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Short communication

Simultaneous determination of nitrendipine and one of its metabolites in plasma samples by gas chromatography with electron-capture detection

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Abstract

A sensitive method for GC-ECD simultaneous determination of nitrendipine and its pyridine metabolite M1 in human plasma is described. Felodipine was used as the internal standard. The plasma samples were extracted with toluene. One microlitre of the extract was injected onto the capillary column (polymethylsiloxane) and measured with electron-capture detector. The developed method showed to be linear over the range 0.25–70 for nitrendipine and 0.3–61 ng/ml for its metabolite M1 with an inter-day and intra-day precision in terms of R.S.D. lower than 8% except the concentrations near lowest limit of quantification (LLOQ) (<11% R.S.D.). The LLOQ for nitrendipine was 0.25 and 0.3 ng/ml for its metabolite, respectively. The analytical recovery was 94% for nitrendipine and 89% for its pyridine metabolite M1. This GC-ECD method was developed for being used in clinical pharmacokinetic studies.

Keywords: Nitrendipine; Felodipine; Plasma; GC-ECD

1. Introduction

Nitrendipine [ethyl methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate] (see Fig. 1a) belongs to the group of calcium channel antagonists, which are often used in the therapy of hypertension and angina pectoris [1–3]. The main route of the nitrendipine metabolism is dehydrogenation to the pyridine analogue [4] by cytochrome P450 3A4/5, then cleavage of ester groups by hydrolysis to carboxylic acids, hydroxylation of methyl groups and glucuronidation [5–7]. Pyridine metabolite M1 (see Fig. 1b) exhibits 1000 times less biological activity than nitrendipine [5]. The concentrations of nitrendipine as well as other dihydropyridine calcium channel antagonists in human blood plasma are relatively low as a consequence of extensive first-pass metabolism, poor bioavailability and also relatively high inter- and intra-individual variability [7,8]. Therefore, the determination of nitrendipine requires very sensitive techniques. Several methods have been described for the determination and quantification of nitrendipine in blood plasma. HPLC with ultraviolet detection [9,10] is a generally available technique, but it has relatively low sensitivity and specificity compared with HPLC–MS or LC–MS–MS [11–13]. Gas chromatographic method with electron-capture detection [7,14,16–20,30] or with MS detection [21–23] ensures best the desired analytical parameters.

Some authors employed nitrendipine as an internal standard for determination of other dihydropyridines [13–16,18–21,24–27,30]. Soons et al. [19] used nitrendipine as an internal standard for GC-ECD determination of felodipine in human plasma. On the contrary, we have applied the easily obtainable felodipine as the internal standard for determination of nitrendipine in human plasma replacing 3-(2-hydroxy-2-methyl)ethyl-5-methyl-2,6-dimethyl-4-nitrophenyl)-3,5-pyridine dicarboxylate in the GC-ECD method of Soons and Breimer [17]. For sample preparation we have chosen the robust method of liquid–liquid extraction with toluene as an

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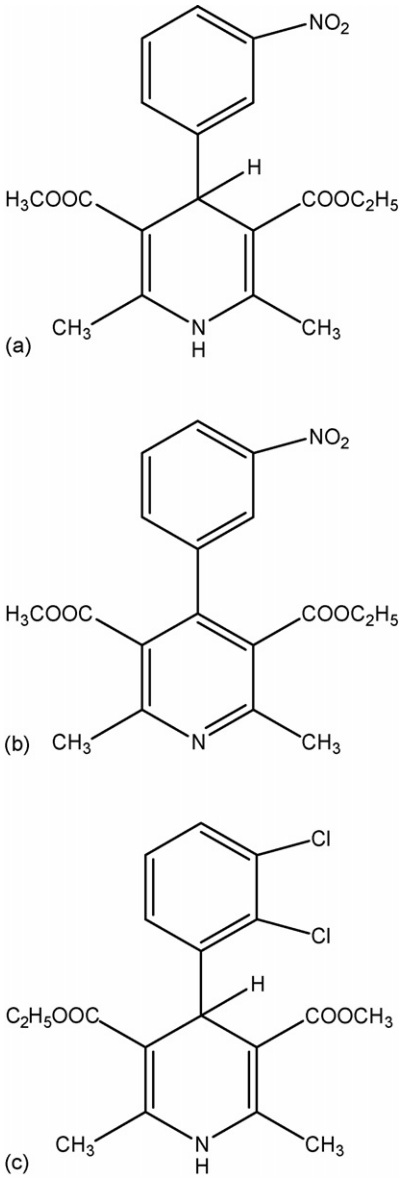

