Detection of the abortifacient mifepristone (Mifegyne, RU-486) in a hair sample after illegal administration

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Aim: Mifepristone (11β-[p-(Dimethylamino)phenyl]-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one) is a synthetic steroid and used as an abortifacient in the first months of pregnancy. In Germany, mifepristone is only available on prescription via institutions responsible for abortion. Mifepristone was supposed to be intentionally administered by food to a pregnant woman without her knowledge. Subsequently, the women suffered from several adverse effects (sickness, vomiting, bleeding). Ten weeks after the occurrence, the analysis of a hair sample should be carried out to verify the supposed intake of mifepristone by segmental hair analysis. **Methods:** The analysis of the hair sample (total length 40 cm, color brown) was processed in four segments (0-3 cm, 3-6 cm, 6-9 cm, and 30-40 cm). The snipped hair material was extracted by methanol. Detection was carried out by a LC-MS/MS instrument (ABSciex QTrap 5500) on a RP-C18 column (LOD 5 pg/mg). In addition, confirmation was realized by LC-QTOF (ABSciex TripleTOF 5600+) to take advantage of fragment identification at high resolution. **Results:** Mifepristone was detected in the two proximal segments (length 0-3 cm: 10 pg/mg, and 3-6 cm: 5 pg/mg, respectively). The analyses of the segments 6-9 cm and 30-40 cm resulted in negative findings. **Conclusion:** The period of the presumed mifepristone uptake (10 weeks before sampling) is covered by the proximal hair segment (length 0-3 cm), and thus characterized by the highest mifepristone concentrations. Therefore, the results of the segmental analysis of the hair strand are in accordance with the supposed circumstances of the offense. However, retrospective temporal interpretations may well be affected by hair incorporation (contamination) via sweat and/or washout, which are known to be common mechanisms in cases of structurally similar compounds, e.g. anabolic steroids.

1. Introduction

Mifepristone (11β-[p-(Dimethylamino)phenyl]-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one; Figure 1) is a synthetic steroid with antiprogesterone as well as antiglucocorticoid properties. Due to its antagonistic interaction at the progesterone receptor, mifepristone is used for terminating pregnancy in the first months. To induce abortion, the application of mifepristone (causes dilatation of the cervical os, shedding of endometrium) is followed by a prostaglandine, e.g. misoprostol (effects uterine contraction), after 36 to 48 hours [1]. Mifepristone is approved in many countries worldwide for abortion, and legally available as a prescription drug (e.g., USA [2], Europe with exception of Ireland and Poland [3]). In Germany, mifepristone is only available on prescription via institutions responsible for abortion (according §47a AMG).

2. Case report

Mifepristone was supposed to be intentionally administered by food to a pregnant woman without her knowledge. Her partner was accused of having illegally obtained mifepristone tablets in Thailand and intentionally applied them to terminate the pregnancy.
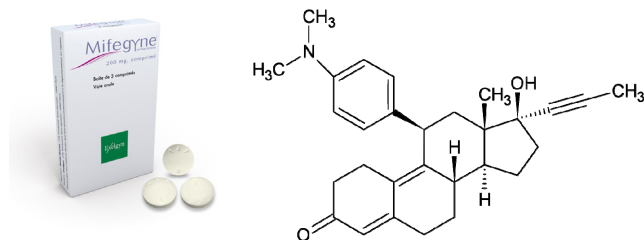
Fig. 1. Mifegyne® and structure of mifepristone.

Following the uptake (mifepristone was added to a Chinese meal), the women suffered from several adverse effects such as nausea, vomiting, discharge and bleeding. The subsequent administration of a prostaglandine drug did not take place, therefore the abortion was unsuccessful.

The affected woman has been informed about the offense afterwards by another person (then-girlfriend of the defendant). Ten weeks after the occurrence, the analysis of a head hair sample should be carried out to verify the supposed uptake of mifepristone by segmental analysis.

