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Simple and validated UHPLC–MS/MS analysis of nimodipine in plasma and cerebrospinal fluid of patients with subarachnoid haemorrhage

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ABSTRACT

We present a simple, fast and validated method for the determination of nimodipine in plasma and cerebrospinal fluid (CSF) of patients with subarachnoid haemorrhage using ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS). Plasma or CSF 250 μL aliquots were pretreated with acetonitrile spiked with lacosamide as internal standard. The chromatographic separation was performed on a Fusion (3 μm) 50 × 2.0 mm I.D. column with gradient elution of 0.1% (v/v) formic acid in water and 0.1% (v/v) formic acid in acetonitrile at a flow rate of 0.35 mL/min. The MS/MS ion transitions were 419.1 → 343 for nimodipine and 251.1 → 91 for the internal standard. The linearity was determined from 2.0 to 40.0 ng/mL in plasma and 40.0–800.0 pg/mL in CSF. The lower limit of quantitation (LLOQ) of nimodipine was 0.4 ng/mL in plasma and 40 pg/mL in CSF. The mean recovery for nimodipine was ≥75% in plasma and ≥90% in CSF at all three considered concentrations. Intra- and interassay precision and accuracy were ≤15% at all quality control concentrations in plasma and CSF. The method was applied to measure plasma and CSF concentrations of nimodipine in a series of patients with subarachnoid haemorrhage treated with intravenous nimodipine. The present procedure, omitting time-consuming liquid-liquid extraction and drying steps, is faster, simpler and cheaper than published LC–MS/MS analytical methods for nimodipine in plasma and the first validated one for nimodipine in CSF.

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1. Introduction

Nimodipine (NIM), [3-*O*-(2-methoxyethyl) 5-*O*-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate], is a calcium channel blocker that crosses the blood-brain barrier with particular affinity for cerebral blood vessels. It is currently used in the prevention and treatment of ischaemic neurological deficits associated with cerebral vasospasm, in particular induced by aneurysmal subarachnoid haemorrhage (SAH) [1–4]. It is subject to extensive first-pass metabolism after oral intake, resulting in poor absolute bioavailability (around 10–15%) and low and highly variable intersubject plasma concentrations [5]. NIM can be administered both by enteral and parenteral route and controversy still remains about the preferential drug administration method in SAH patients [2,3,6]. Although oral administration is recommended by guidelines and reviews even to poor-grade patients [7,8], enteral administration has been related to lower and highly variable plasma drug concentration compared with the parenteral route, especially in patients with a decresead level of consciouness [2,3,6]. It has been suggested that the neuroprotective effect of NIM might be higher after parenteral administration, possibly due to matched higher plasma and cerebrospinal fluid (CSF) drug concentrations [3], but therapeutic plasma concentrations of NIM are not yet defined.

Several methods have been published for quantitation of NIM in human plasma, based on HPLC–MS/MS coupled with laborious sample liquid-liquid extraction [9–13]. To our knowledge, no validated method has been reported so far for the analysis of NIM in human CSF. Here we describe a fast UHPLC–MS/MS method with simple sample pretreatment with acetonitrile for the quantitative analysis of NIM in plasma and CSF, developed and validated for

Abbreviations: NIM, nimodipine; LCS, lacosamide; I.S., internal standard; SAH, aneurysmal subarachnoid haemorrhage; UHPLC, MS/MS ultra high performance liquid chromatography-tandem mass spectrometry; LLE, liquid-liquid extraction; CSF, cerebrospinal fluid; aCSF, artificial cerebrospinal fluid; LLOD, lower limit of detection; LLOQ, lower limit of quantification; QC, quality control; SRM, selected reaction monitoring; IV, intravenous.

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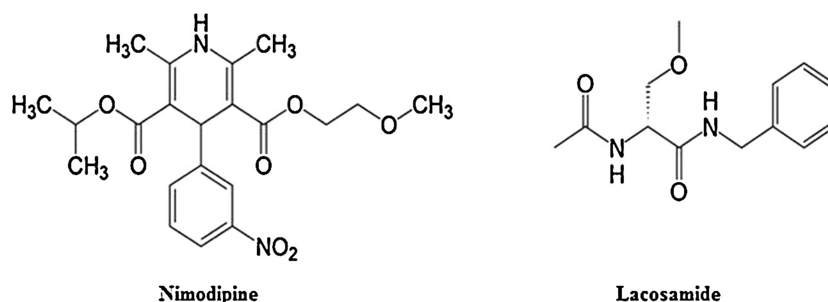

Fig. 1. Chemical structures of nimodipine and lacosamide (internal standard).

application in clinical pharmacological investigations and therapeutic drug monitoring in patients with SAH.

2. Experimental

2.1. Reagents and standards

Nimodipine (NIM) and the internal standard (I.S.) lacosamide (LCS) (1 mg/mL in acetonitrile) were purchased from Sigma Aldrich (St. Louis, MO, USA) (Fig. 1). HPLC grade acetonitrile and formic acid were purchased from Merck Millipore (Darmstadt, Germany). Ultrapure water was obtained from a MilliQ Gradient A10 apparatus (Merck Millipore).

