*Mar. Üniv. Ecz. Der.*,
2 (1), 77—83 (1986)

*J. Pharm. Univ. Mar.*,
2 (1), 77—83 (1986)

# DAPSONUN TABLETLERDE 1,2-NAFTOKİNON-4-SÜLFONİK ASİD SODYUM TUZU İLE SPEKTROFOTOMETRİK MİKTAR TAYİNİ*

# SPECTROPHOTOMETRIC DETERMINATION OF DAPSONE IN TABLETS WITH 1,2-NAPHTHOQUINONE-4-SULPHONIC ACID SODIUM SALT*

Gülsen İSKENDER**, Fatma ORAK**

## SUMMARY

A spectrophotometric method for the assay of dapsone based on the chromophore formation after reaction with 1,2-naphthoquinone-4-sulphonic acid sodium salt was developed. The reaction proceeded quantitatively at pH 2 and 60°C within 30 minutes when the molar ratio of reagent to dapsone was 10. After completion of the reaction, formed derivative (dapsone - NQ) was extracted from the aqueous solution with chloroform/n-butanol (3:1). Dapsone-NQ shows maximum absorbance at 440 nm. The absorbance is linear ($r = 0.9999$) over a 2-30 $\mu g.ml^{-1}$ concentration range. The proposed method has been successfully applied to the determination of dapsone in tablets and the results were compared with those obtained by the USP XX method using t- and F- tests.

## ÖZET

Dapsonun miktar tayini için 1,2-naftokinon-4-sülfonik asid sodyum tuzu ile kromofor oluşturmasına dayanan bir spektrofotometrik yöntem geliştirildi. Reaksiyon pH 2 de ve 60°C de 30 dakikada, belirteç dapson mol oranı 10 olduuğnda kantitatif olarak yürümektedir. Reaksiyonun tamamlanmasından sonra, oluşan türev (dapson-NQ) sulu çözeltiden kloroform/n-butanol (3:1) ile ekstre edildi. Dapson-NQ 440 nm de maksimum absorbans göstermektedir. Absorbans 2-30 $\mu g.ml^{-1}$ konsantrasyon aralığında doğrusaldır ($r = 0.9999$). Önerilen yöntem, tabletlerde dapson miktar tayininde başarı ile uygulanmıştır ve sonuçlar USP XX yöntemiyle elde edilen sonuçlar ile t- ve F- testleri yönünden kıyaslanmıştır.

* A part from the Dissertation of Fatma Orak.

** Faculty of Pharmacy, University of Istanbul, Beyazıt-Istanbul Turkey.

## INTRODUCTION

Dapsone (DDS, 4,4′-sulfonyldianiline) is an important drug in the treatment of leprosy. Various analytical techniques have been investigated for its analysis in pharmaceutical preparations and biological fluids. These techniques can be summarized as follows: titrimetry (1), colorimetry (2, 3), spectrofluorometry (4), TLC (5), GLC (6) and HPLC (7).

NQS (1,2-naphthoquinone-4-sulphonic acid sodium salt) has been widely used to produce coloured derivatives of several primary and secondary amines (8-13).

This report presents a new method for the assay of dapsone and its dosage form with NQS in an aqueous solution.

## EXPERIMENTAL

Instruments: Ultraviolet and visible absorption spectrum was recorded with a Varian Techtron UV-Visible spectrophotometer model G. Quantitative measurements were carried out on a Beckman B model spectrophotometer.

Chemicals: Dapsone (Imperial Chemical Industries PLC, Macclesfield, Cheshire, Great Britain) and NQS (E. Merck, A.G. Darmstadt, G.F.R.) were used. Other chemicals were analytical grade.

Stock solution: 250 mg of dapsone was dissolved in 50 ml of ethanol. Further dilutions were made with chloride buffer (pH 2).

Reagent solution: A $2 \times 10^{-2}$ M aqueous solution of NQS was prepared daily.

Buffer solutions were prepared according to the official procedures (14, 15).

