P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2010,(4), (1) :40-45

Spectrophotometric Determination of Nitrazepam in Pharmaceutical Tablets Using Flow Injection Analysis

Raghad Sinan Abdulsattar

University of Baghdad - College of Science.

ARTICLE INFO

Received: 3 / 11 /2009
Accepted: 17 / 3 /2010
Available online: 14/6/2012
DOI: 10.37652/juaps.2010.43885

Keywords:
Nitrazepam,
Promethazine hydrochloride,
Pharmaceutical tablets,
Flow injection analysis.

ABSTRACT

Nitrazepam (NZP) was determined spectrophotometrically in the pure form and in the pharmaceutical tablets using flow injection analysis (FIA). The method was based on oxidative coupling organic reaction of reduced NZP with promethazine hydrochloride in the presence of sodium periodate to give a green solution having an absorbance maximum at 613 nm. The various chemical and physical variables were optimized. The calibration graph of NZP is linear from 1 to 50 µg mL-1 with detection limit (S/N = 3) was 0.9284 µg mL-1. The method was successfully applied to the analysis of NZP in pharmaceutical tablets. The results obtained by applying the proposed FIA method were in good agreement with those obtained by British Pharmacopoeia method at the 95% confidence.

Introduction

Nitrazepam (NZP) is a yellow, crystalline powder, practically insoluble in water, slightly soluble in alcohol and in ether. It is chemically known as 7-nitro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one, C15H11N3O3[1].

NZP is an anticonvulsant and sedative-hypnotic drug. It is capable of inhibiting the development and spread of epileptiform electrical activity in the central nervous system[2].

Various methods for the determination of NZP in biological and pharmaceutical samples have been reported in the literature including derivative ultraviolet spectrometry[3], high performance liquid chromatography (HPLC)-electrospray tandem mass spectrometry[4], capillary electrophoresis[5], titrimetry -multiwavelength spectrophotometry[6], micellar electrokinetic capillary chromatography[7], thin layer chromatography-densitometry[8], gas chromatography-mass spectrometry[9], HPLC-voltammetry[10], and spectrophotometry[11-15].

A several of flow-injection (FI) methods have also been reported for the determination of NZP, such as FI-voltammetry[16], and FI-fluorimetry[17]. However, the control of such reactions and / or manifolds is still complicated.

The BP recommends a spectrophotometric method for NZP tablets at 280 nm[1].

Most of spectrophotometric methods for determination NZP are time consuming and require heating. In most of these methods, absorbance measurements for both samples and standards must be done either at a constant, fixed time after addition of the colorimetric reagent or waiting for the reaction to proceed to completion in order to attain the required reproducibility.

In this work, the possibility of using flow injection analysis (FIA) to overcome these difficulties was attempted. In FIA, reaction completion is not necessary because measurements for all samples and standards are subjected to the same timing sequence in a precise, automatic manner. FIA technique has found recently wide applications mainly due to reduction of the analysis time and reagent consumption compared with conventional manual procedures.

In this paper, FI method using spectrophotometric detection at 613 nm is described for the determination of NZP. The batch method[14] was adopted as a basis to developed FIA method. The method is based on oxidative coupling reaction of reduced NZP with promethazine hydrochloride in the presence of sodium periodate to form a green solution. The FI method has been successfully applied to the determination of NZP in pharmaceutical tablets.

* Corresponding author at: University of Baghdad - College of Science, Iraq.E-mail address: raghadsinan@yahoo.com

Experimental

Apparatus

A Shimadzu UV-VIS 260 digital double-beam recording spectrophotometer (Kyoto, Japan) was used for all spectral and absorbance measurements with matched 1-cm quartz cells.

The FI system comprised a peristaltic pump (Ismatec, Labortechnik-Analytic, CH-8152, Glatbrugg-Zurich, Switzerland, six channels) with polyvinyl chloride flow tubes of 0.8 mm i.d., an injection valve (Rheodyne, Altex 210, Supelco-USA), a 50 µL flow cells and a Shimadzu UV-VIS 260 spectrophotometer (Tokyo, Japan) as the detector. Flexible Teflon tubes of 0.5 mm i.d. were used for reaction coils and to transport the reagents solutions. T-link was also used to mix two streams of reagents.

Chemicals and reagents

Chemicals and reagents of analytical grade used in present study. The standard material of NZP was provided from the State Company for Drug Industries and Medical Appliances (SDI), Samarra-Iraq.

Pharmaceutical tablets

Pharmaceutical tablets were obtained from commercial sources.

Mogam Tablets: 5 mg Nitrazepam for each tablet (Domina Pharmaceuticals, Damascus-Syria).

