Journal of the Association of Arab Universities for Basic and Applied Sciences (2012) 11, 32-36

تحديد بيرمثامين في المتحضرات الصيدلانية عن طريق المطيافئة باستخدام 2 – 1 نافثوكوينون

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المخلص:

في هذه الدراسة تم وصف طريقة تمتاز بالدقة والبساطة لتحديد بيرمثامين في المستحضرات الصيدلانية. تعتمد الطريقة على تكوين مركب ملون نتيجة لتفاعل بيرمثامين مع 1–2 – نافثوكوينون في درجة حرارة 60 درجة مئوية. تمت متابعة التفاعل عن طريق المطيافية الضوئية بقياس الزيادة في الامتصاصية عند الطول الموجي 483 نانوميتر بدالة زمنية. عند الظروف المثلى للتفاعل كانت العلاقة بين قانون بيرز للامتصاصية والتركيز مطاعة (ممتثلة) في المدى 12 – 40 مايكرو جرام/مل ومعادلة الانحدار للبيانات المعايرة ص = 0.704 + 0.0132 ج مع معامل الارتباط (0.996) وامتصاصية مولية بمقدار 5.8* 10^3 لتر مول $^{-1}$ سم $^{-1}$. وكان الحد من الكشف النوعي والكمي 3.25 و 10.83 مايكرو جرام لكل ملليتر على التوالي. وقد طبقت الطريقة المقترحة بنجاح لتحديد بيرمثامين في أقراص الدواء مع دقة جيدة. كانت النسبة المئوية لبيرمثامين للدفعة 20 هي 101،4 $^{+}$ 0،15%. وكانت النتائج في اتفاق جيد مع طريقة الأداء العالية للفصل الكروماتوغرافي السائل الرسمية.

Journal of the Association of Arab Universities for Basic and Applied Sciences (2012) **11**, 32–36

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Journal of the Association of Arab Universities for Basic and Applied Sciences

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ORIGINAL ARTICLE

Spectrophotometric determination of pyrimethamine (PYM) in pharmaceutical formulation using 1,2-naphthoquinone-4-sulfonate (NQS)

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Available online 24 January 2012

KEYWORDS

UV–visible spectrophotometry;
Pyrimethamine;
NQS;
Pharmaceutical formulation

Abstract A simple and sensitive spectrophotometric method for the quantitative analysis of pyrimethamine (PYM) in pharmaceutical formulations has been described. The method is based on the formation of colored product between PYM and 1,2-naphthoquinone-4-sulfonate (NQS) at 60 °C. The reaction is followed spectrophotometrically by measuring the increase in absorbance at 483 nm as a function of time. Under the optimized reaction condition, Beer's law correlation for the absorbance (A) with PYM concentration (C) was obeyed in the range 12–40 μg mL^{-1} the regression equation for the calibration data was $A = 0.704 + 0.0132C$, with correlation coefficient (0.996). The molar absorptivity (ε) was 5.8×10^3 L mol^{-1} cm^{-1}. The limits of detection and quantification were 3.25 and 10.83 μg mL^{-1}, respectively. The proposed method was successfully applied to the determination of PYM in pharmaceutical tablets with good accuracy and precision; the percentage for PYM was 101.4 ± 1.47% for batch 20 and 100.4 ± 0.51% for batch 13. The results were in good agreement with those obtained with the official high performance liquid chromatography (HPLC) method.

1. Introduction

Pyrimethamine (PYM), 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidineamine (Fig. 1) is a drug widely used in the treatment of parasitic diseases such as malaria and toxoplasmosis (Bosch-Driessen et al., 2002).

The mechanism of action of PYM is the inhibition of dihydrofolate reductase (DHFR), an essential enzyme responsible for the conversion of folic acid into folinic acid in the nucleic acid biosynthesis (Anderson, 2005). Some major drawbacks of PYR therapy include a relatively weak inhibitory activity and severe side effects such as nausea and neutropenia, which are caused by the low selectivity toward the parasite enzyme (Katlama et al., 1996).