Fig. 1. Structure of: (a) nitrendipine; (b) pyridine metabolite M1 of nitrendipine; (c) felodipine (internal standard).

extraction solvent [14,18,21] followed by direct injection in the split–splitless injection device.

2. Experimental

2.1. Chemicals and reagents

The chemical structures of nitrendipine [ethyl methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate], its metabolite M1 [ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate] and the internal standard felodipine [ethyl methyl (4*RS*)-2,3-dichlorophenyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate] are shown in Fig. 1a–c. All chemicals were of analytical grade or better. Both nitrendipine (order code N0905000) and nitrendipine metabolite M1 (nitrendipine impurity A, order code N0905005) were obtained from European Pharmacopoeia Catalogue. Felodipine (internal standard) was extracted from tablet preparation Plendil® ER, 5 mg (Astra AB, Sweden). Methanol SupraSolv® and toluene SupraSolv® were from Merck (Germany). UHQ water was from PRO.MED.CS Praha a.s. (Prague, Czech Republic).

2.2. Equipment

An Agilent Technologies 6890 Series GC System gas chromatograph with a μ-^{63}Ni-ECD detector, equipped with an Agilent Technologies 7683 Series Injector and 7683 Series Autosampler was used (Agilent Technologies, CA, USA) together with a 30 m × 0.32 mm fused silica column coated with polydimethylsiloxane (DB-1) with a film thickness of 0.25 μm (J&W Scientific, USA). Helium at the flow rate of 2.2 ml/min was used as the carrier gas. The inlet temperature was set at 260 °C. Splitless injection (1 μl) was used with a deactivated injection liner, which was replaced after every 80 injections. The electron-capture detector was set at 260 °C with a nitrogen makeup flow of 25 ml/min. The initial column temperature was 100 °C. After 3 min at 100 °C, the temperature was gradually raised to 230 °C at the rate of 50 °C/min, and this temperature was maintained for 15 min. A Hewlett-Packard ChemStation (Agilent Technologies) was used to control the GC apparatus and to acquire and process the data. Calibration curves obtained with a known concentration range of nitrendipine or its M1 metabolite and internal standard (felodipine) were used for quantification of unknown samples.

2.3. Extraction procedure

Fifty microlitres of the internal standard solution (felodipine, concentration: 1 μg/ml) was added to 1.0 ml of the human blood plasma, and the solution whirl-mixed for 10 s. For the extraction, toluene (1.0 ml) was added, and the tube was shaken in the horizontal shaker (90 cycles/min) for 30 min and centrifuged (3000 × *g*) for 5 min. 0.2 ml of the organic layer was transferred into the glass insert tube, which was placed in the brown autosampler vial. One microlitre of the extract was injected into the gas chromatograph.

2.4. Preparation of standards, calibration and clinical samples

The entire treatment and analytical procedures had to be performed in the dark room using yellow sodium light to prevent degradation of dihydropyridine analytes [28]. For sample preparation only glassware was used to prevent the loss of nitrendipine by absorption [30]. The human blood samples were withdrawn from a forearm vein, collected into lithium–heparin tubes, and immediately centrifuged (3000 × *g*, 10 min); blood plasma was transferred into the brown glass tubes for further treatment or storage (at −70 °C).

2.4.1. Preparation of the stock and calibration solutions

One tablet of Plendil® ER containing 5 mg of felodipine was disintegrated and diluted with 100 ml of methanol. Methanol