3. Material and Methods

3.1. Preparation of hair material

To differentiate the period of the suspected application, the analysis of the hair sample (total length 40 cm, color brown) was processed in four segments (0-3 cm, 3-6 cm, 6-9 cm, and 30-40 cm). After a wash step (methanol/water, 1:1, v/v), the hair material was dried and snipped. Deuterated nandrolone (d3-nandrolone) was used as internal standard (10 pg/mg). Extraction of the hair material (weight 50 mg) was realized by methanol in an ultrasonic bath. The residue of the evaporated methanolic phase was reconstituted using 40 µl of the HPLC buffer (2 mmol ammonium acetat /ACN, 20:1, v/v, 0.1% acetic acid). In addition, 1 ml of the wash solution was prepared in accordance.
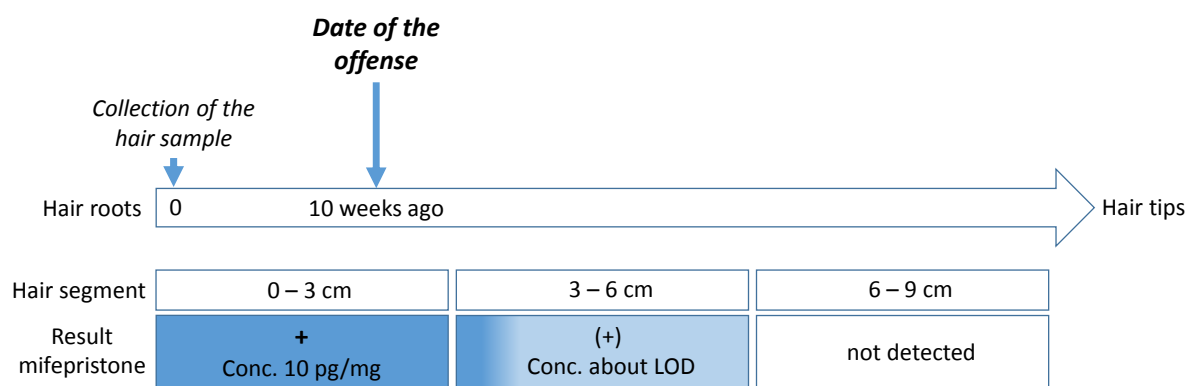

Fig. 2. Chart of the segmentation of the hair sample in relation to the suspected date of the offense, and detection of mifepristone in the proximal segments (segment 30-40 cm not pictured).

3.2. Reference material

Mifepristone was extracted from a tablet (Mifegyne® 200mg, Nordic Pharma, Ismaning). Positive controls were prepared utilizing hair blank material (child) spiked with the following concentrations of mifepristone: 5; 10; 20 pg/mg.