Solutions

Nitrazepam (NZP) reduction solution (500 µg mL-1)

This was prepared by dissolving 0.0500 g of NZP in ethanol. It was transferred into 50 mL volumetric flask, and diluted to the mark with the same solvent. The solution was transferred into beaker of 125 mL. A 20 mL of distilled water, 20 mL of hydrochloric acid (11.64 N), and 3 g of zinc powder were added. The beaker was allowed to stand for 15 min at room temperature (25 ºC), then the solution was filtered into 100 mL volumetric flask, washed the residues with distilled water, and finally the volume was diluted to the mark with distilled water to obtain 500 µg mL-1 of NZP reduction solution[13]. More dilute solution was prepared daily by appropriate dilution using distilled water.

Promethazine hydrochloride (PMH) solution (15 mM)

This was freshly prepared by dissolving 0.4809 g of PMH and diluting to 100 mL with distilled water in volumetric flask.

Sodium periodate (SPI) solution (40 mM)

This was prepared by dissolving 0.8556 g of SPI and diluting to 100 mL with distilled water in volumetric flask.

Solutions of pharmaceutical tablets Tablets samples: Twenty tablets were accurately weighted and finely powdered. An amount of the powder equivalent to 50 mg of NZP was dissolved in 30 mL of ethanol. The solution was filtered into a 50 mL volumetric flask, the residue was washed with ethanol and finally the volume was diluted to the marked with the same solvent to obtain 1000 µg mL-1 of NZP. This solution was transferred into 125 mL beaker and was reduced as previously described. Further appropriate solutions of pharmaceutical tablets were made using distilled water.

Recommended procedure for calibration FI-procedure The FI system is shown in Figure (1). 200 µL aliquots of drug solutions prepared at different concentrations (1 – 50 µg mL-1) were injected into carrier stream which produced from mixing of two channels. The first channel was used to transport PMH solution of 15 mM and second channel was used to transport SPI solution 40 mM. The total flow rate of the two channels was 1.6 mL min-1. The reaction was carried out by passing the solution through a reaction coil (100 cm) and the absorbance of the resulting green color product was measured at 613 nm. Calibration graph of NZP was prepared by plotting the absorbances of the peak maximum versus drug concentrations.

Results and Discussion

Preliminary studies

Preliminary experiments under continuous-flow conditions were carried out to test the manifold configurations and the approximate ranges of the tested parameters. The design of the manifold selected is shown in Figure (1) using total flow rate of 1.6 mL min-1 for two-channels. This design of the manifold gave the maximum absorbance. Therefore, a two-channel FI assembly was adopted, in which the sample (200 µL) was injected into the carrier stream, which was formed from mixing two carrier streams (R1 and R2). The reaction was carried out by passing the solution through a reaction coil (100 cm) and the absorbance of the resulting green color product was measured at 613 nm. The presence of the drug caused an increase in the absorbance, which was proportional to its concentration.

Optimization of the experimental conditions

The effect of various variables on the color development was studied to establish the optimum conditions for the determination of NZP by FI method.

The effect of the concentration of SPI was studied in the range 15 – 50 mM with fixed NZP concentration of 50 µg mL-1. As can be observed from Figure (2) the absorbance was increased as the concentration of SPI was increased up to 40 mM, thus 40 mM SPI was found to be the most suitable concentration for a maximum absorbance and was chosen for further use.

It was found that the reaction between NZP and PMH in the presence of SPI depends on the PMH concentration. Therefore, the effect of different concentrations of PMH (2.5 – 30 mM) was studied [Figure (2)]. The result obtained indicated, that the absorbance increased with the increasing concentration of MPH up to 15 mM, thus a concentration of 15 mM gave the maximum absorbance and was chosen for further use.

The use of FI as an alternative to existing methods for NZP determination is dependent on optimization of the system to achieve maximum absorbance. As a consequence, several experiments were conducted in order to establish the best experimental conditions for operating the FI manifold.

Figure (3) shows the effects of flow rate, reactor length and sample injection volume on the absorbance. The effect of flow rate on the absorbance was studied over the range 0.65 – 4.8 mL min-1. Figure (3) shows that, with increasing flow rate, maximum sensitivity was obtained at 1.6 mL min-1, which was selected, as a compromise between reproducibility and sampling rate. Above this value, the absorbance decreased slightly owing to dispersion effects.

The effect of reactor length was studied in the range 25 – 250 cm in the same experimental conditions selected above. As can be seen from Figure (3), maximum absorbance value was obtained at 100 cm and was selected for further use.

