Employing Ionization Behaviors to Resolve a Trace-Level Impurity: Determination of 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic Acid Di-2-[methyl-(phenylmethyl)amino]ethyl Ester in Nicardipine Drug Substance[1]

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INTRODUCTION

Nicardipine (1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid methyl 2-[methyl(phenylmethyl)amino]ethyl ester), a dihydropyridine calcium channel blocker (Fig. 1), is indicated for the treatment of chronic stable angina and hypertension (1). As part of the evaluation of a parenteral dosage form, a high-performance liquid chromatography (HPLC) assay was developed to quantify the presence of 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid di-2-[methyl-(phenylmethyl)amino]ethyl ester (XE820), the diaminoalcohol ester of nicardipine (Fig. 1), in the nicardipine drug substance.

The synthesis of dihydropyridines proceeds through the Hantzsch synthesis (2). In the case of nicardipine, the substituted acetoacetates react with substituted aldehyde and ammonia. On a theoretical basis, the three resulting products are nicardipine, XE820, and 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester in a yield of 2:1:1. Preliminary studies found a trace-level contaminant with a molecular ion of 613 which corresponded to the free base of XE820 that was obtained by independent synthesis.

A variety of methods has been published for the determination of nicardipine in biological fluids. Most recently, methods have been introduced that eliminate the need for the lengthy extraction procedure (3), permit microdetermination of nicardipine and its pyridine metabolite by capillary gas chromatography (4), or enable enantioselective determinations in human plasma (5). However, the methods are not suitable for the determination of trace-level impurities. The chromatographic purity has been reported with a reversed-phase assay (6). However, the method was unable to resolve trace levels of XE820 from nicardipine. The current work details an ion-exchange method that exploits the ionization behavior of nicardipine and XE820 and results in an improvement in the sensitivity for the detection of XE820.

MATERIALS AND METHODS

Materials

Nicardipine was used as received. Acetonitrile and methanol were HPLC grade (EM Science). The water was house-distilled water that was passed through a Nanopure II ion-exchange cartridge system (Barnstead) and was 18 MΩ. All the other materials were reagent grade.

Synthesis of XE820

XE820 was prepared by the Hantzsch synthesis (7). 2-Chloroethanol was reacted with diketene to yield 2-chloroethyl acetoacetate 2 (Fig. 2). Compound 2 was treated with ammonia in ethanol to give the corresponding aminocrotonate 3, which was reacted with 3-nitrobenzaldehyde and compound 2 in refluxing ethanol to give the desired dihydropyridine compound 4. The reaction of 1 mol of 4 and 2 mol of *N*-methylbenzylamine followed by treatment of HCl to form the dihydrochloride salt, which was recrystallized from 1-chlorobutane to give XE820 as a yellow solid. *Elemental Analysis*. Calculated for $C_{35}H_{42}N_4O_6Cl_2$: C, 61.31; H, 6.17; N, 8.17; Cl, 10.34. Found: C, 61.36; H, 6.16, N, 8.02; Cl, 10.27. ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 6H); 2.60 (m, 6H); 3.20–3.50 (m, 4H); 4.30 (m, 4H); 4.40 (m, 4H); 5.05 (s, 1H); 7.45 (m, 6H); 7.58 (m, 6H); 7.95 (m, 2H); 9.40 (s, 1H); 11.0 (m, 2H). MS: 612. IR(KBr) ν_{max}: 1700 cm^{-1}.

Potentiometric Titration

Titrations were performed at 25°C with 0.1 *N* KOH as the titrant and initial nicardipine or XE820 concentrations of 0.001 to 0.01 *M* (Model 636 Titroprocessor, Metrohm). The titrant was prepared with CO_2-free deionized water. The nicardipine and XE820 solutions were prepared in acetonitrile:CO_2-free deionized water:methanol (50:40:10).

Chromatographic Method

The concentration of XE820 was measured with an isocratic ion-exchange HPLC method using external standard. Separation was performed on a 25-cm Macrosphere SCX 300-Å 7-μm column (Alltech) under ambient conditions. The mobile phase was composed of acetonitrile:0.02 *M* potassium dihydrogen phosphate:methanol (50:40:10) with 0.1% (v/v) triethylamine and was adjusted to pH 4.2 with phosphoric acid. To examine the effect of pH on the resolution of nicardipine and XE820, the pH was adjusted higher with sodium hydroxide, or for the lower pH values potassium dihydrogen phosphate was replaced with phosphoric acid and adjusted with sodium hydroxide. A flow rate of 1.5 mL/min was employed (HPLC Pump, Model 510, Waters Chromatography). Ultraviolet detection was utilized at 240 nm