Frozen, drug-free plasma (blank plasma) for the preparation of calibrators and quality control (QC) samples for NIM plasma assay was obtained from the blood bank of the Maggiore Hospital of Bologna, stored at −20 °C and thawed at room temperature before use.

Artificial CSF (aCSF) for the preparation of calibrators and QCs for NIM assay in CSF was prepared according to a published procedure [14] and stored at 4 °C.

2.2. Instrument and conditions

Quantitative analysis was performed using a triple quadrupole turbo ion-spray mass spectrometer (AB Sciex 4500 QTRAP, Concord, Ontario, Canada) coupled with UHPLC (Nexera X2, Shimadzu Corporation, Kyoto, Japan). The MS/MS analyses were carried out using selected reaction monitoring (SRM) in positive ionization mode. The chromatographic separation was performed with a Fusion, 50 × 2.0 mm I.D. column (Phenomenex, Torrance, CA, USA), protected by a Security guard precolumn (Phenomenex), at a temperature of 40 °C.

Injected samples were eluted over 4 min using a linear binary gradient mobile phase: eluent A was 0.1% (v/v) formic acid in water and eluent B was 0.1% (v/v) formic acid in acetonitrile. The elution was performed with a constant flow rate of 0.35 mL/min as follows: 0–2 min, 10% eluent B; 2–4 min, 10–95% eluent B; 4.1–6 min, 95–10% eluent B; 6.1–6.2 min, 10% eluent B. The ion spray voltage was set at +4500 V. The curtain and collision gas (nitrogen) pressures were set at 35 PSI and medium, respectively, the nebulizer and heater gas (air) pressures were set at 45 PSI for both, ion spray probe temperature was set at 300 °C, whereas the declustering, collision and entrance potentials were 75, 15 and 10 V for NIM; 40, 38 and 10 V for I.S. Detection of the ions was performed in the multiple-reaction monitoring mode, monitoring the transition pair of NIM at m/z 419.1 precursor ion to the m/z 343 and m/z 251.1 precursor ion to the m/z 91 product ions for I.S.

2.3. Preparation of standard solutions and quality controls

To prevent NIM photodegradation, all analytical steps, including standard solutions, calibrators, quality control (QC) preparation, blood and CSF collection and treatment were performed under dim light. Stock solution (1 mg/mL) and subsequent dilutions (1 μg/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL working solutions) of NIM were prepared in aluminum foil covered test tubes by dissolving the pure standards in methanol. All solutions were prepared monthly and stored at 4 °C.

Plasma calibrators at 2.0, 4.0, 8.0, 20.0, 40.0 ng/mL for NIM in plasma and 40.0, 80.0, 200, 400, 800 pg/mL for NIM in CSF were prepared by pipetting suitable amounts of working solutions of the analyte (100 ng/mL and 1 μg/mL for plasma, 1 ng/mL and 10 ng/mL for CSF) to 250 μL aliquots of blank pooled plasma or aCSF. Quality controls for method validation were similarly prepared using suitable volumes of the same NIM working solutions, to yield three drug concentrations (i.e. 2.0, 8.0, 40.0 ng/mL in plasma and 40.0, 200, 800 pg/mL in aCSF) corresponding to the low, middle and high QCs. Calibrators and QCs were prepared fresh for each batch and then treated exactly as patients' specimens.

2.4. Sample processing

Venous blood samples were collected by jugular access via a catheter used for haemodynamic monitoring. CSF was collected by external ventricular drainage inserted in SAH patients for neurointensive procedures. The protocol was approved by the Ethics Committee of Bologna-Imola Local Health Trust. Blood specimens were transferred into heparinized tubes (8 IU heparin/mL blood) and centrifuged at 1500g for 10 min at 4 °C. Separated plasma and CSF aliquots were transferred into aluminum foil covered test tubes and stored at −80 °C until analysis (within 3 months).

250 μL aliquots of plasma or CSF (calibrators, QCs, patient samples) were pretreated by addition of 750 μL (plasma) or 500 μL (CSF) acetonitrile spiked with I.S. (1.25 ng/mL for plasma or 0.625 ng/mL for CSF), vortexed for 30 s and then centrifuged at 3000g at 4 °C for 10 min. The supernatant was filtered (RC membrane filter 0.22 μm, Phenomenex) and 2 μL (plasma specimens) or 4 μL (CSF specimens) injected into the chromatographic system.

2.5. Method validation

Calibration curves for NIM in plasma and CSF were run on each analysis day (n = 6) over two months. The analyte to I.S. peak area ratios were plotted against NIM matched concentration added to the blank plasma or aCSF. The calibration curves were calculated by the least square method. Linearity was assessed by determining the coefficient of correlation (r) of the points of the curves.

The selectivity of the method towards endogenous plasma matrix components was assessed in 10 different pools of blank plasma.

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