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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC AND POLAROGRAPHIC METHODS FOR THE QUANTITATION OF NICARDIPINE HYDROCHLORIDE IN CAPSULES

A. A. MUSTAFA*, K. A. AL-RASHOOD, M. E. M. HAGGA, E. A. GAD-KARIEM AND M. E. AL-AWADY

Department of Pharmaceutical Chemistry, College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh-11451, Saudi Arabia

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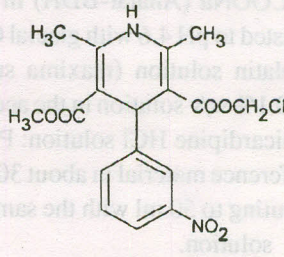
Nicardipine hydrochloride in bulk and in capsule dosage form has been assayed by two proposed independent analytical methods; (i) isocratic reverse-phase high-performance liquid chromatography (RP-HPLC) and (ii) differential pulse polarography (DPP). The RP-HPLC involves employment of μ-Bondapak-C_{18} column of 300 x 3.9mm i.d. dimensions and mobile phase composed of 80% v/v CH_3CN + 20% v/v (0.01M) CH_3COONa buffer adjusted to pH 3.5 with glacial CH_3COOH. The flow rate of the eluent was maintained at 1.5 ml/min. In the polarographic method, nicardipine produces a distinct reduction wave and a diffusion-controlled current which is linearly related to concentration of nicardipine HCl over the range 10-100 μg/ml. The RP-HPLC and DPP methods yielded comparable results when applied for the assay of nicardipine HCl in capsules (Pycarden®/30mg). However, the sensitivity and selectivity of the RP-HPLC can be improved substantially if the detection is carried out amperometrically.

Key words :Nicardipine, Capsules, Quantitation.

Introduction

Nicardipine, 2-(Benzylmethylamino)ethyl methyl-1,4-dihydro-2, 6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate, Fig.1, is a relatively new calcium antagonist [1-3] used clinically for the treatment of hypertension. Gas chromatography, GC, was reported for the determination of nicardipine in biological fluids, with either electron capture detection [4] or mass spectrometric detection [5]. The GC-method is relatively non-specific since it requires oxidation of nicardipine prior to analysis. A more specific method based on reverse-phase high-performance liquid chromatography (RP-HPLC) was utilized for the simultaneous estimation of nicardipine and its pyridine metabolite in human plasma [6]. RP-HPLC methods adopted to assess the purity of nicardipine were those by Fernadez *et al.* [7] and Maurin *et al.* [8]. Several other RP-HPLC procedures were employed for the quantitation of nicardipine in plasma [9-11] and in presence of co-administered drugs [12-14]. Recently polarographic techniques have been applied for the investigation of photodecomposition in nicardipine and in some other calcium antagonists [15] and the determination of intact nicardipine and its photodegradant in pharmaceutical formulations [16].

In the present report, the popular column μ-Bondapak-C_{18} has been utilized as a stationary phase with acetonitrile + 0.01M-CH_3COONa (pH 3.5, adjusted with CH_3COOH) (80:20 v/v) as mobile phase for the separation and quantitation of nicardipine in capsules dosage form. The results of the RP-HPLC and the DPP methods were compared statistically. Both methods are sensitive, precise and accurate for the routine quality control of the drug. However, the RP-HPLC method is more selective than DPP and is therefore recommended in situations of complex matrices. On the other hand, the principal advantage of the DPP methods is the prompt determination since no prior filtration of the admixture containing the pharmaceutical preparation is required.



NICARDIPINE

Fig. 1. Molecular structure of nicardipine.

Experimental

Materials. Nicardipine HCl (M.Wt.515.90, L.N. 118F0492 and certified purity) used as reference substance without further treatment was purchased from Sigma Chemical Company, St. Louis, U.S.A.

The commercial capsules (Pycarden®/30 mg, B.N.PK 307) were obtained from local pharmacies in London, England.

Chromatography. Apparatus. Water liquid chromatography equipped with Waters-U6K Millipore injector, Waters-486 tunable absorbance detector operated at 254 nm, and

* Department of Med. Pharmacology, College of Medicine, P.O. Box 2925, King Saud University, Riyadh-11461, Saudi Arabia.

