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Developing and optimizing a validated isocratic reversed-phase high-performance liquid chromatography separation of nimodipine and impurities in tablets using experimental design methodology

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ABSTRACT

In the present study an isocratic reversed-phase high-performance liquid chromatography was investigated for the separation of nimodipine and impurities (A, B and C) using statistical experimental design. Initially, a full factorial design was used in order to screen five independent factors: type of the organic modifier – methanol or acetonitrile – and concentration, column temperature, mobile phase flow rate and pH. Except pH, the rest examined factors were identified as significant, using ANOVA analysis. The optimum conditions of separation (optimum values of significant factors) determined with the aid of central composite design were: (1) mobile phase: acetonitrile/H_2O (67.5/32.5, v/v), (2) column temperature 40 °C and (3) mobile phase flow rate 0.9 ml/min. The proposed method showed good prediction ability (observed–predicted correlation). The analysis was found to be linear, specific, precise, sensitive and accurate. The method was also studied for robustness and intermediate precision using experimental design methodology. Three commercially available nimodipine tablets were analyzed showing good % recovery and %RSD. No traceable amounts of impurities were found in all products.

1. Introduction

Calcium antagonists are a heterogeneous group of cardiovascular drugs used to block the entry of calcium ions into nerve cells, producing a reduction in peripherical vascular resistance [1]. This class of compounds (dihydropyridines) are 4-aryl-1,4-dihydropyridine 3,5-dicarboxylates. The ester functions in the 3,5-positions may vary widely without a significant reduction in potency [1].

Nimodipine (Nim) is a 1,4-dihydropyridine (DHP) with calcium channel antagonistic activity [2]. It was identified as having cerebrovasodilatory and neuronal effects at doses that had little or no effect on peripheral circulation [3–5]. Experimental studies in animals and humans suggested its effectiveness in the treatment of subarachnoid haemorrhage [6], focal or global ischemia [4,7–9], as well as epilepsy [2].

The chemical structure of nimodipine and its impurities is shown in Fig. 1. The pyridine derivatives (impurities) of nimodipine are [1]: (1) the photo-degradation product of nimodipine impurity A (Imp A): 2-methoxyethyl 1-methylethyl-2,6-dimethyl-4-(3-nitrophenyl) pyridine-3,5-dicarboxylate, (2) impurity B (Imp B): bis (1-methylethyl)-2,6-dimethyl-4-(3-nitrophenyl), 1,4-dihydro-pyridine-3,5-dicarboxy-late and (3) impurity C (Imp C): bis (2-methoxyethyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro pyridine-3,5-dicarboxylate.

There are several literature reports for the determination of nimodipine using high-performance liquid chromatography (HPLC) [10–14]. However, there is only one attempt made for the simultaneous determination of all substances (nimodipine and all impurities) using a preparative HPLC [1].

Developing and optimizing an isocratic HPLC method is a complex procedure that requires simultaneous determination of several factors (e.g. type and composition of the organic phase, column temperature, flow rate, pH, type of the stationary phase, etc.). For decades HPLC separations were based on a trial and error methodology. The traditional approach entails studying the influence of the corresponding factors by Changing One Single (or Separate) factor at a Time (COST), whilst keeping the others constant [15]. The technique, at times, is also known as OFAT (One Factor at a Time) [15–17]. Many years of experience have showed that these COST methods are inefficient and time consuming as they require a great amount of effort (planned experiments) and time without actually (in many cases) being able to identify the optimum conditions [15,18].

A great amount of effort has been made in order to overcome these inefficiency problems. Snyder suggested a systematic methodology for selecting the "best" mobile phase, based on

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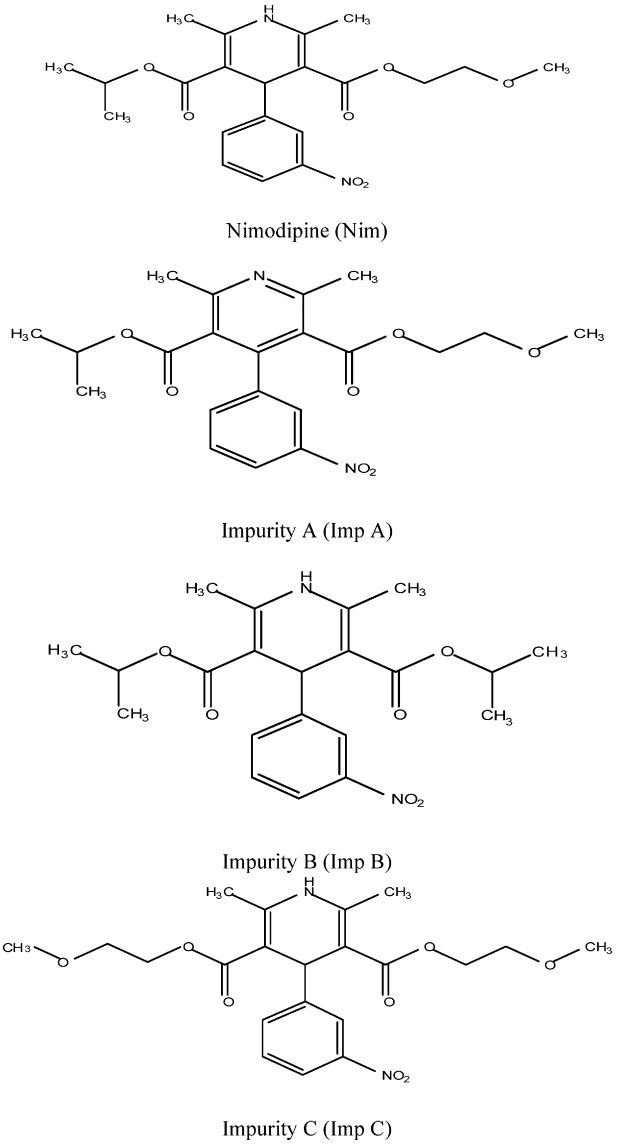

