SPECTROPHOTOMETRIC DETERMINATION OF NITRAZEPAM IN TABLETS

Salwa Rizk El-Shabouri

Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
University of Assiut, Assiut, Egypt.

ABSTRACT

A sensitive spectrophotometric method is reported for the determination of nitrazepam either pure or in tablets. The method is based on the reduction of nitrazepam with zinc dust and calcium chloride followed by reacting the reduced compound with trisodium pentacyanoaminoferate. The reaction gives a violet product having an absorbance maximum at 560 nm. Beer's law is obeyed in the concentration range of 25-500 µg/ml of the reaction mixture. The common excipients in tablets do not interfere. The recovery and precision are similar to those of the official B.P. method.

INTRODUCTION

Nitrazepam is one of the widely used hypnotics and anticonvulsants belonging to the 1,4-benzodiazepines.

A number of methods have been performed for the determination of nitrazepam such as spectrophotometric¹, colorimetric^{2,4}, gas chromatographic^{5,6}, TLC⁷, HPLC⁸, fluorimetric⁹, and differential pulse polarographic^{10,11} methods. The B.P.¹² describes a non-aqueous titration for bulk drug and a spectrophotometric method for tablets.

Trisodium pentacyanoaminoferate (SCAF) reagent has been used to detect aromatic nitro compounds¹³. This reaction was applied in this report to determine nitrazepam either pure or in tablets. Various conditions, such as volume and concentration of the reagent, reduction time and colour development have been standardized. The proposed method is simple, sensitive, accurate and relatively rapid.

EXPERIMENTAL

Apparatus:

Ultraviolet-visible spectrophotometer, Unicam SP 1750 (Pye- Unicam, Cambridge, England) and Ultraviolet-visible spectrophotometer, PM₂ DL (Zeiss, Oberkochen, West Germany) were used with 1-cm matched glass cells.

Reagents and solutions:

- Trisodium pentacyanoaminoferate (SCAF), $\text{Na}_3\{\text{Fe}(\text{CN})_5\text{NH}_3\}$, prepared according to a reported procedure¹⁴.
- Reagent solution, 0.1% in water. To be freshly prepared daily.
- Calcium chloride, solution, 10% in water.
- Zinc dust (Merk).
- Standard nitrazepam solution. prepared by dissolving 50 mg nitrazepam (Hoffman-La Roche) in 25 ml ethanol. This is the stock solution from which appropriate dilutions were made with ethanol. This solution must be freshly prepared daily.

All chemicals used were of analytical grade. Distilled water was used throughout this work.

Procedure

*Spectrophotometric Determination of Nitrazepam in Tablets.*Procedure

1- Powder: Pipette 0.5 ml of the nitrazepam solution, containing 25-500 μg , into a 50-ml conical flask containing 100 mg of zinc dust and 5 ml of calcium chloride solution. Let to stand 5 minutes at room temperature (25°C) with occasional shaking. Filter through Whatman No1 filter paper previously moistened with ethanol into 25-ml standard flask. Wash the residue thoroughly with three 2-ml portions of ethanol and add the washings to the filtrate in the flask. Add 5 ml SCAF reagent solution to the combined filtrate and washings, mix well and let to stand for 10 minutes at room temperature. Adjust the volume with water and measure the absorbance at 560 nm against a blank prepared concomitantly, substituting nitrazepam with ethanol.

2- Tablets: Weigh and powder 20 tablets. Transfer an accurately weighed amount of the powder, equivalent to 50 mg of nitrazepam, into a small beaker. Dissolve the nitrazepam content by triturating with four 5-ml portions of ethanol, decanting the ethanolic extract each time and completing the final volume to 25 ml with ethanol. Mix thoroughly, filter and discard the first portion of the filtrate. Pipette an aliquot of the clear solution containing 25-500 μg of nitrazepam into a 50-ml conical flask and proceed as given under "powder" above, starting with the words "containing 100 mg of zinc dust.....".

RESULTS AND DISCTSSION

Nitrazepam, when reduced with zinc and calcium chloride solution, under the proposed conditions, and subsequently reacted with SCAF in aqueous ethanol, produces a violet chromophore, having an absorbance maximum at 560 nm (Fig.1) with $\epsilon = 1.15 \times 10^4$. The relationship between absorbance and concen-

tration of nitrazepam at λ_{max} 560 nm was found to be linear within a range 25-500 $\mu\text{g}/25$ ml with good correlation coefficient (0.9998) ($n=8$) and small intercept (0.0134). The colour species exhibited stability for 10 hours at room temperature.

Various conditions have been standardized for the proposed analytical determination. The following conclusions were reached, using 25-500 $\mu\text{g}/25$ ml of nitrazepam.

- 1- No difference in the absorption intensity was observed up to the use of 300 mg zinc dust, but further increase in the amount of showed a slight decrease in the absorption intensity. However, 100 mg of zinc dust are used throughout this report.
- 2- The decrease or increase in the volume of calcium chloride solution (1-10 ml) did not affect the absorption intensity.
- 3- Three ml of SCAF reagent solution were sufficient to develop the colour to full intensity. However, 5 ml seemed to be more convenient and are used throughout this work.
- 4- Maximum colour intensity was exhibited after interaction up to 2 minutes reduction time at room temperature, and further increase in time (up to 20 minutes) showed no change in the absorbance value. Five minutes are, however, recommended in this work.
- 5- The intensity of the colour increased up to 10 minutes standing after addition of SCAF solution and remained stable thereafter. Fifteen minutes are recommended in this work.
- 6- Dilution of the coloured product with water, ethanol, methanol, dioxane and isopropanol showed no effect on the position of λ_{max} or on the intensity of absorption, except for isopropanol which gave 25% decrease in the absorption

Spectrophotometric Determination of Nitrazepam in Tablets.

intensity. Water is here chosen as a diluent, and the developed colour is stable for at least 10 hours.