Literature survey reveals that various methods have been reported for the analysis of PYM in pharmaceutical and bio-

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Peer review under responsibility of University of Bahrain.
doi:10.1016/j.jaubas.2011.12.003

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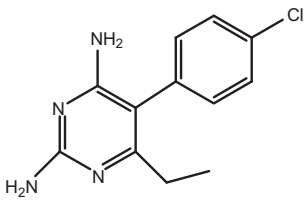

Figure 1 Chemical structure of PYM.

logical samples such as microbiological method (Weidekamm et al., 1982), gas–liquid chromatography (Cala et al., 1972; Jones et al., 1981; Midskov, 1984b), thin layer chromatography (DeAngelis et al., 1975), spectrophotometry (Schmidt et al., 1953; Sastry et al., 1986; Berzas Nevado et al., 1993; Khalil et al., 2000; Onah and Odeiani, 2002; Zayed et al., 2005; Nagaraja et al., 2010), fluorimetry (Parimo, 1988) and HPLC(Midskov, 1984a,b; Bergqvist and Eriksson, 1985; Timm and Weidekamm, 1982; Edstein, 1948a,b) individually and in combination dosage form with other drugs.

Spectrophotometry is considered the most convenient analytical technique, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories.

Spectrophotometric methods have been reported for PYM. These methods were associated with some major drawbacks such as laborious multiple extraction steps in the analysis by ion-pair based methods (Khalil et al., 2000; Nagaraja et al., 2010) Furthermore, the analytical reaction was long and thus the procedure was time-consuming (Onah and Odeiani, 2002).

1,2-Naphthoquinone-4-sulfonate (NQS) has been used for the determination of many compounds containing primary amine group (Gallo-Martinez et al., 1998; Wang et al., 2004; Saurina and Hernández-Cassuo, 1993). Use of NQS as a colored reagent for the determination of PYM by spectrophotometry has not been reported yet. Therefore, the present work was devoted to study the reaction between PYM and NQS and employment of the reaction in the development of a simple method for the determination of PYM in dosage form.

2. Material and methods

2.1. Instrumentation

All absorbance measurements were made with a Double beam UV-1800 (SHIMADZU, Japan) ultraviolet–visible spectrophotometer provided with matched 1-cm quartz cells and also temperature controller was used for the spectrophotometric measurements. pH meter model pH211 (HANNA, Italy). Thermostatically controlled water bath type RE 220 (LAUDA, Germany).

2.2. Reagents and materials

Pyrimethamine (PYM) (AMIPHARMA Laboratories Ltd., Khartoum) were obtained and used as received; its purity was 99.7%. A solution of 0.5% (w/v) of 1,4-naphthoquinone-4-sulfonate (NQS) (Aldrich chemical Co., St. Louis, USA) was prepared by dissolving 125 mg in 25 mL double distilled water. The solution was freshly prepared and protected from light during use. Buffer solution of pH 13.0 was prepared by mixing 25 mL solution of KCl (0.2 M) and 66 mL solution of NaOH (0.2 M) in 100 mL volumetric flask, and adjusted by the pH meter. Other buffer solutions of different pH values were also prepared. Amifan tablets were kindly donated by AMIPHARMA Laboratories Ltd. (Sudan, Khartoum). Milli-Q water was used for preparing all solutions used.

2.3. Preparation of standard and sample solution

2.3.1. PYM standard solutions

An accurately weighed 10 mg of PYM was dissolved in methanol, transferred into a 10 mL standard flask and completed to the mark with methanol. This stock solution was further diluted with the same solvent to obtain working solutions in the range 6–60 μgmL^{-1}.

2.3.2. Tablets sample solution

Twenty tablets were weighed, and finely powdered. An accurately weighed quantity of the powdered tablets equivalent to 4.25 mg of PYM was transferred into a 25 mL calibrated flask, and dissolved in methanol. The prepared solutions were diluted quantitatively with methanol to obtain a suitable concentration for the analysis.

