Contraception 100 (2019) 247–249

Contents lists available at ScienceDirect

Contraception

journal homepage: www.elsevier.com/locate/con

Original research article

A sensitive method detecting trace levels of levonorgestrel using LC-HRMS☆

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ARTICLE INFO

Article history:
Received 22 March 2019
Received in revised form 16 May 2019
Accepted 2 June 2019

Keywords:
Liquid chromatography
High resolution mass spectrometry
Solid phase extraction
Intrauterine device
Lower limit of quantification

ABSTRACT

Objective: To develop a high resolution mass spectrometry (HRMS) method to quantify levonorgestrel (LNG) in serum.
Study design: Levonorgestrel was extracted using solid phase extraction and measured using liquid chromatography (LC) HRMS.
Results: Low limit of quantification (LLOQ) was 25 pg/mL and low limit of detection (LLOD) was 12.5 pg/mL. Precision and accuracy bias were <10%. LNG in serum samples from Mirena® users ranged between 37 to 219 pg/mL (n=12). In eight out of 22 patients with suspected intrauterine device (IUD) expulsion LNG was detected (26–1272 pg/mL).
Conclusion: A sensitive, fast and simple LC-HRMS method was developed to detect trace levels of LNG.

1. Introduction

Levonorgestrel (LNG) is a synthetic birth control drug used in intrauterine devices (IUD). Well known complications with LNG-IUD include when the device becomes embedded in the myometrium, perforates the wall of the uterus or becomes expulsed [1]. If LNG-IUD is located in its normal position it can be visualized with trans-vaginal ultrasound (TVUS), but if perforation has occurred it is not easily seen with TVUS but can usually be localized by X-ray or magnetic resonance imaging (MRI).

Low resolution triple quadrupole mass spectrometry (MS/MS) is routinely used in clinical settings and modern high resolution mass spectrometry (HRMS) has started to gain more attention for use in the clinic [2–4]. There are MS/SM methods utilizing liquid–liquid extraction (LLE) (low limit of quantification, LLOQ of 49.6 pg/mL) [5] and supported liquid extraction (LLOQ of 20 pg/mL) [6] to measure LNG in plasma and serum, respectively. There are also radioimmunoassay (RIA) (LLOQ of 50 pg/mL) [7] and commercial enzyme immunoassay kits (EIA) are available to measure LNG (LLOQ of 6 pg/mL), but the EIA kit is not for clinical use.

Here we present a fast and simple method using solid phase extraction (SPE) in combination with LC-HRMS to detect trace levels of LNG in serum. At Uppsala University Hospital, Sweden, we use this approach as a complementary assay in cases of suspected LNG-IUD expulsion.

2. Materials and methods

We prepared 400 μL of serum using SOLA® SCX 10 mg/1 mL (Thermo Scientific™) and analyzed using an Ultimate 3000 HPLC (Dionex, Thermo Scientific™) equipped with an Accucore™ C18 column (2.6 μm: 100 mm×2.1 mm) and coupled to a high-resolution Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer (Thermo Scientific™). Detailed method description is found in supplemental information.

To investigate method performance around LLOD and LLOQ, LNG concentration was measured up to 78 h after removal of a Mirena® LNG-IUD. To establish a typical serum LNG range in Mirena® users the levels were measured in 12 self-reported users. Finally, serum LNG was measured in 22 patients that had undergone gynecological examination including a vaginal ultrasound for suspected LNG-IUD expulsion.

☆ Declarations of interest: none

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https://doi.org/10.1016/j.contraception.2019.06.001
0010-7824/

Table 1
Precision (%CV) and accuracy (%bias) data for LNG (inter- and intra-assay statistics) measured at two levels; QCL 40 pg/mL and QCH 100 pg/mL. Stability data of LNG for processed and unprocessed samples under different conditions; freeze–thaw cycles (unprocessed samples), bench-top (unprocessed samples) and autosampler stability (processed samples). All the stability conditions were evaluated using freshly prepared calibration curve.

