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Simultaneous HPLC determination of nitrendipine and impurities of the process of synthesis

V. Marinkovic [a,*], D. Agbaba [b], S. Vladimirov [b], S. Stankovic [a]

[a] *A.D. 'Zdravlje', Pharmaceutical and Chemical Industry, 16000 Leskovac, Serbia, Yugoslavia*
[b] *Faculty of Pharmacy, Department of Pharmaceutical Chemistry, PO Box 146, 11000 Belgrade, Serbia, Yugoslavia*

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Abstract

A method has been developed for separation of nitrendipine and its impurities of reaction partners and side reaction products by high-performance liquid chromatographic method on a RP-18 column and detection at 238 nm. The mobile phase composition that provided an acceptable nitrendipine resolution, in large excess and possible impurities, in a short elution time, is methanol:water (70:30) and pH 3. Linearity ($r \geq 0.999$), reproducibility (RSD = 0.8–1.4%), determination limit (0.5–2%) and recovery (99.8–102.3) were validated and found to be satisfactory. This method enables monitoring of the process of synthesis, as well as the choice of the synthetic design.

Keywords: Nitrendipine; Impurities of the synthesis; Side reaction; High-performance liquid chromatography

1. Introduction

Nitrendipine, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid ethyl, methyl ester is a calcium-channel blocker with vasodilatory properties, present in several commercial preparation administrated in the treatment of hypertension [1].

On exposure to light, nitrendipine decomposes to give dehydrogenated pyridine derivative which also is the main metabolite.

Stability studies have been mostly performed by using HPLC [2–4]. GC [5,6], TLC [3,7], polarographic [8] and UV derivative spectroscopic methods [5]. Enantiomer separation of nitrendipine was also evaluated by HPLC method [9–11].

According to the scientific [12,13], and patent [14–17] papers, 1,4-dihydropyridine derivative with nonidentical ester functions has been synthesized by Michael's reaction of cyclocondensation of β-crotonic acid ester and benzyliden intermediary. Reaction partners of the final Michael addition in the synthesis of nitrendipine were methyl-3-amino crotonate (I) and 2-ethyl 2-(3-nitrobenzylidene) acetoacetate (II), shown in Fig. 1.

Inadequate conditions of synthesis have involved in appearance of side reactions and formation of 1,4-dihydropyridines with identical esters functions: 1,4-dihydro-2,6-dimethyl-4-(3-nitro-

* Corresponding author. Fax: +381-16-51297.

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phenyl)-3,5-pyridinedicarboxylic acid diethyl ester (III) and 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester (IV). These by-products, as impurities in nitrendipine, are requested to be tested according to British Pharmacopoeia [18], but there is not official method for that analysis.

Good resolution between the dihydropiridine clevidipine and its symmetric ester were performed by packed-column supercritical fluid chromatography [19].

However, there are no published reports of the quantification assay of these impurities involved in the process of synthesis. Therefore, the present paper will focus on assay of nitrendipine and the isolation and quantification assay of the residue of methyl-3-amino crotonate (I) 2-ethyl-2-(3-nitro-benzylidene) acetoacetate (II), 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid diethyl ester (III) and 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester (IV) in nitrendipine during the different conditions of the synthesis by HPLC method and to justify the choice of the synthetic design.

2. Experimental

2.1. Solvents and chemicals

Water and methanole were of HPLC grade, Merck, Darmstadt, Germany.

Nitrendipine, standard substance was obtained from PRO.MED, Praha, Chech Republic.

Methyl-3-amino crotonate (I) and 2-ethyl-2-(3-nitro-benzylidene) acetoacetate p.a. substance were obtained from ROHS CHEMIE, Germany.

1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid diethyl ester (III) and 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester (IV) were obtained from PRO.MED, Praha, Czech Republic.