2.3.3. General recommended procedure

Accurately measured 1 mL of PYM solution containing 12–40 μgmL^{-1} was transferred into a 10 mL volumetric flask. 3 mL of buffer solution pH (13.0) was added, followed by 1 mL of NQS solution (0.5%, w/v). The reaction solution was allowed to proceed at 60 °C for 15 min. The reaction mixture was completed to volume with water, and the resulting solution was measured at 483 nm against reagent blank.

2.4. Determination of PYM by HPLC

According to the United States Pharmacopoeia, PYM was determined in two batches number (batch number 20 and 13). The analysis was performed on a Phenomenex C18-column; 5 lm (250 mm 4.6 mm i.d.) with a guard column

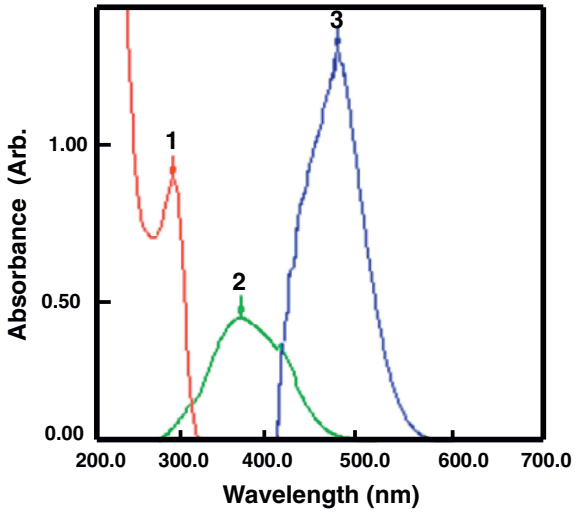

Figure 2 Absorption spectra of Pyrimethamine (20 μgml^{-1}) against methanol (1). NQS (0.5% w/v) against water (2). And the reaction product of primethamine (30 μgml^{-1}) with NQS against reagent blank (3).

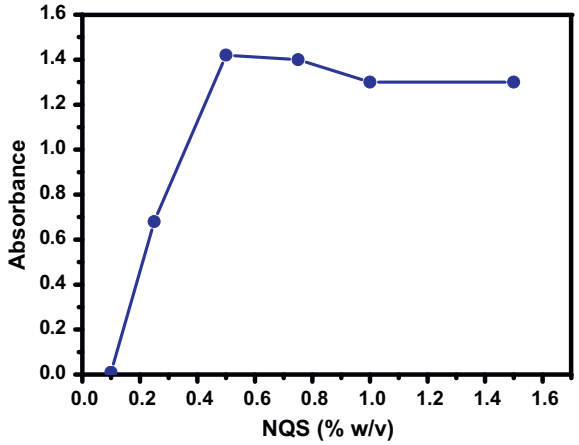

Figure 3 Effect of NQS concentration on the reaction of PYM with NQS. PYM (40 μg/ml): 1 ml; buffer solution (pH 13.0); 3 ml; temperature 60 °C; reaction time: 15 min.

(4.0 mm × 3.0 mm i.d.). The mobile phase consisting of 80% glacial acetic acid (1:100), 20% acetonitrile and 500 μL triethylamine was delivered as an isocratic elution at a flow rate of 2 mL/min and 10 μL of the sample was injected. Before use the mobile phase was degassed by an ultrasonic bath and filtered by a Millipore vacuum filter system equipped with a 0.45 ml HV filter.

3. Results and discussion

3.1. Optimization

The absorption spectrum of the reaction product between NQS and PYM with an absorption maximum at 480 nm is shown in Fig. 2. Clearly from this figure a broad absorption band at longer wavelengths is obtained for the reaction between NQS and PYM.