Waters-746 data module attached to Waters-600 E system controller was employed. To get satisfactory reproducibility, solvent degassing with He is recommended.

Column. A μ-Bondapak-C_{18} (300x4 mm, i.d.) packed with 10 μm size particles column (Waters) was used at ambient temperature with a mobile phase flow rate of 1.5 ml/min.

Mobile phase. It consisted of 20% v/v aqueous 0.01M CH_3COONa-CH_3COOH buffer (pH 3.5) and 80% v/v acetonitrile.

Standard solutions. A stock solution of 0.03% w/v of nicardipine HCl reference material in the mobile phase was prepared and working solutions were made by appropriate dilution.

Internal standard. A stock solution of 0.02% w/v papaverine HCl (Analar-BDH) was prepared in the mobile phase and the diluted working solutions were made accordingly.

DIFFERENTIAL PULSE POLAROGRAPHY

Instrumentation. A Metrohm polarecord assebly (unit 626) consisting of three electrodes, namely, a silver-silver chloride reference electrode, a platinum auxiliary electrode and a dropping mercury working electrode (DME) was used. The polarograph stand was model E505 and the DME was a fine cappillary with a drop controller supplying a steady stream of mercury droplets at frequency of 0.5/s and a flow of approx. 3 mg/s under a corrected head pressure of 80 cm Hg column.

Reagents. (i) Acetate buffer of pH 4.6 prepared by dissolving 10.8 g CH_3COONa (Analar-BDH) in 100 ml of distilled water and adjusted to pH 4.6 with glacial CH_3COOH (Analar-BDH). (ii) Gelatin solution (maxima suppressor). Prepared fresh daily as 0.1% w/v solution in the acetate buffer pH 4.6. (iii) Standard nicardipine HCl solution: Prepared by shaking 50 mg of the reference material in about 30 ml acetate buffer of pH 4.6 and diluting to 50 ml with the same buffer to produce 1 mg/ml stock solution.

PROCEDURES

A. *The chromatographic method.* Preparation of standard curve: Appropriate final dilutions of 3, 6, 9, 12, 15 and 18μg/ml nicardipine HCl were made; each standard solution containing 2 μg/ml of internal standard. Three injections, each of 10 μl, were made for each standard solution and the mean ratios of peak-area responses of nicardipine HCl to papaverine HCl (i.e. peak area of Std./peak areas of int. Std.) were computed. To establish the standard curve, the calculated peak-area response ratios were plotted vs concentration of nicardipine HCl. Alternatively a linear regression equation could be worked out by regression analysis.

Assay of capsules (Pycarden®/30 mg). Twenty capsules were accurately weighed and the net fill weight per capsule was calculated. A quantity of mixed powder equivalent to about 30 mg of nicardipine HCl was accurately weighed and transferred into 100 ml volumetric flask. The admixture was shaken for 10 min. after addition of 60 ml mobile phase. The volume was adjusted with same solvent and finally filtered. Three ml. of the filtrate were transferred quantitatively into 10ml volumetric flask, 1ml of internal standard solution (0.001% w/v) was added and the volume was adjusted with mobile phase. Six 10 μl injections were made and the average peak-area response ratio was calculated. The quantity, in mg, of the drug component in the portion of the powder sample taken is obtained from the formula:

$$\frac{Ru}{Rs} \times C \times 100,$$

where C is the concentration, mg/ml, of nicardipine HCl standard stock solution, Ru and Rs are the average peak-area response ratios of the drug component to the internal standard obtained from the sample and the standard preparation respectively.

Alternatively, the quantity of the drug component can be computed by a linear regression equation.

THE POLAROGRAPHIC METHOD.

B. Establishment of the calibration curve. A standard series of nicardipine HCl solution in acetate buffer of pH 4.6, namely, 10, 20, 40, 60, 80, and 100 μg/ml was prepared in 100 ml volumetric flasks. Each solution contained 0.001% w/v gelatin as maxima suppressor. About 30 ml of each solution were transferred to the polarographic vessel and de-aerated for 5 min. with a stream of oxygen-free nitrogen. The differential pulse polarogram for each solution was recorded under the set of conditions listed in Table 1 and the peak current, i_p, was measured from the base-line of each polarogram.

The measured i_p values were plotted vs concentration. Alternatively a linear equation relating i_p to concentration, C, can be calculated by regression analysis.