	Nominal concentration (pg/mL)	Mean (pg/mL)	SD (pg/mL)	CV (%)	Bias (%)	n	Temp °C	Time (hours)
Interday								
LLOQ	25	24.8	0.6	2.5	−0.7	6	NA	NA
QCL	40	38.7	3.4	8.7	−3.2	13	NA	NA
QCH	100	93.4	5.3	5.7	−6.6	12	NA	NA
Intraday								
LLOQ	25	NA	NA	NA	NA	NA	NA	NA
QCL	40	39.2	2.5	6.3	−2.1	3	NA	NA
QCH	100	95.1	2.8	3.0	−4.9	3	NA	NA
Stability test								
Freeze–thaw	91.7	89.2	0.4	0.4	−3.0	2	−20	NA
		89.5	0.7	0.8	−2.7	2	−70	NA
Bench-top	48.9	46.7	2.0	4.3	−4.5	3	25	NA
Autosampler	39.2	43.7	1.4	3.2	11.5	3	10	24
		38.4	1.1	2.8	−2.0	3	10	72
	95.1	106.4	1.7	1.6	11.9	3	10	24
		93.4	2.1	2.2	−1.9	3	10	72

3. Results

The linear range was between 25 and 500 pg/mL with the coefficient of determination (R^2) ≥0.991. The LLOD and LLOQ were 12.5 pg/mL and 25 pg/mL, respectively. Precision expressed as %CV and the accuracy expressed as %bias for intraday and interday were within ±10% at quality control low (40 pg/mL, QCL) and quality control high (100 pg/mL, QCH). The stability tests show that unprocessed samples were stable after three freeze–thaw cycles; the % bias was within ±5% from the nominal value at 91.7 pg/mL. Bench-top stability was demonstrated over 4 h with the largest deviation of −4.5% from the nominal concentration at 48.9 pg/mL. Processed serum samples demonstrated stability for at least 72 h at 10 °C, with the greatest deviation of −11.9% from the first result (95.1 pg/mL, QCH). Storage of serum samples for longer time periods (here tested for 11 months) needs to be kept at −70 °C, while keeping them at −20 °C caused a higher background and interference. A summary of the results and chromatogram examples at LLOD and LLOQ are found in Table 1 and Fig. 1. A more elaborate description of the validation results is found in the supplemental information.

After removal of a Mirena® LNG-IUD, the level decreased from 150 to 43.9 pg/mL within 30 h and after 54 h it was below the LLOQ of 25 pg/mL (detectable but not quantifiable). After 78 h, LNG could no longer be detected. The results of self-reported Mirena® LNG-IUD users (n=12), demonstrated that the LNG concentrations ranged between 37 and 219 pg/mL (121±66.9 pg/mL) (supplemental Fig. 1). Finally, in eight patients out of 22 who had undergone gynecological examination for suspected IUD expulsion LNG was detected at (26, 113, 117, 136, 219, 450, 600 and 1272 pg/mL). Two of these patients were pregnant at the time of sample collection.

4. Discussion

Here we present a highly sensitive, fast and simple LC-HRMS method to detect trace levels of LNG in serum. The presented method is as sensitive as previously reported methods using LLE and supported liquid extraction in combination with low resolution triple quadrupole mass spectrometry [5,6]. SPE is practical to use since it is fast, consumes limited amounts of solvent and the extracts can be more concentrated and specific compared with LLE. The retention mechanism used for the SPE here was strong cation exchange which helped reducing the interferences from the matrix. The advantage of HRMS is that it can detect minute differences in mass allowing quantification based on precursor and/or product ions. Here, the lowest LLOQ and LLOD were achieved based on precursor ion quantification. The LNG levels found in users of Mirena® is in line with previous results [5] [6], showing a high degree of interindividual variation and no apparent correlation between LNG levels and the number of carrying months. In 14 out of 22 cases of suspected IUD-LNG expulsion, we could not detect any LNG. These findings suggested that the IUD had been expulsed and limited the need for further investigations. In two of the cases where the patients in fact were pregnant, the assay helped clinicians avoid the use of X-ray for diagnosis.

A single case report has reported that an intra-peritoneal dislocated Mirena® LNG-IUD resulted in plasma levels 10 times higher (4.7 nmol/L, corresponding to 1470 pg/mL) than the plasma level of LNG observed with LNG-IUD placed *in utero* [8]. Here we found that in the suspected LNG-IUD expulsion cases the levels can differ up to 50 times between the lowest (26 pg/mL) and highest observed concentrations (1272 pg/mL). The main limitation of this assay as a clinical test is thus that the lowest level of LNG in circulating blood where the LNG-IUD has been embedded or perforated the wall of the uterus is unknown. In current study we have no clinical data on outcomes of individual patients. At Uppsala University Hospital, we use this analysis as a complementary assay in cases of suspected LNG-IUD expulsion. This will increase our knowledge regarding LNG levels in circulating blood when the LNG-IUD has been dislocated, which is an important aspect regarding usefulness of the assay. Also, further studies that correlate LNG levels to clinical outcomes are needed to assess the general utility of the now proposed assay.

Funding sources

This work was supported by Region Uppsala (ALF-grant and R&D funds). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.contraception.2019.06.001.