Fig. 1. Chemical structures of nimodipine and impurities A, B and C.

organic solvent's selectivity (Snyder's solvent-selectivity triangle) [19]. Although this method reduced (to some extend) the deficiency of COST methodology (using a systematic approach to identify optimum mobile phase type and composition) the rest of the factors were still being determined by trial and error. These drawbacks forced scientists to consider more efficient systematic optimization techniques such as experimental design.

The principles behind these techniques (known as Design of Experiments (DoE)), encompasses the use of experimental design, generation of mathematical equations and graphic outcomes [15]. Employing various rational combinations of factors, statistical experimental design fits experimental data into mathematical equations (known as models) in order to predict and optimize the examined responses. Examples of HPLC development and optimization attempts with the aid of DoE have shown important advantages [20–23].

The aim of the present paper was to develop and optimize an isocratic high-performance liquid chromatography method for the determination of nimodipine and impurities, using experimental design. The significance of the studied factors was evaluated with the aid of a full factorial design (full FD) whilst the optimum chromatographic conditions were estimated by a central composite design (CCD) using both a graphical (overlay contour plots) and a mathematical (Derringer's desirability function) global optimization approach. Finally, the proposed method was tested for linearity, specificity, inter and intra-day precision, accuracy, robustness and intermediate precision (using experimental design). Three commercially available nimodipine tablets were analyzed in order to check the validity of the proposed method.

2. Experimental

2.1. Apparatus

Experiments were performed on a Shimadzu Prominence HPLC system (HPLC 1) consisted of: degasser (Model DGU-20A5), pump (Model LC-20AD), total-volume injection-type auto-sampler (Model SIL-20AC), variable wavelength UV–vis detector (Model SPD-20A), and column oven (Model CTO-20AC). Chromatographic analyses were done on an Interchrom analytical column C8 (5 μm particle size, 250 mm × 4.6 mm I.D.). A C18 analytical column (150 mm × 4.6 mm I.D. and 3.6 μm particle size) was also tested but was unable to separate the examined substances. The entire HPLC system was controlled using LC solutions, ver. 1.21 SP1 PC software (Shimadzu Corporation, Kyoto, Japan). All eluents were filtered though 0.45 μm membrane filter (Whatman). The volume injected into the chromatographic system was 10 μl. UV detection was performed at 236 nm. A second Shimadzu Prominence HPLC system (HPLC 2) was used for intermediate precision, consisting of: communication bus module (Model CBM-20A), diode array detector (Model SPD-M20A), degasser (Model DGU-20A5), pump (Model LC-20AD), total-volume injection-type auto-sampler (Model SIL-20AC), and column oven (Model CTO-20AC).

2.2. Materials and reagents

Nimodipine and all impurities (A, B and C) were supplied by Union Quimico Farmaceutical S.A. (Barcelona, Spain). Acetonitrile and methanol (HPLC-grade) were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). The excipients used for specificity were: microcrystalline-cellulose (Avicel PH101, FMC International, Little Island, Cork, Ireland), starch (Sigma Chemical Co., Steinheim, Germany), povidone and crospovidone (BASF Co., Ledgewood, NJ), magnesium stearate (Katayama, Osaka, Japan), hydroxypropylmethyl-cellulose (Dow Chemical Co., Midland, MI, USA), polyethylene (CLARIANT, Sulzbach, Germany), iron oxide and titanium dioxide (Aldrich Chemical Co., Milwaukee, WI). Nimodipine tablet products (30 mg nominal content) were purchased form a local pharmacy store in Greece: Nimotop® (Bayer, Germany), Nimovac-V® (Pharmathen S.A., Greece) and Nortolan® (Anfarm, Greece). Double-distilled water was used during the analysis.

2.3. Standard solution

Stock standard solution of nimodipine and impurities (A, B and C) were prepared in methanol at a concentration of 2 mg/ml. The prepared stock solution was stored at 4 °C and protected from light. In the development and optimization phase, working mixture solutions of all compounds, containing 20 μg/ml of nimodipine and 300 ng/ml of each impurity, were freshly prepared by diluting the stock standard solution with methanol during the day of analysis.

2.4. Validation

The proposed method was validated according to ICH guidelines "Q2(R1), Validation of Analytical Procedures: Text and Methodology" [24]. For specificity study, placebo containing microcrystalline-cellulose, maize starch, povidone, crospovidone,