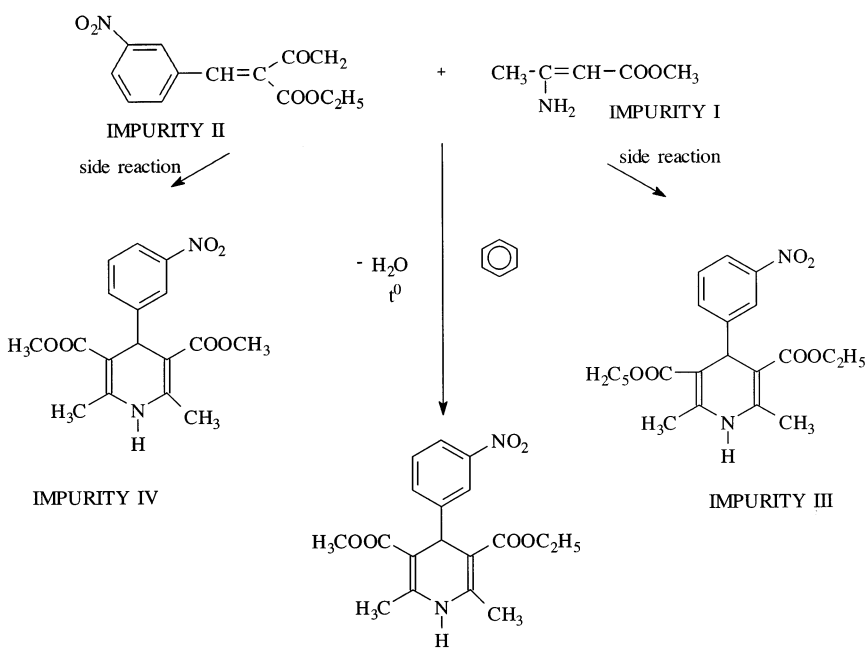

Fig. 1. Synthesis of nitrendipine. Michael's addition.

Different samples of nitrendipine, raw material, A, B, C and D were synthesized in ZDRAVLJE — Leskovac, Serbia.

The conditions of reaction were improved in such a way the influence of side reaction was minimized:

- Sample A: Heating of reaction partners at the reflux temperature in absolute methanol for 10 h. Recrystallization was done several times from absolute ethanol.
- Sample B: Heating of reaction partners on the reflux temperature in absolute methanol for 10 h. Recrystallization was done several times from absolute ethyl acetate.
- Sample C: Heating of reaction partners at the reflux temperature in absolute methanol for 8 h. Crystallization was done from absolute ethanol.
- Sample D: Heating of reaction partners at the reflux temperature in absolute methanol for 8 h. Crystallization was done from ethyl acetate.

2.2. Equipment

HPLC system HEWLETT PACKARD with UV detector (wavelength set up at 238 nm). The chromatographic column (250×4 mm) was packed with 5 μ Lichrosorb RP-18 (Merck, Darmstath, Germany). The mobile phase of methanol–water (70:30) Ph 3 (pH adjusted to three with phosphoric acid) was filtered through a 0.45 μ membrane filter and degassed in an ultrasonic bath prior to use. The injection volume was 20 μl, elution was performed at a flow rate of 1.5 ml/min and the column was maintained at ambient temperature.

2.3. Solutions

2.3.1. Stock solutions

A stock solution of 0.5 mg/ml nitrendipine standard substance was prepared in methanol.

A stock solution of 0.5 mg/ml syn. interm. I and II, and by-products III and IV were prepared in methanol.

2.3.2. Preparations of standard curve

Calibration solutions for nitrendipine were prepared by diluting the stock solution to obtain 0.05–0.15 mg/ml. Calibration solutions for impurities were prepared by diluting the stock solutions to obtain 0.02–0.1 mg/ml of I and 5–50 g/ml of II, III and IV.

2.3.3. Sample preparation

50 mg of nitrendipine raw material was dissolved in 50 ml of methanol. After filtration, the aliquots of 50 μl sample were subjected to HPLC for impurities assay. 1 ml of the diluted sample solution to 10 ml were subjected to HPLC for nitrendipine assay.

2.3.4. Standard preparation

Working standard solution contained 0.1 mg/ml of nitrendipine, 0.02 mg/ml of impurity I and 5 μg/ml of impurities II, III and IV.

3. Results and discussion

3.1. Optimum conditions for chromatographic procedure

The combined effect of pH and mobile phase composition on reverse-phase liquid chromatographic behavior of nitrendipine and possible impurities during the process of synthesis were studied. The effects of these factors were examined in the range of conditions where they provided acceptable retention and resolution.

The composition of methanol and water in mobile phase of 70:30 was optimal, because higher concentration of methanol disturbed separation and resolution. In the same time, concentration of water higher then 30% produced nonsymmetrical peaks and provided long separation period.

Optimum pH was 3.0, because higher pH provided lower sensitivity of the method.