Synthesis of the derivative: 2 mmol of NQS in 9 ml of water and 10 ml of buffer solution (pH 2) were added to 0.5 mmol of dapsone in 20 ml of ethanol/water (1:1) solution. The mixture was heated for 30 min. at 60°C. The dapsone-NQ derivative was extracted with chloroform/n-butanol (3:1). Combined organic phases were dried over anhydrous sodium sulfate and distilled to dryness in vacuo. The derivative was crystallised from the methanol/water (1:1).

The dark red crystals melted at 227-232°C. The Rf value of the derivative was 0.36 on a silica gel plate using chloroform methanol (9 : 1) as the solvent system. The yield of the reaction was found as 88.1 %. Ultraviolet and visible absorption spectrum of dapsone - NQ is shown in Figure 1. IR (KBr) $cm^{-1}$ : 3300 (OH), 1690 (C = 0), 1610 (C = N), 1580 (aromatic C = C), 1150 and 1105 (= $SO_2$).

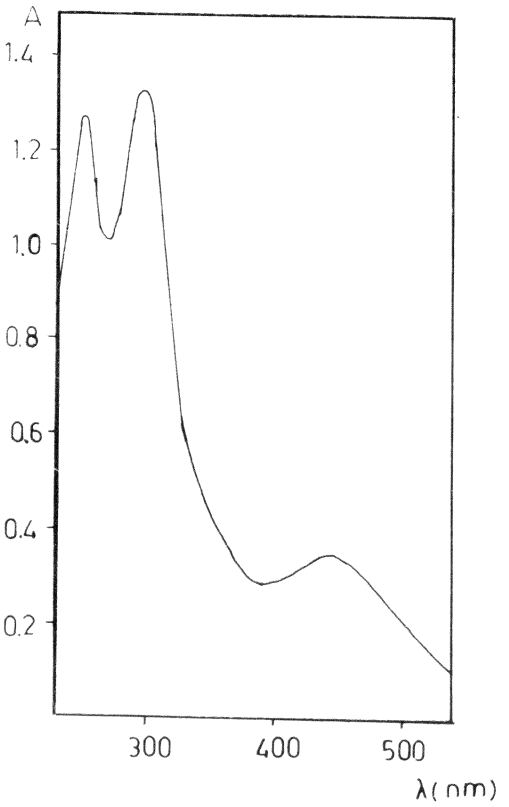


Figure 1. Ultraviolet and visible absorption spectrum of dapsone - NQ in chloroform/n-butanol (3:1).

Assay procedure : A 1 ml of an aqueous solution containing 20 - 300 μg. $ml^{-1}$ of dapsone was pipetted into a 10 ml test tube. After addition of 0.65 ml of NQS solution, the tube was incubated at 60°C for 30 min. The dapsone - NQ derivative was extracted with 5 ml of chloroform/n-butanol (3 : 1). The aqueous phase was removed and discarded. The organic phase was dried over an-

hydrous sodium sulfate, centrifuged and transferred into a 10 ml calibrated flask. The residue in the tube was washed twice with 2 ml of chloroform/n-butanol (3 : 1) and organic solutions were combined. Then the volume was made up to 10 ml with the same organic solvent mixture. The absorbance of the solution was read at 440 nm against a blank prepared similarly. Calibration curve was prepared by plotting concentration of dapsone ($\mu g.\ ml^{-1}$) versus absorbance values.

Assay procedure for dapsone tablets : A quantity of thoroughly mixed powder of the tablets (Udolac$^R$ Tablet) equivalent to about 100 mg of dapsone was accurately weighed and transferred into a 100 ml calibrated flask. A 20 ml of ethanol and 30 ml of water were added and shaken for 15 min. The volume was adjusted to 100 ml with water, mixed and filtered. The first portion (20 ml) of the filtrate was discarded. Then 2 ml of the filtrate was transferred into a 10 ml calibrated flask and diluted to volume with chloride buffer (pH 2). One ml of this solution was pipetted into a test tube and the assay procedure above was followed.