3.3. Detection methods

Detection was carried out by a LC-MS/MS instrument (ABSciex QTrap 5500) in +MRM mode. The following fragmentation reactions were monitored for mifepristone: 430.2/372.2; 430.2/415.2; 430.2/288.2; 430.2/236.0; 430.2/172.0. Chromatographic separation was carried out on a RP-C18 column (Zorbax C18, 2.1 x 50 mm, 3.5 µm; Agilent). The gradient was composed of 2 mmol ammonium acetat and ACN (with 0.1 % acetic acid): starting at 25% ACN, increasing to 100% ACN at 6 min (held for 2 min), flow rate 250 µl/min. Mifepristone was detectable at a retention time of 5.75 min (Figure 3). The LOD was estimated at 5 pg/mg, taking the S/N ratio greater than 3 into account (evaluating the fragmentation 430.2/288.2). In addition, confirmation was realized by LC-QTOF (ABSciex TripleTOF 5600+) to take advantage of fragment identification at high resolution.
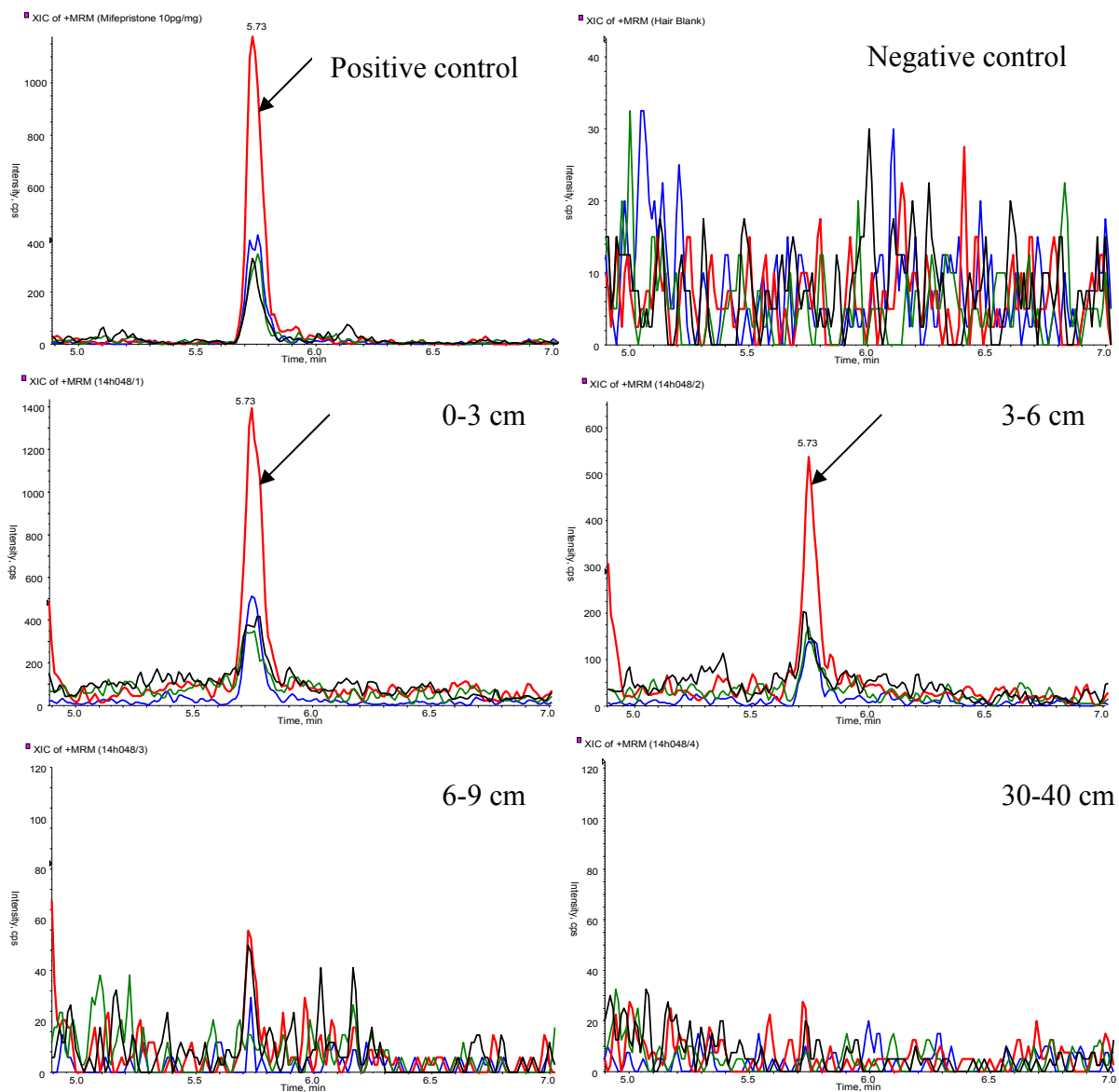
Fig. 3. HPLC-MS/MS detection of mifepristone in the hair extract (red line: fragment 430.2/372.2; blue: 430.2/415.2; green: 430.2/288.2; black: 430.2/236.0). First row: positive hair control sample (spiked with 10 pg/mg mifepristone) and negative control (hair blank child). 2nd and 3rd row: Hair segments (length 0-3; 3-6; 6-9; 30-40 cm) of the suspicious sample, and identification of mifepristone in the proximal segments 1 and 2.