Assay of capsules (Pycarden® /30 mg). A quantity of powder containing an amount of nicardipine HCl equivalent

TABLE 1. POLAROGRAPHIC EXPERIMENTAL CONDITIONS.

Modulation amplitude	50 mV
Initial voltage	-0.100 V
Final voltage	-0.600 V
Sweep rate	-5 mV/S
t (drop/s)	0.5
Sensitivity	10 nA/mm
Chart speed	100 mV/cm
Damp	2

to 100 mg was accurately weighed and transferred into 100 ml volumetric flask. Thirty millilitres of acetate buffer (pH 4.6) were added; the admixture was shaken for 10 min and finally the volume was adjusted. 10 ml of the supernatant solution were pippetted into a 100 ml volumetric flask, diluted to 100 ml and about 30 ml of the final solution were transferred into the polarographic vessel and analysis was completed as prescribed in the establishment of the calibration curve. The concentration of nicardipine HCl in the sample was read for the calibration graph of calculated from the linear regression equation.

Results and Discussion

The HPLC method. Under the optimized chromatographic conditions nicardipine HCl eluted at 4.93 in (Fig. 2) which is a satisfactory retention time for analysis. Papaverine HCl, employed as internal standard, eluted at 3.36 min, a fact which indicates a good separation of the two components from each other.

The test the suitability of the adopted chromatographic system, twelve replicate injections from the 9 µg/l standard solution of nicardipine HCl yielded a relative standard deviation (R.S.D. or coefficient of variation, C.V.) of 1% indicating excellent reproducibility.

The calculated linear regression equation relating average peak-area response ratio (Y) to concentration (C, µg/ml) was $Y = -0.01 \pm 1.93 \times 10^{-1} C$, concentration varies in the range 3 - 18 µg/ml, n = 6 and correlation coefficient, r, is 0.9999 suggesting adequate linearity between Y and C.

To assess the accuracy of the HPLC method, recovery experiments were conducted by spiking varying known quatities of nicardipine HCl to sample solutions. The obtained responses with and without spiking were compared to find out the equivalent increases due to the known added amount. These recovery experiments gave mean percent results of 99.84 ± 0.070 (n = 6), a proof that the HPLC methods is accurate. Table 2 shows the mean percent results of 99.97 ± 1.59 (n = 6) for the assay of nicardipine capsules (Pycarden®/ 30 mg) by the chromatographic method.

The DPP method. Polarography of organic compounds is influenced by pH since the hydrogen ions participate in the electrode reaction [17]. With acetate buffer of pH 4.6, well-defined direct-current polarogram (Fig.3) is produced most probably due to the reduction of the aromatic nitro group of nicardipine on the DME surface under the applied potential. A neat pek-shaped polarogram (convenient for measurmeent) is obtainable by superimposing 50 mV pluse voltage on the direct current ramp as depicted by Fig. 3.

To investigate the reversibility or otherwise of the reduction reaction of nicardipine on the DME surface, loga-

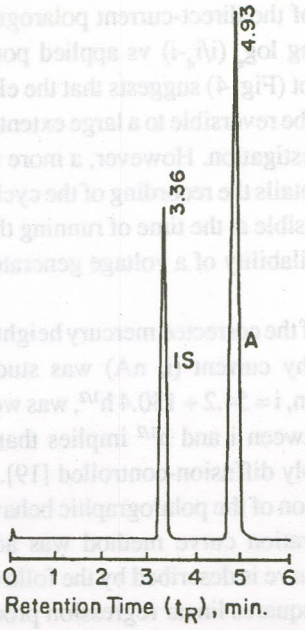


Fig. 2. HPLC chromatogram of nicardipine HCl (A), 120 ng, t_R = 4.93 min and papaverine HCl (IS), 20 ng, t_R = 3.36 min, 20 ng; a.u.f.s. = 0.1

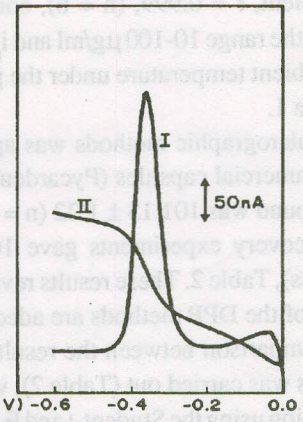


Fig. 3. Differential pulse (I) and direct-current (II) polarograms of nicardipine HCl (20µg/ml) in acetate buffer pH 4.6 and ambient temperature.