The volume of sample injected was varied in the range 50 – 250 µL by changing the length of the sample loop in the injection valve, while the other variables were held constant. The absorbance increased with increasing volume of sample injected [Figure (3)]. Best sensitivity was obtained by using 200 µL as a volume of sample injected, which was selected.

The flow system selected provided a sampling rate of 72 samples h-1.

Analytical characteristics of FI spectrophotometric method

For FI method, the calibration graph for NZP was obtained by the procedure described previously in which a series of standard solutions were analyzed in triplicates to test the linearity. The slope (a), the intercept (b), the correlation coefficient (r) and the correlation of determination (r2) were evaluated by a least-squares regression analysis and are included in Table (1). The obtained r value is highly significant.

Statistical evaluation[18] of the regression line gave the values of standard deviations for residuals (Sy/x), slope (Sa), intercept (Sb) at 95% confidence and limit of detection (LOD) are shown in the same Table. These small figures point out to the high precision of the proposed method.

Accuracy and precision of FI spectrophotometric method

The accuracy and precision of the proposed method was tested by analyzing of three different concentrations of NZP for five replicate. The values of the percentage errors (E%) and percentage relative standard deviation (RSD%) are summarized in Table (2). These values indicate the high accuracy and precision of the proposed method.

Pharmaceutical applications

In order to demonstrate the applicability of the proposed method for the determination of NZP, the method was successfully applied to the analysis of NZP in pharmaceutical tablets. The results are summarized in Table (3). When pharmaceutical tablets of NZP were analyzed by the proposed method, interference from the sample matrix caused no problem. For pharmaceutical tablets of NZP examined, the assay results of proposed method were in good agreements with the declared contents. In Mogam tablets, quantitative recoveries between 99.220 and 99.605% were obtained [Table (3)].

Evaluation of the proposed method

For evaluating the competence and the success of the proposed method, the results obtained were compared with those obtained by standard BP method[1].

The same pharmaceutical tablets for NZP were analyzed by standard BP method. The results obtained by the two different methods [Table (4)] were

statistically compared, using the Student t-test and variance ratio F-test at 95% confidence level[18]. In all cases, the calculated t- and F-values [Table (4)] did not exceed the theoretical values, which indicate that there is no significant difference between either methods in accuracy and precision in the determination of NZP in pharmaceutical tablets.

The FI spectrophotometric method proposed for the determination of NZP in pure and pharmaceutical tablets has the advantages of simplicity, speed, accuracy and the use of inexpensive equipment.

The speed of analysis and the precision render this method are also suitable for the quality control of formulations containing this drug.

The proposed method advantages over the standard BP method (spectrophotometric method) are more selective, as they depend on the presence of the nitro group, and less prone to interferents, which are normally encountered in single wavelength UV measurements.

The proposed method was applied to analysis of NZP in tablets solutions, suggesting that it is used as a reliable and advantageous alternative to the other previously exported methods for routine analysis of NZP in these samples. There is no significant difference between the proposed method with respect to precision and accuracy.

