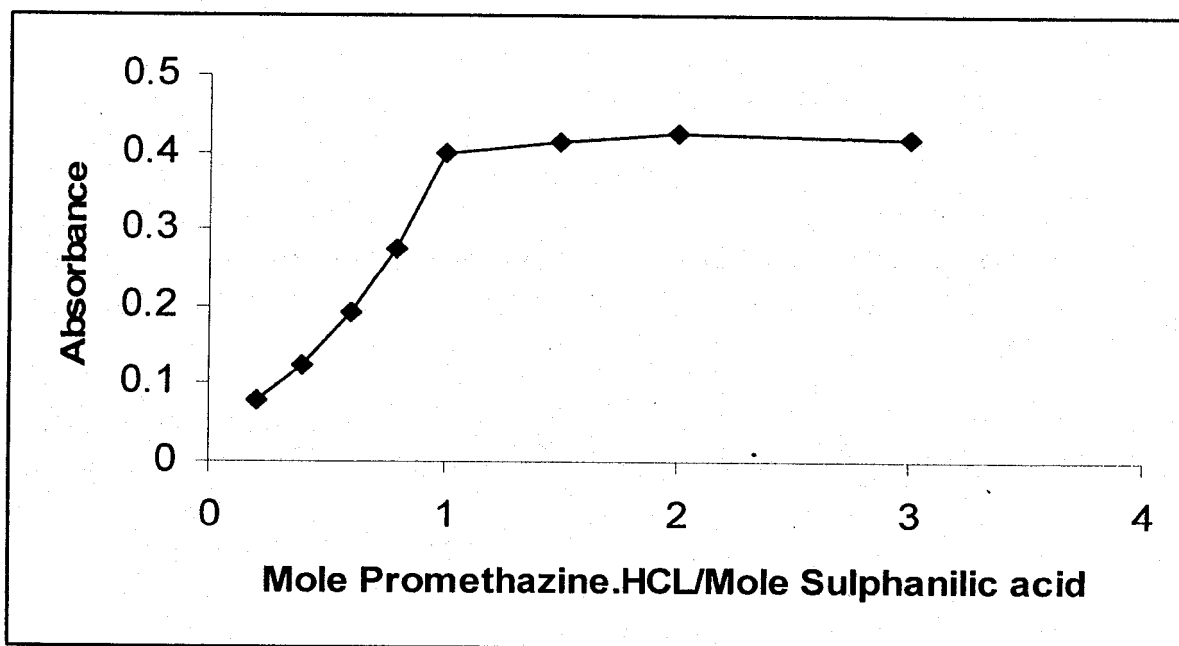
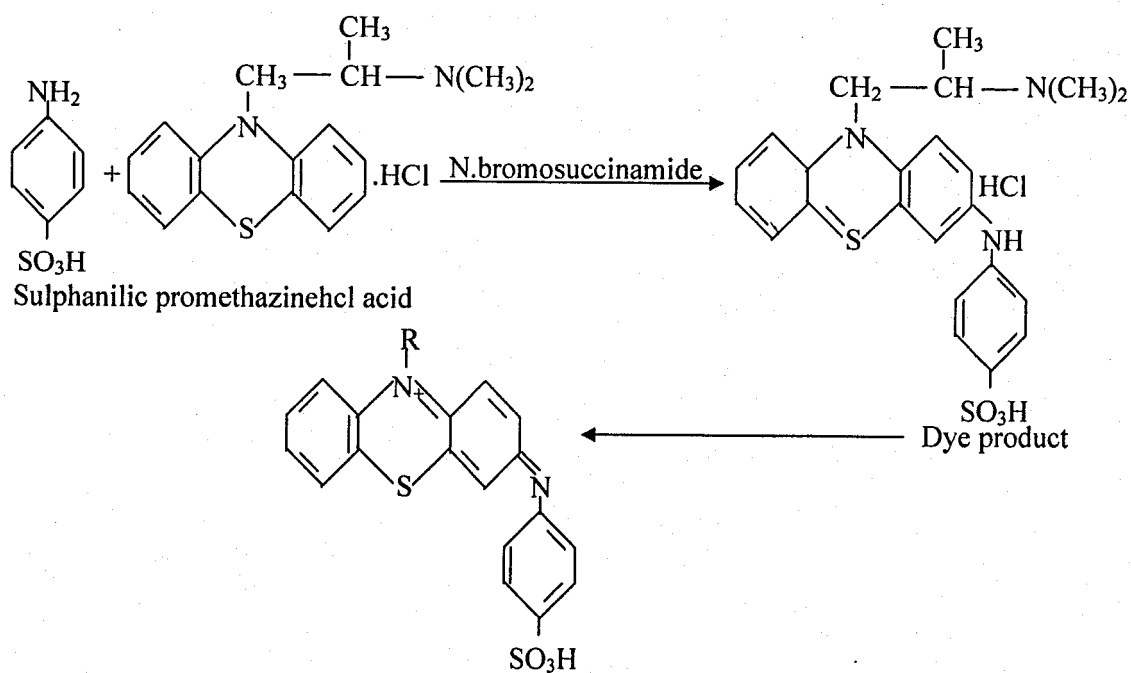