The optimum conditions for the development of method were established by varying the parameters one at a time while keeping the others fixed and observing the effect produced on the absorbance of the colored product. In order to establish experimental conditions, the effect of various parameters such as concentration of NQS, pH, temperature, and time of heating were studied.

The effect of NQS concentration was studied over the range 0.1–1.5 w/v% as shown in Fig. 3. Increasing the concentration of NQS results in more products up to an amount of 0.5% after which the absorbance remained almost constant. Therefore a concentration of 0.5% NQS was considered optimum.

Table 1 Parameters for the performance of the proposed method.

Parameter	Value
Measurement wavelength (nm)	483
Linear range (μg mL^{-1})	12–40
Intercept	0.704
Standard deviation of the intercept	0.014
Slope	0.013
Standard deviation of the slope	0.001
Correlation coefficient (r)	0.996
Limit of detection, LOD (μg mL^{-1})	3.25
Limit of quantification, LOQ (μg mL^{-1})	10.83
Molar absorptivity, ε (1 mol^{-1} cm^{-1})	5.8×10^3

The influences of pH on the absorbance of product I was investigated in the range 1.0 to 14. At pH 1.0–7.0, the absorbance of the product is close to 0, indicating that under high acidity, PYM does not react with NQS. The possible reason may be that the amino group ($-NH_2$) of PYM is protonated (pk_a = 7.26 G.S. Lázaro et al., 2008) and converted into protonated amine salt ($-NH_3^+$). So it loses nucleophilic capability for 4-sodium sulfonate of NQS, and the nucleophilic substitution reaction cannot take place easily. At pH > 10.0, the absorbance of the solution increases rapidly up to pH 13.0. It may be that protonated amine salt ($-NH_3^+$) of PYM turns into amino group ($-NH_2$) again when the acidity of the solution becomes low. The higher the pH, the more effectively the protonated amino group removes the proton, and more easily the nucleophilic substitution reaction happens. At pH 13.0, the absorbance reaches its maximum; in other words, the degree of the nucleophilic substitution reaction is also maximal. At pH > 13.0, the absorbance of solution decreases sharply again. Presumably it may be that the increase of hydroxide ion holds back the nucleophilic substitution reaction between PYM and the chromogenic reagent. Consequently, the absorbance of the solution reduces. In order to keep the high sensitivity for the determination of PYM, pH 13.0 was selected for optimal experimental conditions. Reaction of NQS with compound bearing primary amines at pH 13.0 was reported (Li and Zhang, 2008).

The effect of temperature on the reaction was studied in the range 20–80 °C. About 60 °C was found to be optimal for maximum color development.

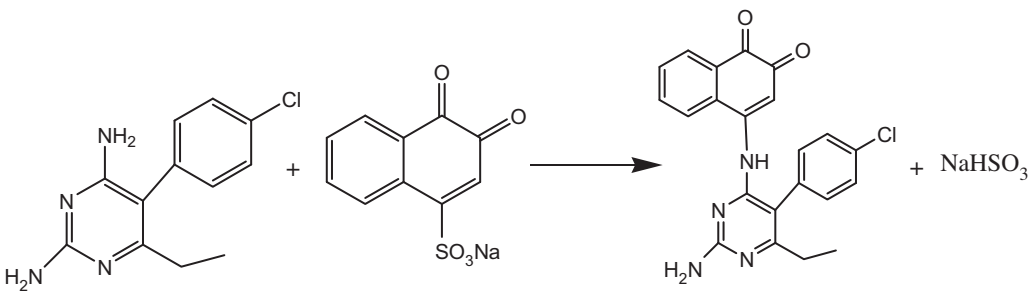

Figure 4 Scheme for the reaction pathway of PYM with NQS.

Table 2 Recovery studies for the determination of PYM, by the proposed method.