References

1. "British Pharmacopoeia", (2001). 303, 832, The Stationery Office, London.
2. B. G. Katzung, (2007). "Basic and Clinical Pharmacology". 475, 10th Ed., The McGraw-Hill Companies, Inc., USA.
3. A. M. Rubio, J. V. J. Ortiz, A. Salvador, and M. D. L. Guardia, (1994). Hydrolysis of benzodiazepines in a microwave oven and ultraviolet derivative analysis of their benzophenones. Microchem. J., 49 (1): 12-19.
4. M. Kleinschnitz, M. Herderich, and P. Schreier, (1996). Determination of 1,4-benzodiazepines by high-performance liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. B, 676 (1): 61-67.
5. M. Tomita, and T. Okuyama, (1996). Application of capillary electrophoresis to the simultaneous screening and quantitation of benzodiazepines. J. Chromatogr. B, 678 (2): 331-337.
6. R. I. Allen, K. J. Box, J. E. A. Comer, C. Peake, and K. Y. Tam, (1998). Multiwavelength spectrophotometric determination of acid dissociation constants of ionizable drugs. J. Pharm. Biomed. Anal., 17 (4-5): 699-712.
7. M. E. C. Peiró, D. Bose, A. M. Domínguez, M. G. Agustí, and J. E. Romero, (2002). Direct injection micellar liquid chromatographic determination of benzodiazepines in serum. J. Chromatogr. B, 780 (2): 241-249.
8. M. Bakavoli, and M. Kaykhaii, (2003). Quantitative determination of diazepam, nitrazepam and flunitrazepam in tablets using thin-layer chromatography-densitometry technique. J. Pharm. Biomed. Anal., 31 (6): 1185-1189.
9. T. Gunnar, K. Ariniemi, and P. Lillsunde, (2005). Determination of 14 benzodiazepines and hydroxyl metabolites, zaleplon and zolpidem as tert-butyldimethylsilyl derivatives compared with other common silylating reagents in whole blood by gas chromatography-mass spectrometry. J. Chromatogr. B, 818(2): 175-189.
10. K. C. Honeychurch, G. C. Smith, and J. P. Hart, (2006). Voltammetric behavior of nitrazepam and its determination in serum using liquid chromatography with redox mode dual-electrode detection. Anal. Chem., 78 (2): 416-423.
11. S. R. El-Shabouri, (1986). Spectrophotometric determination of nitrazepam in tablets. Talanta, 33 (9): 743-744.
12. M. I. Walash, M. Rizk, and A. El-Brashy, (1988). Spectrophotometric determination of chlordiazepoxide and nitrazepam. Talanta, 35 (11): 895-898.
13. S. M. Hassan, F. Belal, M. S. El-Din, and M. Sultan, (1988). Spectrophotometric determination of some pharmaceutically important nitro compounds in their dosage forms. Analyst, 113 (7): 1087-1089.
14. R. S. Abdul Satar, (2009). Spectrophotometric determination of nitrazepam in pharmaceutical tablets. Iraqi J. Biotech, 8 (1): 252-262.
15. R. Sinan, and M. Q. Al-Abachi, (2009). Spectrophotometric determination of nitrazepam in pharmaceutical tablets by oxidative coupling reaction with pyrocatechol. J. of University of Anbar for Pure Science, 3 (3): 6-12.
16. E. Ruiz, M. H. Blanco, E. L. Abad, and L. Hernández, (1987). Determination of nitrazepam

and flunitrazepam by flow injection analysis using a voltammetric detector. Analyst, 112 (5): 697-699.

17. J. Dolejs'ova´, P. Solich, Ch. K. Polydorou, M. A. Koupparis, and C. E. Efstathiou, (1999). Flow-injection fluorimetric determination of 1,4-benzodiazepines in pharmaceutical formulations after acid hydrolysis. J. Pharm. Biomed. Anal., 20 (1-2): 357-362.
18. J. N. Miller and J. C. Miller, (2001). "Statistics and Chemometrics for Analytical Chemistry". 116, 117, 121, 47, 52, 4th Ed., Pearson Education Limited, London.

Table (2): Accuracy and precision of the proposed method

Concn. of NZP, µg mL^{-1}		E%	Rec.%	RSD%
Present	Found			
5.000	4.971	– 0.580	99.420	1.066
20.000	20.059	+ 0.295	100.295	0.521
40.000	40.039	+ 0.098	100.098	0.221

Table (3): Application of the proposed method for determination of NZP in pharmaceutical tablets

Pharmaceutical tablets	Concn. of NZP, µg mL^{-1}		E%	Rec.%	RSD %
	Present	Found *			
Mogam Tablets	5.000	4.961	– 0.780	99.220	1.386
	20.000	19.921	– 0.395	99.605	0.697
	40.000	39.822	– 0.445	99.555	0.323

* Average of five determinations.

Table (4): The comparison of the proposed FI method with standard BP method using t- and F-statistical tests

Pharmaceutical tablets	FI method		BP method		s	Value	
	Rec.% * $(x_i)_1$	$(x_i-\bar{x})^2_1$	Rec.% * $(x_i)_2$	$(x_i-\bar{x})^2_2$		T (theor.)	F (theor.)
NZP pure	100.000	0.0729	100.000	0.0576	0.361	0.083 (4.303)	1.266 (161.4)
Mogam Tablets	99.460	0.0729	99.520	0.0576			
	$\bar{X}_1$ = 99.730	Σ = 0.1458	$\bar{X}_2$ = 99.760	Σ = 0.1152	$(n_1 + n_2 - 2) = 2$	$(n_1 - 1) = 1$ $(n_2 - 1) = 1$	

* Average of five determinations.

