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# LC–MS/MS determination of the HIV-1 protease inhibitor indinavir in brain and testis of mice

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**Abstract**

A rapid and sensitive method for the determination of indinavir in mice brain and testis is described and validation data are provided. Indinavir and the internal standard (IS) amprenavir were isolated from homogenized tissue matrices using a mixed-mode solid-phase extraction (SPE) procedure and were then analyzed by reversed-phase liquid chromatography/tandem mass spectrometry (LC–MS/MS). The mass spectrometer in the positive-ion multiple reaction monitoring mode used pairs of ions at m/z of 614.1/421.3 for indinavir and of 506.1/245.3 for IS. The calibration curves were linear over the range 0.0012–0.0390 μmol/kg for brain and 0.39-12.50 μmol/kg for testis. Linearity, repeatability and accuracy were validated. The applicability of the method was demonstrated by assessing indinavir in brain and testis of three mice dosed with intravenous bolus administration of indinavir (16.3 μmol/kg).


*Keywords:* Indinavir; Amprenavir; LC–MS/MS; Solid-phase extraction; Brain; Testis

## 1. Introduction

Protease inhibitors (PI), such as indinavir (Fig. 1a), constitute a major advancement in the treatment of infections by Human Immunodeficiency Virus (HIV), the etiologic agent of acquired immunodeficiency syndrome [1]. The incomplete eradication of HIV in brain and testis, and other so-called "viral sanctuaries", attributed to a poor distribution of anti-HIV drugs in these tissues, results in the impossibility to cure patients [2,3] and in the selection of resistant mutants [4]. The restricted penetration of PI into brain and testis is attributed to the P-glycoprotein (P-gp) [5,6], an ATP-dependent drug efflux pump encoded by the human MDR1 genes, which transports structurally-unrelated compounds out of the blood-brain and the blood-testis barriers [7,8]. Increased indinavir diffusion into these viral sanctuaries might be achieved by co-administration of P-gp inhibitors or by new drug formulations [9]. All these approaches require preliminary validation in laboratory animals by indinavir assessments in brain and testis. Chromatographic determinations of protease inhibitors have only been reported in liquid biological matrices like plasma [10–12], urine [13], saliva [14], cerebrospinal fluid [15] or semen [16,17], using UV [13–15,17] or spectrofluorimetric [16] detection or more sensitive mass spectrometric methods [10–12]. No report deals with indinavir determination in solid tissues. The paper presents a rapid solid-phase extraction procedure and liquid chromatography–tandem mass spectrometric (LC–MS/MS) assay validation for indinavir determination in mice brain and testis.

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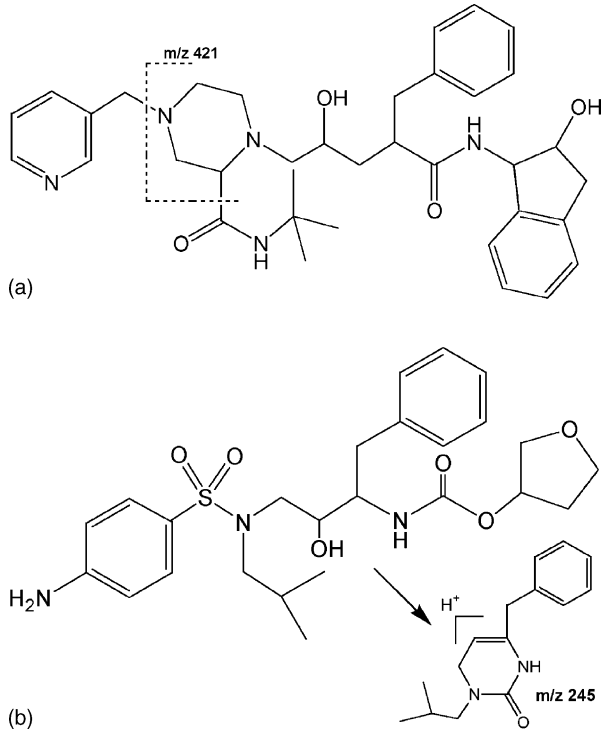


Fig. 1. Chemical structures of indinavir (a) and of the internal standard (IS) amprenavir (b) with their proposed product ions.