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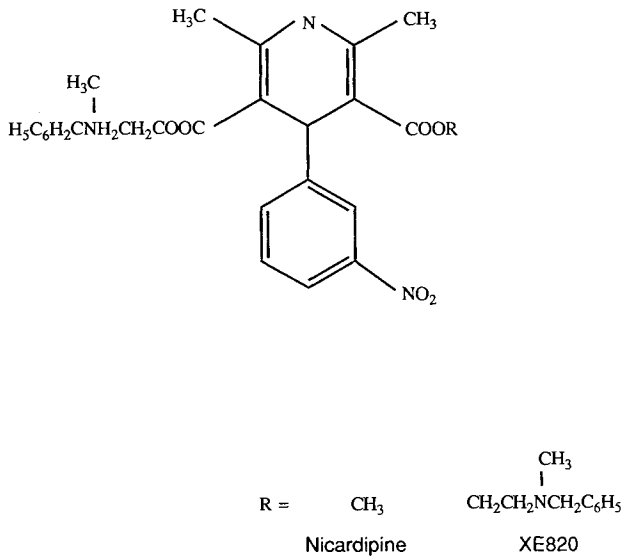

Fig. 1. The chemical structure of nicardipine and XE820.

(1050 Series Variable Wavelength Detector, Hewlett Packard). Data acquisition was completed with a VAX-based program that calculated the sample concentrations from a standard curve based on the XE820 or nicardipine peak areas (Multichrom software, VG Instruments). The standards were freshly prepared before each analysis.

RESULTS AND DISCUSSION

Potentiometric Titration

Titration of nicardipine permitted the determination of the pK_a at 6.65, while XE820 revealed only one ionization at 6.53. The single pK_a for XE820 represented the macroionization constant for the two ionizable sites. The value for the pK_a was consistent with that of nicardipine. The resulting single macroionization constant was also consistent with the fact that the two ionizable sites on XE820 are effectively identical, such that at pH values below 4.5, nicardipine had a charge of +1 while XE820 had a charge of +2. Thus the differences in ionization behavior provided a feature that was employed to optimize the separation of nicardipine and XE820 via ion-exchange chromatography.

Table I. Accuracy and Precision for the Validation of the HPLC Assay of XE820

Theoretical amount (μg)	Measured amount (μg)[a]	Coefficient of variation	
		Intraday[b]	Interday[c]
4.00	4.12	0.25	1.3
	4.12	2.0	
	4.18	0.77	
1.00	0.984	0.66	2.3
	0.988	2.4	
	1.02	2.2	
0.200	0.199	3.5	3.3
	0.206	3.1	
	0.200	1.8	

[a] Mean of $n = 6$.
[b] $n = 6$.
[c] $n = 18$.

Chromatographic Method

The UV detector response for XE820 was found to be linear in the range from 0.004 to 8 μg on column. The detection limit for XE820 was calculated to be 4 ng on column with a quantitation limit of 20 ng on column. The results of the assay validation are summarized in Table 1. The short-term precision study of the assay resulted in an intraday coefficient of variation that ranged from 0.25 to 3.5% and an interday coefficient of variation that ranged from 1.3 to 3.3%.

Three lots of nicardipine were analyzed and the XE820

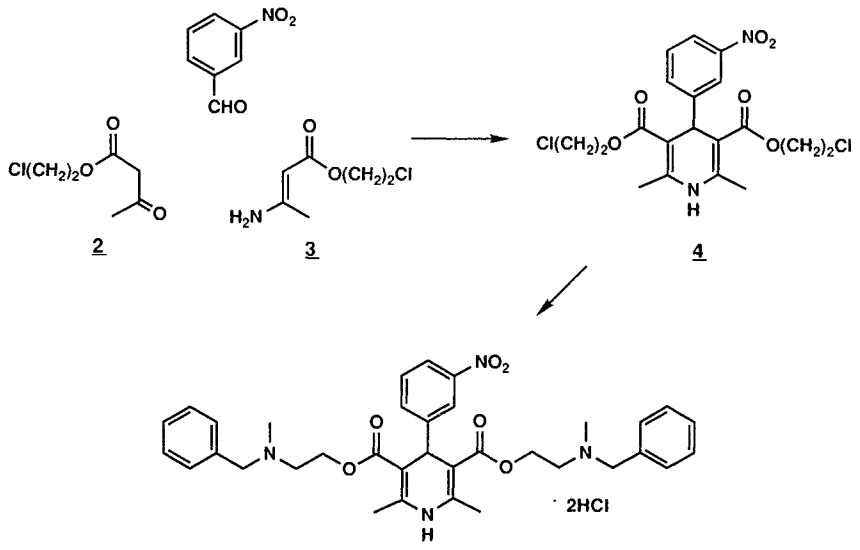

Fig. 2. Synthesis of XE820 via the Hantzsch synthesis.

content was determined. The XE820 level was determined with the mean and standard deviations from three replicates at 0.034 ± 0.0003, 0.41 ± 0.006, and 0.075 ± 0.001% for lots, A, B, and C, respectively.

