High Throughput determination of Levonorgestrel in human plasma using a Sensitive LC-MS/MS method

Cédric Hubert (1), Bruno Streel* (2), Renilde Sibenaler (3), Joëlle Widart (4), Attilio Ceccato (5), Philippe Hubert (1)

(1) Laboratory of Analytical Chemistry, CIRM, Institute of Pharmacy, University of Liège, B 36, B-4000 Liège, Belgium,
(2) Galephar, 39, rue du Parc Industriel, B-6900 Marche-en-Famenne, Belgium,
(3) SMB Laboratories, 26-28, rue de la Pastorale, B-1080 Bruxelles, Belgium,
(4) Department of Pharmacy, University of Liège, B 36, B-4000 Liège, Belgium,
(5) Odyssea Pharma, 16, Rue du Travail, B-4460 Grâce-Hollogne, Belgium.

The present LC method was developed for the monitoring of plasmatic concentration of levonorgestrel (LNG) after insertion of a hormonal intra-uterine device (IUD). In this study, very small plasmatic concentrations of LNG were expected, ranging from 500 pg/mL to 100 pg/mL. Consequently, the present method must be as sensible as possible. For this kind of concentration the liquid chromatography coupled to tandem mass spectrometry detection (LC-MS/MS) has proved its efficiency and usefulness. In this scope, the solid phase extraction (SPE) is generally recommended to avoid matrix effect as well as ion suppression.

Due to the hydrophobic character of the compound of interest, an end-capped octyl silica (C8(EC)) disposable extraction cartridge (DEC) was selected. The plasma sample was first diluted with orthophosphoric acid. Methyltestosterone, the internal standard (IS), was then added and the resulting sample was treated using a GILSON Multiple Probe 215 SPE system. This system processes eight samples simultaneously allowing a high throughput sample preparation. The elution was made in two times using 450 µL of methanol. 100 µL of 0.1 % formic acid in water were then added in order to reconstitute mobile phase.

The separation was carried out on a Phenomenex phenyl-hexyl column (150 x 4.6, 5 µm) using a mobile phase consisting in a mixture of methanol and formic acid 0.1 % in water (90/10, v/v). Using these LC conditions, a short run time was obtained. The mass transition 313.4/245.1 and 303.0/97.1 for levonorgestrel and IS was respectively selected.

The coupling of the GILSON Multiple Probe 215 SPE system with the LC-MS/MS conditions allows a high throughput determination of levonorgestrel in a complex matrix (> 100 samples per day).

Afterwards, the validation of the method was considered using an approach based on the accuracy profile [1-2] allowing to manage the risk associated to the use of these methods in routine analysis [3].