Separation of nitrendipine from its possible impurities is shown in Fig. 2.

3.2. Quantitative determinations

The HPLC method was tested for selectivity, linearity, precision and accuracy.

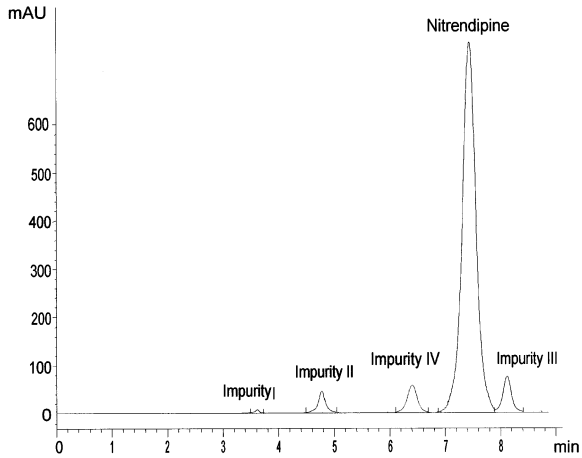

Fig. 2. Chromatogram of test mixture of nitrendipine at concentration of 0.1 mg/ml and impurity I at concentration of 0.02 mg/ml and impurities II, III and IV at concentration of 5 μg/ml.

The response (peak area) was proportional to the concentrations over the range tested; between 0.5 and 1.5 mg/ml for nitrendipine; 0.02–0.1 mg/ml for impurity I and 5–50 μg/ml for impurities II, III and IV.

The regression equations were:

$y = 13992x + 6.81 \quad r = 0.9993$ for nitrendipine

$y = 18821x + 20.4 \quad r = 0.9997$ for impurity I

$y = 13866x - 986.2 \quad r = 0.9952$ for impurity II

$y = 236.1x + 24.5 \quad r = 0.9998$ for impurity III

$y = 145.8x + 24.9r \quad = 0.9999$ for impurity IV

The precision of analytical system was investigated by using working standard solution. Six consecutive replicate injections of each sample gave a relative standard deviation (RSD) of 1.9–0.4%.

The accuracy of the method was provided by determination of impurities I, II, III and IV in presence of nitrendipine. A solution ($c = 1$ mg/ml) containing nitrendipine with no detectable amount of impurities was spiked with aliquots of the impurities I, II, III and IV at concentration of 0.02 mg/ml (corresponding to 2%) Recoveries obtained were shown in Table 1.

Limit of determination (LOD) was measured as the lowest amount of analyte that may be detected to produce a response which is significantly different from that of blank.

Limit of quantification (LOQ) was measured as the lowest amount of analyte that can be reproducibly quantified above baseline noise, for which duplicate injection resulted in a RSD $\leq 3\%$.

The present method can quantify impurity I at the 2% level, and impurities II, III and IV at the 0.5% level.

The method was used to screen the raw material of nitrendipine on impurities of the process in synthesis.

The results obtained for samples of nitrendipine (A–D) for the different conditions of the synthesis for samples are shown in Table 2, and chromatograms in the Fig. 3.

The results obtained for sample A (recrystallization from absolute ethanol) can confirme high content of 99.98% of nitrendipine. Under the conditions of developed method, impurities (I–IV) were not observed.

Inadequate conditions of the synthesis (cyclisation period of 8 h and crystallization) can produce

Table 1
Validation parameters of HPLC method

Sample	RSD %	Recovery %	LOD μg/ml	LOQ μg/ml
Impurity I	1.9	101.2	3.0	10.1
Impurity II	1.3	100.8	1.2	4.0
Impurity III	1.4	98.9	0.9	3.0
Impurity IV	0.4	100.5	0.9	3.1