## 2. Experimental

### 2.1. Reagents and materials

Indinavir was extracted from Crixivan® tablets (Merck) as previously described by Li et al. [18]. The internal standard (IS) amprenavir (Fig. 1b) was kindly given by GSK (Hertfordchire, UK). Solvents were of HPLC grade and all other chemicals were of analytical grade. Highly purified water was produced using a MilliQ gradient® Plus Millipore system (St Quentin-en-Yvelines, France). Oasis® mixed-mode MCX 1 cc (30 mg) solid-phase extraction (SPE) cartridges were supplied by Waters (St Quentin-en-Yvelines, France).

### 2.2. Animals

The use of animals in this study was approved by the Animal Ethics Committee of the Poitiers Faculty of Pharmacy (BHE/2001/12/AF). Swiss mice (30 ± 2 g) from Janvier Laboratories (Le Genest St Isle, France) were housed in the animal breeding facility for 5 days before experiments. They were maintained in a light-controlled (12/12 h light-dark cycle) and temperature-controlled environment with water and food ad libitum.

### 2.3. LC–MS/MS procedure

#### 2.3.1. Apparatus

The chromatography system consisted of a Waters Alliance separations module 2695 (pump and injector) and a Micromass® Quattro micro API triple quadrupole mass spectrometer (Waters, St Quentin-en-Yvelines, France). Quantitative and qualitative analysis were performed using Masslynx® version 4.0 software.

#### 2.3.2. MS/MS parameters

The mass spectrometer was operated in the positive-ion mode with an electrospray capillary potential of 3 kV. The cone potential and collision energy settings for indinavir and amprenavir were 45 V/28 V and 32 V/19 V, respectively. The pressure of argon collision cell was $4.30 \times 10^{-3}$ mbar. The desolvation and cone gas flows were 200 L/h. The desolvation temperature was set at 300 °C. The mass spectrometer was operated in MS/MS mode using a multiple reaction monitoring to detect specific precursor and product ions of each analyte. The mass spectral Q1/Q3 transitions monitored for indinavir and amprenavir were 614.1/421.3 and 506.1/245.3 (*m*/*z*), respectively, according to Chi et al. [11]. The proposed chemical identity of the monitored product ions for indinavir [19] and amprenavir [20] are presented on Fig. 1. Instrument tuning parameters were optimized by infusing separately 100 ng/mL methanol solutions of each analyte into the LC–MS/MS interface using a built-in syringe pump set at a 10 μL/min flow rate in order to achieve the best signal-to-noise (S/N) ratio for each analyte.

#### 2.3.3. Liquid chromatography parameters

The column was a Nucleosil 100-5C18 (5 μm, 150 mm × 1 mm i.d., Macherey-Nagel, Hoerdt, France). A linear gradient was used to separate compounds. Solvent A was a 10 mmol/L ammonium formate aqueous solution, adjusted to pH 4.1 with formic acid, and solvent B a 0.1% (v/v) solution of formic acid in acetonitrile. They were filtered and degassed under vacuum. The flow rate was 0.1 mL/min. Injection volume was 10 μL. At each analysis, the A:B ratio, initially 45:55 (v/v), linearly ramped to 35/65 over 5 min and returned to 45:55 over 0.1 min. This condition was held for 5 min prior to the next injection.

### 2.4. Indinavir and amprenavir stock solutions

Stock solutions of indinavir (1 mM) and of IS (1 mM) were prepared in 10 mL volumetric flask by dissolving accurately weighed amounts in methanol. All stock solutions were stored at +4 °C and used within 6 months. The indinavir stock solution was appropriately diluted in methanol for spiking tissue samples (0.0061–0.1950 μmol/L range for brain and 0.78–25.00 μmol/L range for testis) in order to elaborate the calibration standard extracts. Concentration ranges were selected according to the concentrations expected in tested organs after the administration of usual doses of indinavir to animal experiments (16.3 μmol/kg) and were confirmed in preliminary studies in mice.