



FACILE SPECTROPHOTOMETRIC DETERMINATION OF NIMODIPINE AND NITRAZEPAM IN PHARMACEUTICAL PREPARATIONS

H.D. Revanasiddappa *, H.N. Deepakumari, S.M. Mallegowda and K.B. Vinay

abstract: A simple, accurate and sensitive visible spectrophotometric method for the determination of nimodipine (NMD) and nitrazepam (NTZ) is described. The method is based on the diazotization of reduced drugs with nitrous acid followed by coupling with resorcinol to form colored azo-dye in an alkaline medium. The resulting colored azo-dye exhibits maximum absorption peak at 480 nm for both NTZ and NMD. Beer's law was obeyed over the concentration ranges 0.5-10 and 1.0-40.0 $\mu\text{g/mL}$ for NTZ and NMD, respectively. The calculated molar absorptivities are 1.84×10^4 and $0.67 \times 10^4 \text{ L mol}^{-1}\text{cm}^{-1}$ for NTZ and NMD, respectively. All the variables such as effect of acid, alkali and reaction time were studied in order to optimize the reaction conditions. No interference from the excipients added to tablets was found. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed method and the literature method. The accuracy and validity of the methods were further established by recovery studies through the standard addition technique.

key words: Nitrazepam; nimodipine; spectrophotometry; diazotization; pharmaceutical formulation.

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1. Introduction

Nimodipine (NMD), chemically known as 3-(2-methoxyethyl) 5-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Fig. 1 a) is a dihydropyridine calcium channel blocker and was originally developed for the treatment of high blood pressure. Nitrazepam (NTZ), is a hypnotic drug used in the treatment of insomnia which has sedative and motor impairing properties [1], as well as anxiolytic, amnesic, anticonvulsant and skeletal muscle relaxant properties. NTZ, chemically known as 9-nitro-6-phenyl-2, 5-diazabicyclo [5.4.0] undeca-5, 8, 10, 12-tetraen-3-one (Fig. 1 b) and it was a member of 1, 4-benzodiazepine class of tranquilizers [2]. In brain, NTZ acts on benzodiazepine receptors, which are associated with Gamma amino butyric acid (GABA) receptor [3].

Several techniques have been utilized for the assay of NMD and NTZ in body fluids and pharmaceuticals. HPLC has been employed for the determination of drugs in plasma [4-7]

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and urine [8]. LC-MS [9], UPLC-MS [10] and spectrofluorimetric [11] methods have also been utilized to determine NMD and NTZ in human plasma. Methods based on various techniques such as polarography [12-13], complexometry [14], GC with electron capture detection [15] and spectrophotometry [16-21] have already been used for the estimation of the studied drugs in pharmaceutical samples. Chromatographic techniques involve expensive experimental set ups and the reported spectrophotometric methods suffer from several limitations such as tedious procedure and the use of costly reagents. Considering these demerits, there was a need to develop more advantageous spectrophotometric method for the determination of NMD and NTZ in pure sample and in tablets.

The present investigation reports the development of simple, accurate, sensitive and economically viable procedure for the estimation of NMD and NTZ in both pure and in pharmaceutical preparations based on the diazo-coupling reaction.

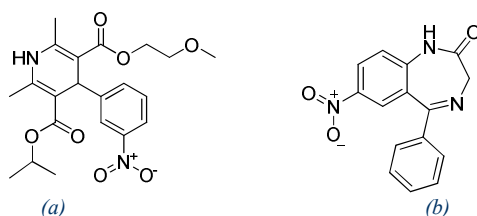


Fig. 1 Structures of NMD (a) and NTZ (b).

2. Experimental details

2.1. Apparatus

All absorbance measurements were performed using a Systronics Model 166 digital spectrophotometer provided with 1-cm matched quartz cells.

2.2. Reagents and standards

Analytical grade chemicals were used. Sodium nitrite, hydrochloric acid and sodium hydroxide were obtained from E. Merck, sulfamic acid (Qualigens) and resorcinol (BDH chemicals, Poole, England). Double distilled water was used for dilution and preparation of all reagents. Nitrazepam pure drug was obtained from Cipla, Ltd., Mumbai India and nimodipine from Cadila Pharmaceuticals Ltd. Ahmedabad, India. Nitrovet (Anglo French) and Nmodip [USV (Corvette)] tablets were purchased from the market.