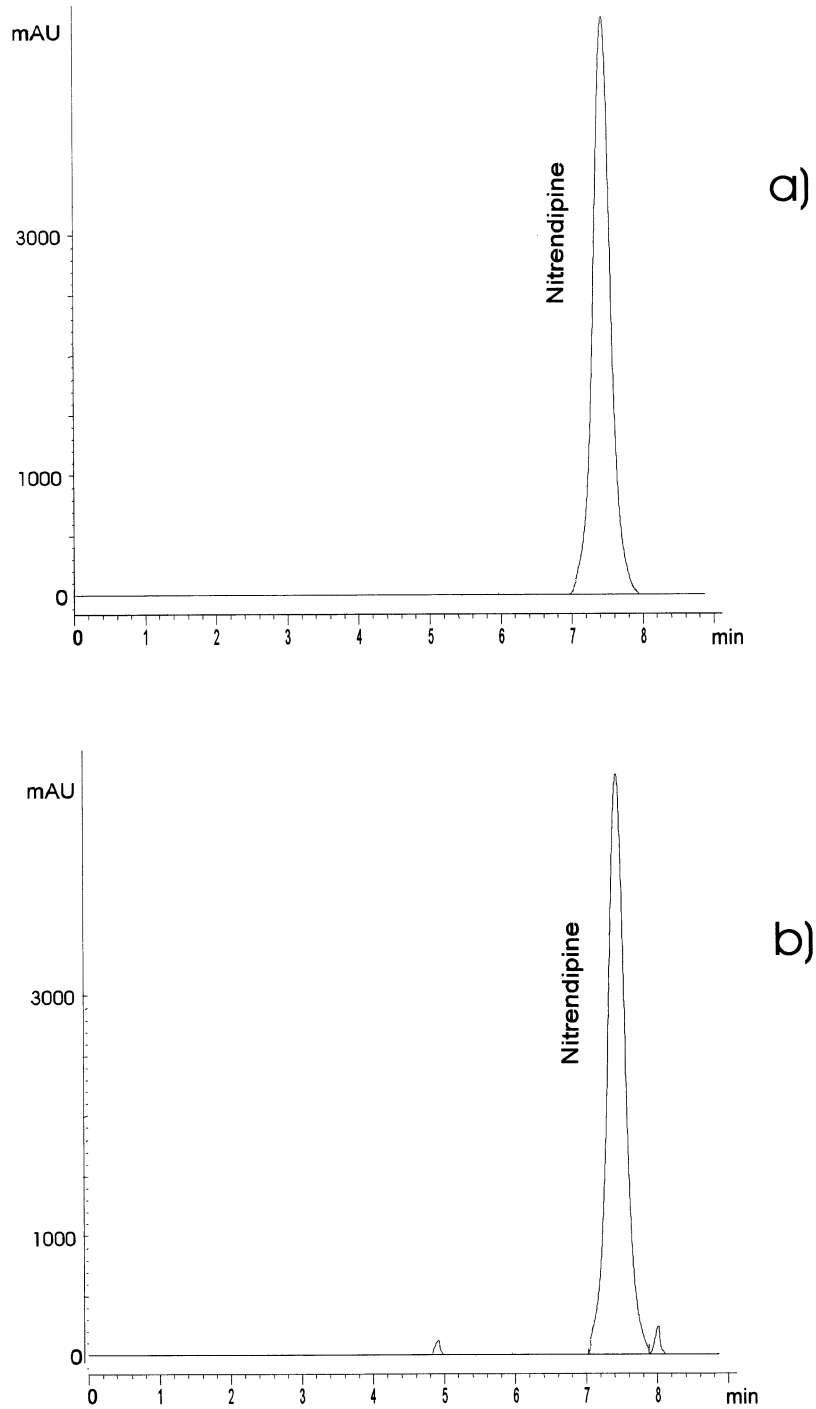

Fig. 3. Chromatogram of nitrendipine substance A (a) and nitrendipine substance D (b).

Table 2
Assay of nitrendipine and its impurities

Sample	Nitrendipine % ± SD	Impurity I % ± SD	Impurity II % ± SD	Impurity III % ± SD	Impurity IV % ± SD
A	99.98 ± 0.13	–	–	–	–
B	99.23 ± 0.09	–	–	–	–
C	98.82 ± 0.11	–	–	1.1 ± 0.02	–
D	97.81 ± 0.12	–	0.5 ± 0.01	2.1 0.21	–

sample D, bellow the declared limit of nitrendipine (98.0%) and certain amount of impurities II and III (Table 2).

4. Conclusions

These results suggest that HPLC is efficient method for separation and quantitative determination of nitrendipine and its reaction partners, as well as by products in raw material. The method provided *ng* sensitivity adequate linearity and repeatability. Simple isocratic system used for separation is found to be suitable for routine purity control and monitoring of the process of the synthesis of nitrendipine.

This method should ensure that the synthetic and purification procedures are working as expected and designed.

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