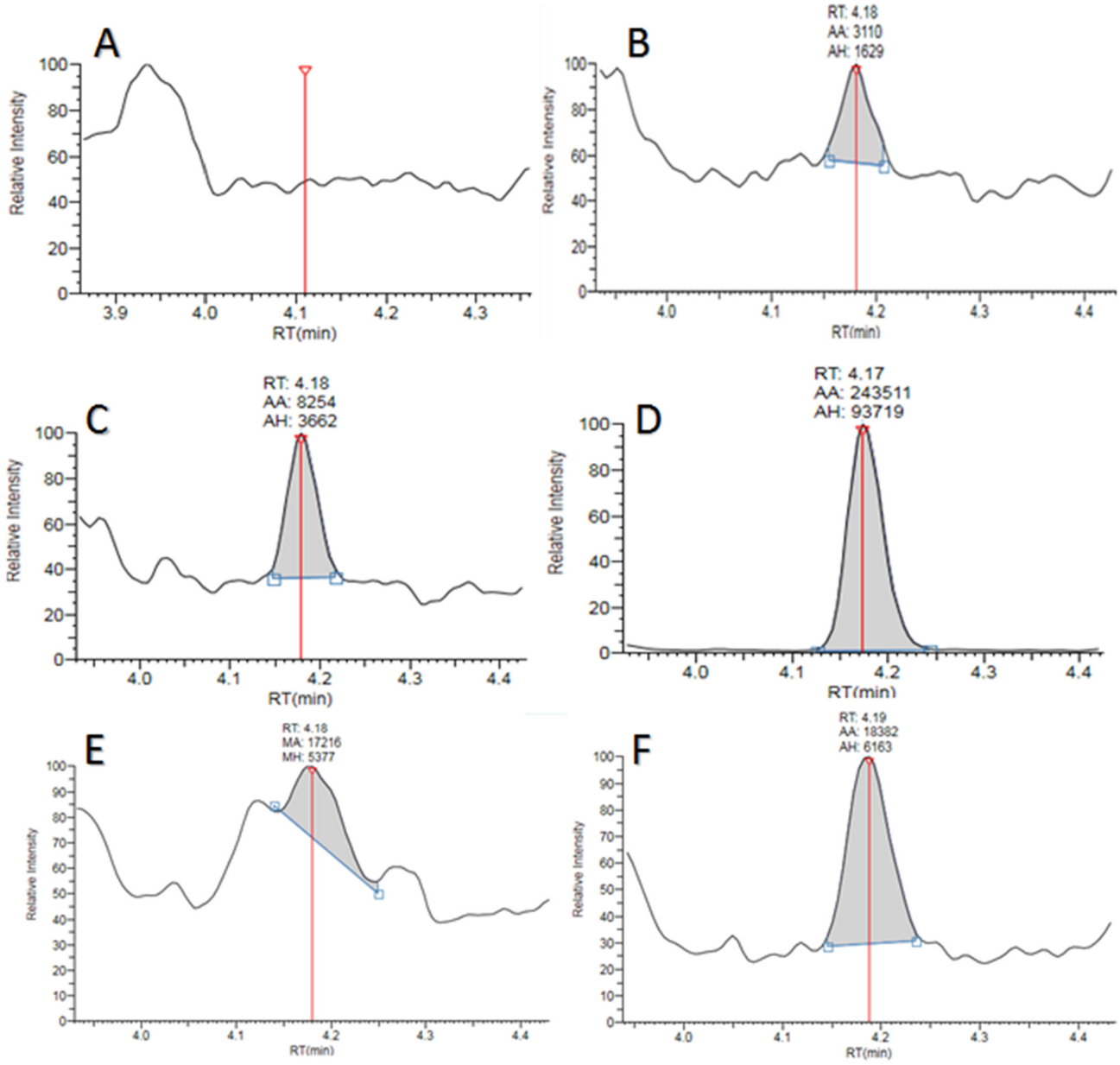

Fig. 1. Levonorgestrel (LNG) chromatograms of (A) a blank sample, the amount of background interference at the analyte retention time was less than 20%; (B) a spiked sample at the low limit of detection LLOD; 12.5 pg/mL; (C) a spiked sample at the low limit of quantification LLOQ, 25 pg/mL. (D) A typical LNG sample from a patient. (E&F) demonstrate results of long-term stability for the same sample when stored at −20 °C and −70 °C for 11 months. In (E), the sample kept at −20 °C shows high background and interference, while in (F), the storage was at −70 °C, demonstrating low background and good peak shape.

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