2.3. Working standard solutions of NTZ and NMD

Accurately 20 mg each of NTZ and NMD (Cipla, Ltd., Mumbai India and Cadila Pharmaceuticals Ltd. Ahmedabad, India as a gift sample and used as received.) was weighed into a separate 100 mL beakers and dissolved NTZ in 5 mL acetone and NMD in 5 mL methanol. To this, 5 mL 4 N hydrochloric acid and 1 g of zinc dust were added and shaken thoroughly for about 15 min and then diluted up to the mark with water in a 100 mL calibrated flasks (200 µg/mL of both NTZ and NMD), and filter through Whatman No.41 filter paper. Working solutions were prepared as required by dilution.

2.4. Standard procedure

Aliquots of standard drug solution in the range 0.0, 0.5, 1.0....2.5 mL of 40 µg/mL NTZ and 0.0, 1.0, 1.5, 2.0,.....4 mL of 100 µg/mL NMD were transferred into a series of separate 10 mL calibrated flasks. To each flask, 1 mL each of sodium nitrite (0.1 % w/v) and 1 M hydrochloric acid were added. After 3 min, 0.5 mL of sulfamic acid (3 % w/v) was added. Then, volumes of 1.5 mL resorcinol (1 % w/v) and 2 mL 4 M sodium hydroxide were added. The contents were made up to the mark with distilled water and mixed well. The absorbance of the colored azo-dye was measured at 480 nm after 5 min for both the drugs against the corresponding reagent blank prepared similarly omitting the drug content.

2.5. Procedure for tablets

Ten tablets were weighed accurately and ground into fine powder. A quantity of the powder equivalent to 20 mg of NTZ or NMD was weighed accurately into a separate 100 mL calibrated flasks. 5 mL acetone was used for NTZ, and 5 mL methanol for NMD was added. Then, 5 mL 4 N hydrochloric acid and 1 g of zinc dust were also added into 100 mL calibrated flasks and shaken thoroughly for about 30 min to extract the drug completely. Then, the volume was diluted to the mark with water and mixed well and filtered using a Whatman No.41 filter paper. The filtrate containing 200 µg/mL of both NTZ and NMD, and the appropriate dilute solution of each drug was subjected to analysis by the procedure described above.

3. Results and discussion

3.1. Absorption spectra and Reaction scheme

In the method developed the presence of the aromatic amino group in the reduced NTZ and NMD, enable the use of diazotization of the drug with nitrous acid and coupling the resulting diazonium salt with resorcinol to form colored azo-dye with a maximum absorption at 480 nm for both drugs. The absorption spectra of the above dye are presented in Fig. 2. Two steps are involved in the reaction that produces the colored dye. In the first step, reduced NMD or NTZ is treated with nitrite solution in hydrochloric acid medium, undergoes diazotization to give diazonium ion. In the second step, the diazonium ion is coupled with the coupling agent resorcinol, to form orange-red colored azo-dye in an alkaline medium for both NTZ and NMD. The proposed chemical reactions are shown in Scheme 1.

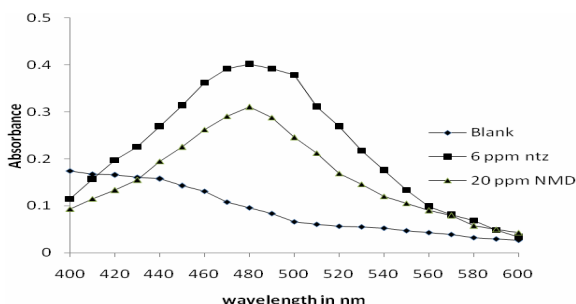
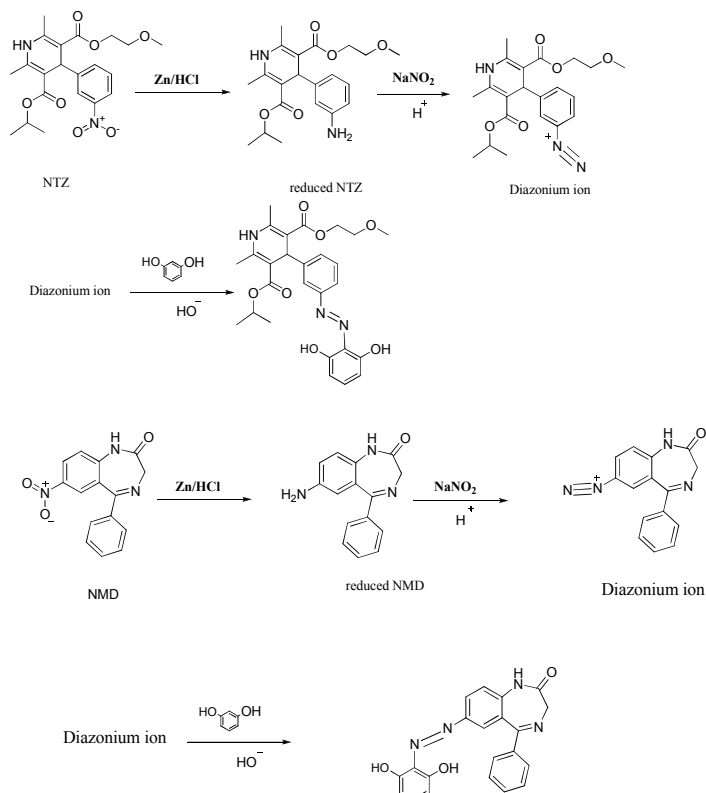