Sample No. +SD	Sample content (μgmL^{-1})	Pyr. added (μgmL^{-1})	Found	Recovery (%)
1	10	14	14.0	100.0 ± 0.01
2	–	18	18.3	101.7 ± 0.03
3	–	26	26.1	100.5 ± 0.01
4	–	32	42.8	101.9 ± 0.09

The influence of the time of heating was also investigated in the range 5–30 min. The experimental results show that heating in the range 15–20 min gave the optimal values in kinetic studies. The color product was stable for at least two days at room temperature.

From the above experiments, the optimized conditions used for the assay were: NQS concentration 0.5% w/v, buffer (pH 13.0), reaction time 20 min and temperature 60 °C.

Under the optimum conditions, the stoichiometry of the reaction between PYM and NQS was studied by the Job's (1964) method. The symmetrical bell shape of Job's plot indicated that the ratio of PYM:NQS is 1:1. Based on this ratio, the reaction pathway was proposed to proceed as shown in Fig. 4.

3.2. Analytical methods validation

As can be seen from Table 1, linear relationship was found between absorbance at λ_{max} (483) and the concentration of the PYM in the range 12–40 μg mL^{-1}. The regression equation was found as $A = 0.0132C + 0.704$ ($r^2 = 0.996$). The limits of detection (LOD) and limit of quantitation (LOQ) were determined using the formula:LOD or LOQ = *K*S.D.a/b, where $k = 3$ for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope.

The accuracy of the proposed method was carried out by applying standard addition technique. A different amount of standard solution was added to a known concentration of the drug sample. The average percent recoveries obtained in range 100.04–101.9. Table 2.

Robustness was examined by evaluating the influence of small variation of method variables including the concentration of analytical reagent and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation of method variables did not significantly affect the procedures; recovery values were 98.43−101.40 (Table 3).

Table 3 Influence of small variation in the assay conditions on the analytical performance of the proposed spectrophotometric method for the determination of pyr. using NQS reagent.

Parameters	Recovery (% ± SD)
Recommended conditions	101.4 ± 1.5
NQS concentration (%, w/v) 0.45	100.2 ± 0.1
NQS concentration (%, w/v) 0.55	99.7 ± 0.1
Buffer (pH) 12.75	100.8 ± 0.1
Buffer (pH) 13.25	99.7 ± 0.3
Temperature (°C) 58	99.6 ± 0.2
Temperature (°C) 60	99.9 ± 0.1
Reaction time (min) 14	98.4 ± 0.2
Reaction time (min)	100.5± 0.1

3.3. Application to the pharmaceutical dosage forms

PYM tablets were subjected to the analysis by the proposed as well as with the official HPLC method (United States pharmacopeia, 2006) and the obtained results were statistically compared with each other. The label claim percentage was 101.40 ± 1.47 and 102.38 ± 0.51 for batch 20 and batch 13 respectively (Table 4). With respect to *t*- and *F*-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicated similar accuracy and precision in the analysis of PYM in tablets. Common tablet excipients such as talc, lactose, starch, avisil, gelatine and magnesium stearate did not interfere with the assay.

4. Conclusions

The present paper described the evaluation of NQS as an analytical reagent in the development of simple, sensitive, and accurate spectrophotometric methods, for the determination of PYM in pharmaceutical formulations. The described method is superior to the previously reported spectrophotometric methods in terms of simplicity and sensitivity. The proposed method has comparable analytical performances and is devoid of any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Therefore, this method can be recommended for the routine analysis of PYM in quality control laboratories.

Table 4 Analysis of PYM containing dosage forms by the proposed and official HPLC methods (United States Pharmacopoeia).

Dosage form	Recovery % + RSD ($n = 5$)		*t*-value	*f*-value
	Proposed	Official		
AMIFAN batch 20	101.4 ± 1.5	102.5 ± 1.4	1.21	1.09
AMIFAN batch 13	100.4 ± 0.5	99.7 ± 0.5	2.40	1.18

The tabulated values for *t* and *f* at 95% confidence limit are 2.78 and 6.26, respectively.

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