The developed method was applied successfully for the determination of nitrazepam in bulk drug and in tablets. Table 1 illustrates the suitable range which gives the best accuracy for nitrazepam. Very low absorbances were obtained upon using concentrations less than 25 $\mu\text{g}/25\text{ ml}$, while unreplicable absorbances were given upon using concentrations higher than 500 $\mu\text{g}/25\text{ ml}$. Commonly encountered tablet excipients such as starch, talc, lactose, and magnesium stearate do not interfere. The statistical analysis of the results obtained by the proposed and the BP methods revealed that both procedures are equally accurate and precise (Table 2).

The chemistry of the colour formation may be suggested on the basis of a previously reported mechanism¹³. The nitro compound (nitrazepam in this case) seems to be reduced under the proposed reaction conditions to the corresponding hydroxylamine derivative which interacts further with SCAF producing the chromogen.

Table 1. Determination of Nitrazepam Powder by the proposed method

Amount taken $\mu\text{g}/25 \text{ ml}$	Recovery, %
25	98.4
75	99.3
125	100.2
200	98.7
250	100.1
325	99.2
400	98.1
450	99.6
500	98.9

Mean $\pm$ confidence limit (P = 0.05) 99.1 ± 1.06

Table 2. Determination of Nitrazepam in tablets by the Proposed Method and B.P. Method

Sample ^a	Found, $\bar{x}\% \pm \text{SD}$	Proposed method		B.P. method
		Nitrazepam added mg	Recovery, $\bar{x}\% \pm \text{SD}$	
A	98.94 ± 1.05 $t=1.69$ $F=2.96$	5	99.72 ± 0.63	99.34 ± 0.61
B	99.02 ± 1.23 $t=1.65$ $F=2.69$	10	98.69 ± 0.72	98.99 ± 0.75
C	99.21 ± 0.95 $t=1.36$ $F=2.89$	15	99.87 ± 0.75	99.62 ± 0.56

x - Average of 10 determinations.

Theoretical Value $t=2.26$ at the 95% Confidence Level

Theoretical Value $F=3.18$ at the 95% Confidence Level

a - Different batches of Mogadon tablets: each tablet contains 5 mg 1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-one. Made in Switzerland by F. Hoffmann-La Roche.

Spectrophotometric Determination of Nitrazepam in Tablets.

Fig. 1 . Absorption spectrum for the reaction coloured product of reduced nitrazepam (250 μ g 25 ml) with SCAF reagent.

REFERENCES

- 1) O.L. Grom, N.S. Komar and N.S. Ladyzhinshaya, *Pharm. Zh. (Kiev)*, 1976, 31, 82.
- 2) H. Raber and J. Gruder, *Sci., Pharm*, 1972, 40, 35.
- 3) N.M. Sanghavi and N.G. Jivani, *Talanta*, 1979, 26, 63.
- 4) Y.A. Beltagy, A.S. Issa and M.S. Mahrous, *Egypt. J. Pharm. Sci.*, 1978, 19, 115.
- 5) K.M. Jensen, *J. Chromatog.*, 1975, 111, 389.
- 6) H. Ehrsson and A Tilly, *Anal. Letters*. 1973, 6, 197.
- 7) P. Haefelfinger, *J. of high Resolution Chrom. and Chrom. Comm.*, 1979, 1, 39.
- 8) H.B. Hanekamp, W.H.Voogt, P. Bos and R.W. Frei, *J. Liqui. Chrom.*, 1980, 3, 1205.
- 9) J. Reider, *Arzneimittel-Forsch.*, 1973, 23, 207.
- 10) P. Van Doorne, *Pharm. Weekblad*, 1975, 110, 149.
- 11) S. Halvorsen and E. Jacobson, *Anal. Chem. Acta.*, 1972, 59, 127.
- 12) *British Pharmacopoeia*, 1980, pp305,793, HMSO, London.
- 13) F. Feigl, "Spot Tests in Organic Analysis", 7th Ed., pp. 290,297-299. Elsevier, Amsterdam, 1966.
- 14) A.I. Vogel, "A Text Book of Quantitative Inorganic Analysis", 3rd Ed., p. 727. Longman London, UK, 1975.

طريقة طيفية لتعيين النيترازيبام فى الاقراص

سلوى رزق الشـابورى

قسم الكيمياء الصيدلية - كلية الصيدلة - جامعة اسيوط

استحدثت فى هذا البحث طريقة حساسة لتعيين النيترازيبام اما فى حالته النقية او فى الاقراص .

وفى هذه الطريقة يعتمد على اختزال النيترازيبام بمسحوق الزنك فى وجود كلوريد الكالسيوم وبعد ذلك يتفاعل ناتج الاختزال مع ملح الصوديوم الثلاثى لخماسى سيانوامينو الحديدات . وهذا التفاعل يعطى لون بنفسجى له درجة امتصاص قوى عند ٥٦٠ نـم ويتبع قانون بيير فى درجة تركيز مـم ٢٥ - ٢٥٠ مـم / مـم وقد وجد ان المواد المضافة فى الاقراص لا تتداخل فى طريقة التعيين ووجد ايضا ان معدل الاستعادة والدقة لتعيين النيترازيبام بالطريقة المقترحة متطابق مع طريقة الدستور البريطانى .

received in 7/11/1985 & accepted in 4/6/1986