Fig. 2 Absorption spectra for NTZ and NMD.



Scheme 1 Proposed reaction scheme.

3.2. Optimization of parameters

The various experimental parameters, which influence the formation of the colored dye, were optimized.

3.2.1. Effect of reagents

In order to study the effect of concentration of resorcinol, a fixed concentration of NTZ and NMD (5 $\mu\text{g/mL}$) was taken in a series of 10 mL calibrated flasks, and to that 1-5 mL of resorcinol (1% w/v) was added and studied by measuring the absorbance at specified wavelengths in the standard procedure. Optimization is done by varying one parameter, keeping other constant. A volume of 1.5 mL resorcinol (1 %) in a total volume of 10 mL was found to be sufficient for both the drugs, leading to maximum color stability of the azo-dye.

The constant absorbance readings were obtained in the range 0.5 - 3 mL of 0.1 % sodium nitrite. Thus, 1 mL of sodium nitrite solution was used (both drugs) in a total volume of 10 mL of reaction mixture. The excess of nitrite could be removed by the addition of 0.5 mL of 3 % sulfamic acid.

3.2.2. Effect of acid concentration

Diazotization was carried out at room temperature and cooling to 0-5 °C was not necessary. The hydrochloric acid concentration was studied and 0.1 M hydrochloric acid concentration was fixed for getting a stable diazonium ion at room temperature (27 ± 3 °C).

3.2.3. Effects of alkali

The optimum concentration of sodium hydroxide leading to a maximum intensity of the azo-dye was found to be 2 mL of 4 M i.e. 0.8 M in the final solution. Higher concentrations of alkali may lead to partial decolorization of the dye. In the developed method, the orange-red colored azo-dye formed was stable and gave maximum absorbance values when reaction mixture with 0.8 M sodium hydroxide.

3.2.4. Effect of reaction time

The colored azo-dye developed rapidly after addition of the reagents and attained maximum intensity after about 5 min at room temperature. The formed azo-dye was stable for a period of more than 1 h for both NTZ and NMD.

3.3. Method validation

3.3.1. Linearity, detection and quantitation limit

Under the optimized experimental conditions a linear relationship was observed between the absorbance and concentration of drugs from 2.0 - 35 and 0.5 - 8.0 µg/mL for NMD and NTZ, respectively. The calibration graph is described by the equation: $Y = a + bx$, where y = absorbance, a = intercept, b = slope and x = concentration, obtained by the method of least squares. The correlation coefficient (r), intercept (a) and slope (b) for the calibration data and sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection and quantitation calculated as per the current ICH [22] guidelines are compiled in Table 1.

Table 1 Optical characteristics and precision.

Parameter	NMD	NTZ
$\lambda_{\max}$, nm	480	480
Linear range (µg/mL)	2.0 - 35	0.5 - 8.0
Molar absorptivity (ϵ), ($L \text{ mol}^{-1} \text{ cm}^{-1}$)	0.67×10^4	1.84×10^4
Sandell sensitivity ($\mu\text{g cm}^{-2}$)	0.0622	0.0153
Intercept (a)	-0.0141	-0.0187
Slope (b)	0.0172	0.0716
correlation coefficient (r)	0.999	0.999
S_a	0.0623	0.0751
S_b	0.0019	0.0119
LOQ(µg/mL)	0.1169	0.0620
LOD(µg/mL)	0.0386	0.0205

* $y = bc + a$ where c is the concentration of drug in µg/mL and y is the absorbance at the respective $\lambda_{\max}$, S_a is the standard deviation of intercept, S_b is the standard deviation of slope.