Fig.3 Mole ratio plot of the reaction between promethazine.HCl and sulphanilic acid

The regression equation obtained, from a series of (PMH) 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 (PMH) determination in order to make an effective comparison between the two approaches.

In order to assess the possible analytical applications of the proposed methods, the effect of some common excipients frequently found with PMH drugs in pharmaceutical formulations, such as sucrose, glucose, fructose, lactose, starch, talc and magnesium stearate was studied by analyzing synthetic sample solutions containing 20 µg/ml of PMH and excess amounts (10-fold excess) of each excipient, none of these substances interfered seriously.

FIA Spectrophotometric determination

The batch method for determination of (PMH) was adopted as a basis to develop FIA procedure, using the manifold indicated in Fig.1. The absorbance intensity of the coloured product at 600 nm has been improved by studying the effect of the different FIA parameters on the reaction between (PMH) and sulphanilic acid in the presence of N-bromosuccinimide such as sulphanilic acid concentration (from 0.1-1.0 mM), N-bromosuccinimide (from 0.1-1.0 mM), flow rates of reagents (from 0.15-2.5 ml/min. in each channel), length of the reaction coil (from 25 -125 cm) and injection volume (from 50-250 µL). The results obtained showed that a concentration of 0.5 mM of each of sulphanilic acid and N-bromosuccinimide were optimum. A flow rate of 1.5 ml/min. in each channel, a reaction coil of length of 50cm and an injection volume of 100 µL were the best conditions which provided the highest absorbance at 600nm with the lowest blank value.

A standard calibration line, obtained for a series of (PMH) 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 at 600nm and caused a degradation of the coloured product and low sensitivity and stability of the reaction products.

Analytical application

The developed methodology is very adequate for the determination of (PMH) in aqueous solution and in pharmaceutical preparation samples at a concentration level of traces (ppm) and requiring neither any previous separation step nor a temperature or pH control. Moreover the proposed procedures are very economical when compared to other methods such as those based on the use of HPLC.

In comparison of the batch with FIA procedure, the later is more convenient than the former method because of its speed (sample through-put of 120 injection/hr.) and wider linear range of the calibration graph (Table1).

The precision of the method was evaluated by analyzing pure sample of (PMH) and a good recovery was obtained (Table1). Finally the proposed method was applied successfully to the analysis of some pharmaceutical preparations containing (PMH). The results in Table2 are in accordance with those obtained by the official method (7).

Table.1 Analytical features of the procedures developed for the determination of (PMH).

Parameter	Batch procedure	FIA procedure
Regression equation	$Y=0.031x+0.11$	$Y=0.0022x+0.0032$
Linear range ($\mu\text{g ml}^{-1}$)	2-30	2-100
Correlation coefficient	0.9987	0.9998
Limit of detection ($s/n=3$) $\mu\text{g ml}^{-1}$	0.20	0.43
Reproducibility % for 20 $\mu\text{g ml}^{-1}$	0.25 (n=5)	1.10 (n=5)
Recovery,% for 20 $\mu\text{g ml}^{-1}$	98.95 (n=5)	98.82 (n=5)
Sample through-put (hr^{-1})	2	120

Table.2 Application of the proposed methods to the determination of (PMH) in pharmaceutical formulations.

Drug sample	Batch method		FIA method		Official method
	Recovery*,% RSD*,%	Recovery*,% RSD*,%	Recovery*,% RSD*,%	Recovery*,% RSD*,%	Recovery, %
Histazine (Tablets 25mg)	98.66 0.92	101.50 0.72	98.30		
Histazine (Syrup 1mg/ml)	101.34 1.80	100.57 0.74	98.02		

*For five measurements of $20\mu\text{g ml}^{-1}$

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