

1 Objectives

In the framework of the monitoring of the plasmatic concentration of levonorgestrel (LNG) during the use of an hormonal intra-uterine device (IUD), there is a need to develop a highly sensitive method in the pg/mL range. Consequently, the objectives of this work are :

- Optimization of the solid phase extraction (SPE) conditions, in order to obtain a specific and quantitative retention of the LNG from endogenous compounds
- Optimization of chromatographic (LC) and ionization (MS/MS) conditions
- Validation of the method using the β -expectation tolerance interval of the total error as decision tool [1].

2 Sample preparation by SPE

Conditions of SPE

- Sorbent: C2 – 50 mg (1 mL)
- DEC first conditioning: 1.0 mL of methanol (MeOH)
- DEC second conditioning: 1.0 mL of water LC grade (H₂O)
- DEC Load: 2.0 mL of diluted plasma (1:1)
- DEC first wash: 1.0 mL of H₂O
- DEC second wash: 1.0 mL of H₂O/MeOH/NH₃ (90/10/0.2)
- DEC first elution: 200.0 μ L of MeOH
- DEC second elution: 250.0 μ L of MeOH

Quantitative extraction

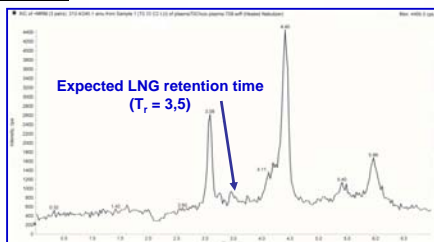
The Disposable Extractive Cartridge (DEC) with C2 sorbent (50 mg) was selected on basis of its excellent recovery and its god selectivity.

Concentration of LNG (pg/mL)	Number of repetition (n)	Recovery (%)	Standard deviation (%)
53.0	4	100.2	16.1
106.6	4	81.9	14.1
266.5	4	81.5	4.4
533.0	4	79.3	4.3
1066.0	4	77.2	8.2

Table 1 : Recovery obtained with C2 DEC at five concentration levels in plasmatic matrix.

Global LNG recovery : **83.4 \pm 11.8 %** (IS: 94.9 \pm 6.0 %)

Specific extraction Figure 1 : Chromatogram obtained for plasmatic matrix.



3 LC/MS-MS

Optimization of the mobile phase composition and of the APCI ionization source temperature

The proportion of the organic modifier and the concentration of an ionic compound (formic acid or ammonium acetate) influence the ionization of the analyte. The influence of the APCI ionization source temperature was also investigated.

Reference: [1] The AAPS Journal 2007; 9(1) Article 4 (<http://www.aapsj.org>)
Themed Issue: Bioanalytical Method Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays

Composition of mobile phase				Temp. of ionization source (°C)	Intensity of response (counts)
% of acetonitrile	% of methanol	% of HCOOH 0.1 %	% of NH ₄ Ac (pH = 4)		
45	35	0	20	300	4241
45	35	20	0	300	23500
45	35	20	0	350	29000
45	35	20	0	400	35667
70	0	30	0	400	28000
0	90	10	0	400	56000

Table 2 : Intensity of response considering the effect of the mobile phase composition and the ionization source temperature. The concentration of LNG was set to 99.13 ng/mL.

Conditions of chromatographic separation and tandem mass spectroscopic detection

- Column: Luna® Phenyl-Hexyl Phenomenex 150 mm x 4,6 mm (5 μ m)
- Mobile phase: Methanol/formic acid 0.1 % (water) at 90/10 (v/v)
- Flow: 0.8 mL/min.
- Temperature: 35 °C.
- Injection volume: 100 μ L.
- Run time: 7 minutes.
- Ionisation source: APCI in positive mode
- Acquisition mode: MRM (Multiple Reaction Monitoring)
- LNG transition: 313,4 / 245,1
- Internal standard (Methyltestosterone) transition: 303,0 / 97,1
- Source temperature: 400 °C

4 Pre-validation

The dosing range considered in the pre-validation phase (based on accuracy profile) was 50 pg/mL to 1000 pg/mL in order to largely cover the plasmatic concentration of LNG attempted in a clinical trial (ranging 300 pg/mL to 500 pg/mL).

The adequate standard curve was the weighted (1/X²) linear regression. The risk α was set to 5 % with acceptance limits at 20 % [1].

Results

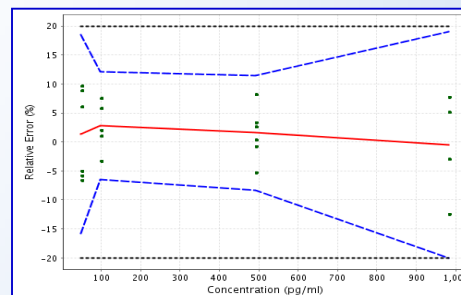


Figure 2 : Accuracy profile

Conc. of LNG (pg/mL)	Intermediate precision (RSD%)	Relative bias (%)
49.05	7.4	1.3
98.10	4.0	2.8
490.50	4.3	1.6
981.00	8.0	-0.5

Table 3 : Results of intermediate precision and trueness

5 Conclusions

The results obtained in the pre-validation phase augur the promising adequacy of the method regarding to its objective. A formal validation will be considered as soon as possible in order to confirm these results and allow its routine use.