3.3.2. Accuracy and precision

The intra-day precision and accuracy of the methods developed were evaluated by replicate analysis of drug samples at three different concentrations (low, medium and high) (Table 2) within the working limits, each being repeated five times. The RE (%) and RSD (%) values of intra-day studies were satisfactory and showed that the best appraisal of the procedures in daily use. The analytical results obtained from this investigation are summarized in Table 2 and showed the high accuracy of the methods.

Table 2 Evaluation of accuracy and precision (within-day).

DRUG STUDIED	DRUG added, $\mu\text{g/mL}$	DRUG found*, $\mu\text{g/mL}$	RE %	SD $\mu\text{g/mL}$	SEM $\mu\text{g/mL}$	RSD %	ROE** %
NMD	10	9.934	0.66	0.026	0.010	0.258	± 0.258
	20	19.992	0.04	0.085	0.032	0.427	± 0.426
	30	29.876	0.41	0.044	0.017	0.146	± 0.146
NTZ	2	1.993	0.34	0.009	0.003	0.462	± 0.461
	6	5.923	0.71	0.078	0.029	1.304	± 1.303
	8	7.991	0.11	0.019	0.007	0.243	± 0.242

RE: Relative error; SD: Standard deviation; SEM: Standard error of mean; RSD: Relative standard deviation; ROE: Range of error, * Mean value of five determinations, **At the 95% confidence level for 4 degrees of freedom.

3.4. Application to analysis of pharmaceutical samples

To check the validity of the proposed method according to the ICH guidelines [22], NTZ and NMD was determined in some pharmaceutical formulations. The results obtained by the proposed methods are in close agreement with the results obtained by the reference methods [17,21]. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed method and the reference methods [17,21] at the 95% confidence level with respect to accuracy and precision (Table 3). The calculated t- and F- values (Table 3) did not exceed the tabulated values ($t = 2.77$ and $F = 6.39$).

3.4.1. Principle of reference methods

For NTZ, the reference method [17] was used for comparison. In this method ethyl acetoacetate reagent was used as a coupling agent. In another method [21], *N*-(1-naphthyl)ethylene diamine dihydrochloride reagent was used as a coupling agent and the same was utilized for comparison with NMD.

Table 3 Results of the determination of NMD and NTZ in tablets and statistical comparison with the reference method.

Tablet studied	Nominal amount, mg/tab	Found**(% of nominal amount $\pm$ SD)		
		Reference methods [17,21]	NTZ	NMD
Nimodip ^a	30 mg	99.2 $\pm$ 0.18	–	99.98 $\pm$ 0.38 $t=2.22$, $F=4.47$
Nitravet ^b	5 mg	101.1 $\pm$ 1.3	100.34 $\pm$ 0.53 $t=2.58$, $F=6.09$	–

a- Marketed by: [USV (Corvette)], b-(Anglo French); **Mean value of five determinations Tabulated t and F-values at 95 % confidence level are 2.77 and 6.39, respectively.

3.5. Recovery study

The accuracy and precision of the proposed method were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure drug at three different concentrations and the total was found by the proposed method. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that frequently encountered common ingredients of formulations were found not to interfere. The results of recovery study are compiled in Table 4.

Table 4 Results of recovery by standard-addition method.

Tablet studied	Labeled amount, mg/tab	Tablet added $\mu\text{g/mL}$	Pure drug added, $\mu\text{g/mL}$	Total found* $\mu\text{g/mL}$	% Recovery $\pm$ S.D
Nimodip [USV (Corvette)]	30	10	5	14.98	99.73 $\pm$ 0.67
		10	10	19.96	99.69 $\pm$ 0.23
		10	15	25.08	100.54 $\pm$ 0.24
		4	2	6.02	100.87 $\pm$ 0.75
Nitrovet (Anglo French)	5	4	4	7.96	99.07 $\pm$ 0.61
		4	6	10.06	101.08 $\pm$ 0.22

*Mean of three determinations

4. Conclusions

The proposed spectrophotometric method is simple, sensitive, accurate, precise, reproducible and economical and can be successfully applied to the routine estimation of nitrazepam and nimodipine in bulk and in pharmaceutical preparations. The value of relative standard deviation was satisfactorily low and recovery was close to 100 % which indicates the reproducibility and accuracy of the method (Table 2). The added advantage of the proposed method is that it does not require cooling (0-5 °C) for diazotization. The possible applicability of the developed method for routine quality control analysis is well established by the assay of NTZ and NMD in bulk form as well as in pharmaceutical preparations.

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