The strong cation-exchange HPLC column contained a sulfonated acid resin stationary phase capable of selectively separating nicardipine from XE820 on the basis of charge. The cation-exchange assay provided a rapid and reliable method for quantification of XE820 in nicardipine that was highly pH dependent. The effect of pH on the resolution of nicardipine and XE820 is presented in Fig. 3. At pH 8, where greater than 95% of the nicardipine and XE820 existed in their uncharged forms, neither compound was retained and both eluted with the solvent front. The resolution of nicardipine and XE820 increased from 6.32 at pH 6.5, where nicardipine was 60% protonated and XE820 was 36% diprotonated and 33% monoprotonated, to 15.9 at pH 4.2, where nicardipine was >99.5% protonated and XE820 was >99.5% diprotonated. The resolution at pH 4.2 was necessary to resolve trace levels of XE820 from nicardipine (Fig. 4).

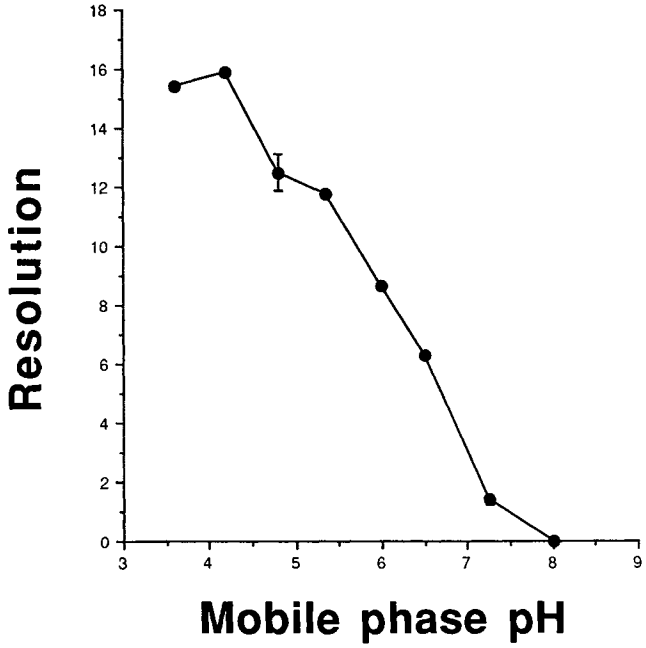

Fig. 3. The effect of pH on the resolution of nicardipine and XE820 in an isocratic cation-exchange HPLC method using a mobile phase that was composed of acetonitrile:0.02 *M* phosphate buffer:methanol (50:40:10) with 0.1% (v/v) triethylamine.

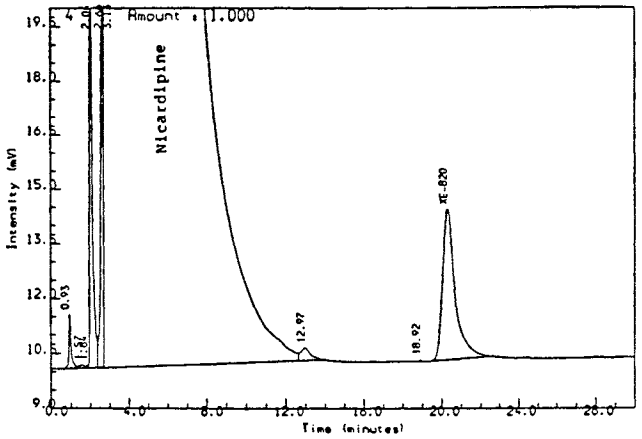

Fig. 4. Representative chromatogram of nicardipine drug substance from an injection of 400 mcg of nicardipine. The sample contained 0.3 μg of XE820 (0.075%).

The cation-exchange assay provided a rapid and reliable method for identification and quantification of XE820 in nicardipine. Thus, the differences in ionization behavior were exploited to optimize the separation of trace levels of XE820 from nicardipine.

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