## RESULTS and DISCUSSION

The optimum conditions of the reaction between dapsone and NQS were investigated at the concentration of 20 $\mu g.\ ml^{-1}$ of dapsone.

It has been found that chromophore obtained shows maximum absorbance at 440 nm in chloroform/n-butanol (3 : 1) organic solvent mixture.

The dapsone-NQ formation is dependent on the pH of the medium. The results on the pH study shown in Table 1 indicated that maximum absorbance was obtained at pH 2 chloride buffer.

Table 1. Effect of pH on the reaction of dapsone with NQS

| pH | 1.5 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |
|---|---|---|---|---|---|---|
| Absorbance | 0.496 | 0.530 | 0.500 | 0.435 | 0.420 | 0.392 |

The effect of varying the temperature and the reaction period have been studied. The reaction was completed at 60°C within 30 min. (Figure 2).

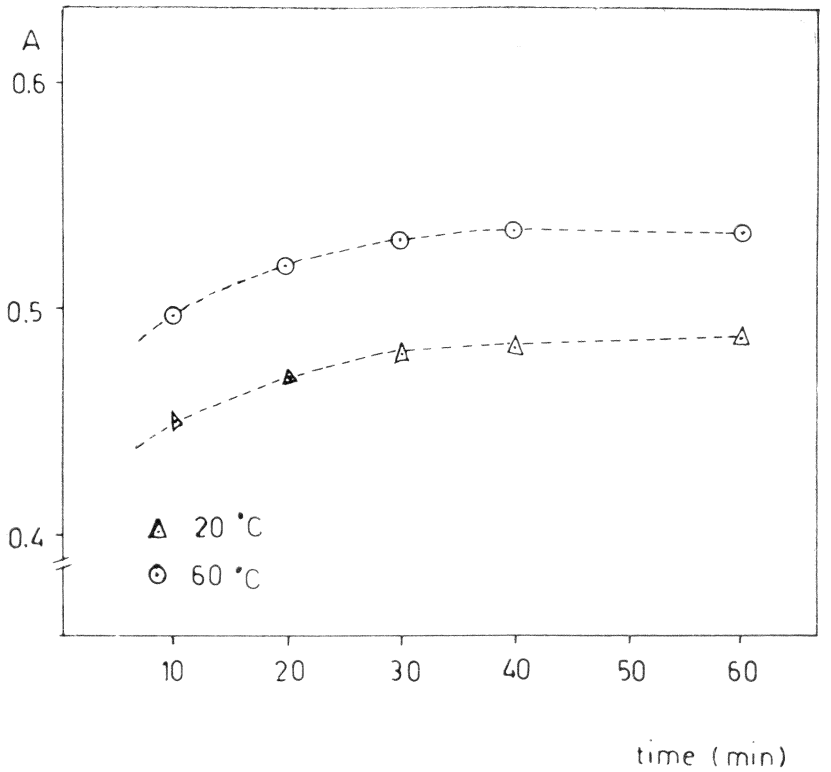


Figure 2. Effect of heating time on absorbance of chromophore

To determine the amount of reagent, varying concentrations of the NQS were added to a fixed quantity of amine. The results indicated that 10 times reagent excess has been enough to complete the reaction (Table 2).

Table 2. Effect of reagent concentration on the reaction of dapsone with NQS

| Mole ratio of dapsone/NQS | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|
| Absorbance | 0.452 | 0.582 | 0.600 | 0.622 | 0.635 | 0.618 | 0.615 | 0.610 |

The dapsone-NQ derivative was found to be stable at + 4°C in chloroform/n-butanol (3 : 1) for at least one week.

Under the experimental conditions, a linear relationship existed between absorbance and concentration over the 2-30 $\mu g.\ ml^{-1}$

concentration range. The regression equation was $A = 0{,}251\ C + 0.0068$ ($r = 0.9999$).