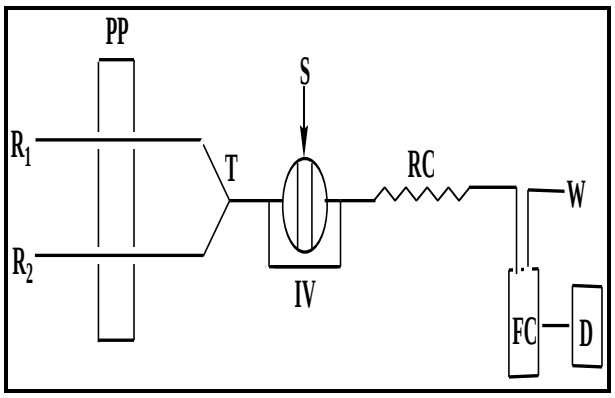

Figure (1): FI manifold for determination of NZP (R_1 = PMH, R_2 = SPI, S = Sample injection, PP = Peristaltic pump, IV = Injection valve, T = T-link, RC = Reaction coil, FC = Flow cell, D = Detector and W = Waste)

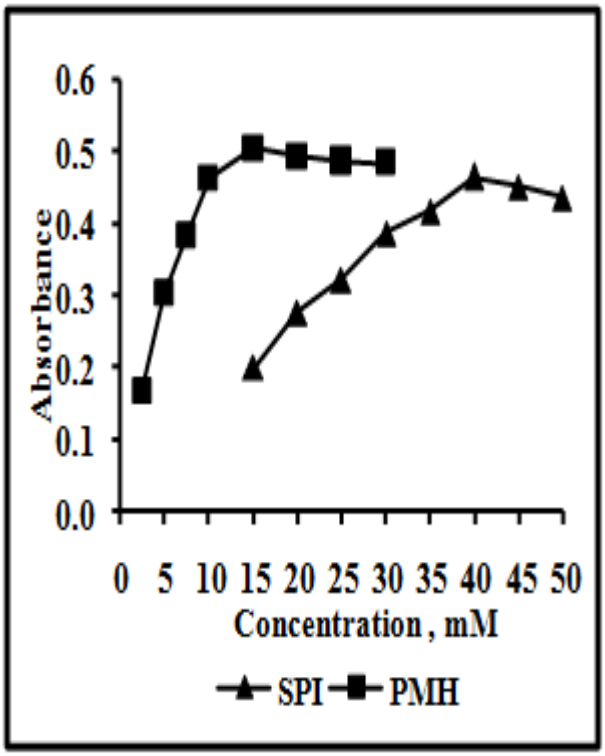

Figure (2): Chemical conditions of FI procedure for determination of NZP

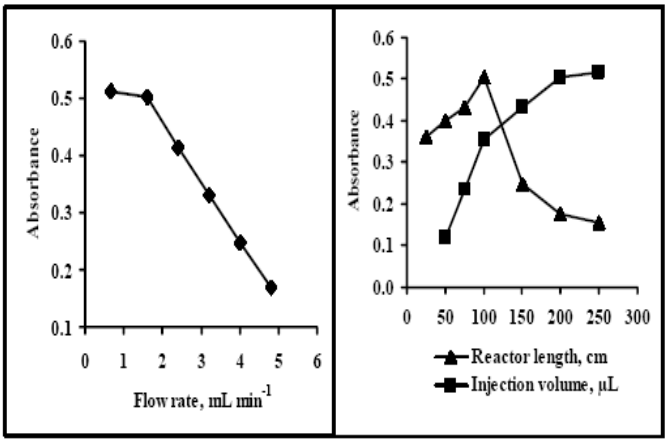

Figure (3): Physical conditions of FI procedure for determination of NZP

التقدير الطيفي لنايترازيبام في الأقراص الصيدلانية باستخدام تحليل الحقن الجرياني

رغد سنان عبد الستار

E-mail: raghadsinan@yahoo.com

الخلاصة

تم تقدير النايترازيبام طيفياً في الشكل النقي و في الأقراص الصيدلانية باستخدام تحليل الحقن الجرياني. تعتمد الطريقة على تفاعل الازدواج التأكسدي العضوي لدواء النايترازيبام المختزل (بوساطة مسحوق الخارصين وحامض الهيدروكلوريك) مع هيدروكلوريد البروميثازين بوجود بيريودات الصوديوم، إذ يتكون ناتج أخضر يمتلك أقصى امتصاصية عند طول موجي 613 نانومتراً. تم تثبيت المتغيرات الكيميائية والفيزيائية للحصول على أفضل حساسية و تطابقية للنتائج. تم الحصول على منحني معايرة خطي للنايترازيبام من 1 – 50 مكغم مل-1 مع قيمة حد كشف 0.9284 مكغم مل-1. طبقت الطريقة بنجاح في تقدير النايترازيبام في الأقراص الصيدلانية و كانت النتائج المستحصلة متوافقة مع النتائج المستحصلة من الطريقة القياسية المعتمدة من قبل دستور الأدوية البريطاني و عند مستوى ثقة 95 %.