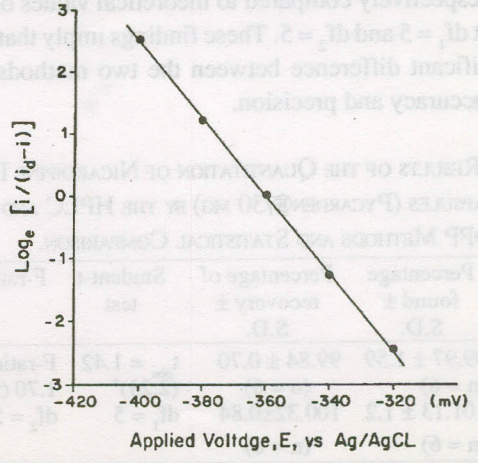


Fig. 4. Variation of $\log_e [i/(i_d - i)]$ with respect to applied voltage; i stands for the polarographic diffusion-current and i_d for the limiting diffusion current.

rithmic analysis of the direct-current polarography was performed by plotting $\log_e (i/i_d-i)$ vs applied potential, E. The linearity of the plot (Fig. 4) suggests that the electrode reduction reaction may be reversible to a large extent [18] under the conditions of investigation. However, a more rigorous proof for reversibility entails the recording of the cyclic polarogram which was not feasible at the time of running this experiment due to the nonavailability of a voltage generator in our laboratory.

The effect of the corrected mercury height (h, cm) on the direct polarography current (i, nA) was studied. A linear regression equation, $i = 54.2 + 130.4\ h^{1/2}$, was worked out. The linear relation between i and $h^{1/2}$ implies that the recroded current i is probably diffusion-controlled [19]. Based on the preceding discussion of the polarographic behaviour of nicardipine, the carlibration curve method was adopted for its evaluation. This curve is described by the following equation obtained by least squares linear regression procedure.

$$i_p\ (nA) = 0.81 + 5.10C,$$

correlation coefficient, r = 0.999, (n = 6), concentration of nicardipine HCl in the range 10-100 µg/ml and i_p standards for peak current at ambient temperature under the polarographic conditions of Table 1.

When the polarographic methods was applied for the analysis of the commercial capsules (Pycarden®/30 mg), the mean percentage found was 101.13 ± 1.22 (n = 6 determinations), whereas recovery experiments gave 100.32 ± 0.84 (n=6 determinations), Table 2. These results reveal that precision and accuracy of the DPP methods are adequate.

Statistical comparison between the results of the DPP and HPLC methods was carried out (Table 2), with regard to accuracy and precision using the Student-t and F-ratio tests. At 95% confidence level, the observed t and F-ratio were 1.42 and 1.70 respectively compared to theoretical values of 2.23 and 5.05 at $df_1 = 5$ and $df_2 = 5$. These findings imply that there is no significant difference between the two methods with regard to accuracy and precision.

TABLE 2. RESULTS OF THE QUANTITATION OF NICARDIPINE HCL IN CAPSULES (PYCARDEN®/30 MG) BY THE HPLC AND DPP METHODS AND STATISTICAL COMPARISON.

Method	Percentage found ± S.D.	Percentage of recovery ± S.D.	Student-t test	F-ratio test
HPLC	99.97 ± 1.59 (n = 6)	99.84 ± 0.70 (n = 6)	t_{obs} = 1.42 (2.23)*	F-ratio$_{obs}$ = 1.70 (5.05)*
DPP	101.13 ± 1.2 (n = 6)	100.32±0.84 (n = 6)	$df_1 = 5$	$df_2 = 5$

S.D. = Standard deviation; n = number of separate determination; df = degrees of freedom; * = tabulated values at 95% confidence level.

In conclusion it can be stated that the DPP method is equally precise and accurate as the HPLC method for the quantitation of nicardipine HCl in capsules dosage form; however, the former is simple and convenient for the assay of nicardipine in pharmaceutical laboratories. Furthermore, the sensitivity of the HPLC method can be enhanced substantially if amperometric detection is adopted.

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