Results obtained by applying the proposed spectrophotometric procedure to commercially available dapsone dosage form are presented in Table 3. The results were compared with those obtained by the USP method (16) in terms of t-and F-tests of significance at 85 % confidence level (Table 4).

The proposed spectrophotometric method is easy to perform and stability indicating. It can be successfully applied to dapsone in pure form or in pharmaceuticals.

Table 3. Results of analysis of commercially available dapsone tablets (100 mg dapsone/tablet)

| Assay Number | Spectrophotometric method | Titrimetric method |
|---|---|---|
| | mg/tablet | mg/tablet |
| 1 | 101.43 | 100.20 |
| 2 | 101.83 | 100.82 |
| 3 | 101.83 | 100.82 |
| 4 | 102.03 | 100.82 |
| 5 | 102.03 | 102.07 |
| 6 | 102.43 | 102.07 |
| Mean | 101.96 | 101.13 |

Table 4. Statistical evaluation of the results given in Table 3

| Statistical values | Spectrophotometric method | Titrimetric method |
|---|---|---|
| Number of determinations | 6 | 6 |
| Mean | 101.96 | 101.13 |
| Standard deviation | 0.35 | 0.76 |
| Relative standard deviation | 0.34 | 0.76 |
| Confidence limits | 101.59-102.33 | 100.33-101.93 |
| t test of significance | 2.42* | ($p = 0.05$ $t = 2.23$) |
| F test of significance | 4.76 | ($p = 0.05$ $F = 5.05$) |

* Significant difference.

ACKNOWLEDGMENTS

We are grateful to Imperial Chemical Industries PLC, Macclesfield, Cheshire, Great Britain and Bilim İlaç Sanayii ve Tic. A.Ş., Istanbul, Turkey for supplying the substances used in this work.

REFERENCES

1. Chien, N.T., Quyen, L.N.: *Duoc Hoc*, **2**, 31 (1979) - Ref., *C.A.*, **93**, 54083 t (1980).
2. Higgins, T.N., Taylor, J.D.: *Clin. Biochem.*, **6**, 295 (1973).
3. Sanghavi, N.M., Sathe, V.H., Padki, M.M.: *Indian Drugs*, **20**, 341 (1983).- Ref., *Anal. Abstr.*, **45**, 4E3 (1983).
4. Peters, J.H., Gordon, G.R., Colwell, W.T. Jr.: *J. Lab. Clin. Med.*, **76**, 338 (1970).
5. Gordon, G.R., Peters, J.H.: *J. Chromatogr.*, **47**, 269 (1970).
6. Burchfield, H.P., Storrs, E.E., Wheeler, R.J., Bhat, V.K., Green, L.L.: *Anal. Chem.*, **45**, 916 (1973).
7. Zuidema, J., Modderman, E.S.M., Hilbers, H.W., Merkus, F.W.H.M., Huikeshoven, H.: *J. Chromatogr.*, **182**, 130 (1980).
8. Feldman, E.G.: *J. Am. Pharm. Ass. Sci. Ed.*, **48**, 197 (1959).
9. Tulus, R., Güran, A.: *J. Fac. Sci., Univ. Istanbul*, Serie C, **28**, 108 (1963).
10. Bartos, J., Pesez, M.: *Bull. Soc. Chim. Fr.*, **4**, 1627 (1970).
11. Gürkan, T.: *J. Fac. Pharm. Istanbul*, **16**, 73 (1980).
12. Salama, R.B., Omer, A.I.H.: *J. Pharm. Sci.*, **69**, 346 (1980).
13. Iskender, G., Prof. Thesis, Istanbul (1982).
14. Küster, F.W., Thiel, A., Fischbeck, K.: *Logaritmische Rechentafeln*, Walter De Gruyter & Co., Berlin (1947).
15. *The British Pharmacopeia (BP)*, Vol. II, p. 755, The University Press, Cambridge (1980).
16. *The United States Pharmacopoeia*, Twentieth Revision (USP XX), p. 197, United States Pharmacopoeial Convention, Inc., Rockville (1980).

*(Received January 29, 1986)*