4. Results and Discussion

Mifepristone was detected in the proximal segment (0-3 cm) with a concentration of 10 pg/mg. Additionally, the analysis of segment no. 2 (3-6 cm) resulted in a positive mifepristone finding, with a significant lower concentration (closed to the LOD). The segment no. 3 (6-9 cm) as well as the distal segment no. 4 (30-40 cm) were both tested negative (Fig. 2 and 3).

Furthermore, the identification of mifepristone has been carried out by LC-QTOF technique (Fig. 4). The fine mass 372.226 representing the fragment resulting from dealkylation and loss of water of the intact precursor (m/z= 430.2) was recorded for confirmation.
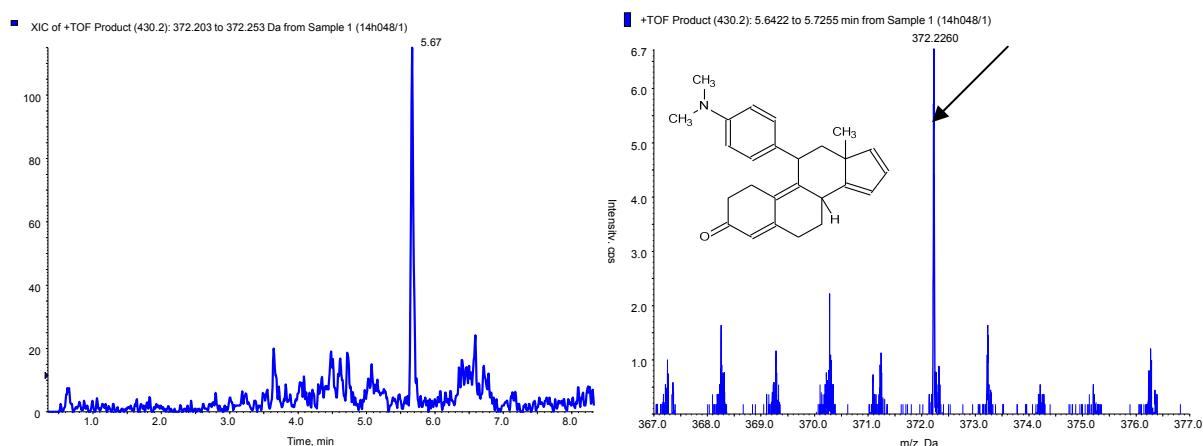

Fig. 4. HPLC-MS/HRMS recording of the fragmentation 430.2/372.23 to confirm the presence of mifepristone in the proximal segment.

The finding of mifepristone in the proximal hair segment verified the ingestion within a period of about 3 months prior sampling, taking an average hair growth rate of 1 cm per month into account [4]. The detection of a significant lower concentration of mifepristone (about LOD) in the adjacent segment may be compatible with the excretion by sweat and sebum, and the subsequent incorporation into the hair shaft. Due to the structural similarities to steroids, this well-known incorporation pathway may also be likely for mifepristone [5-8].

Another interpretation for the occurrence of mifepristone in both segments would be the individual hair growth rate of the subject, which may vary compared to the population's average.

5. Conclusions

The period of the presumed mifepristone uptake is covered by the proximal hair segment (length 0-3 cm), and thus characterized by the highest mifepristone concentrations. Therefore, the results of the segmental analysis of the hair strand are in accordance with the supposed circumstances of the offense. However, retrospective temporal interpretations may well be affected by hair incorporation (contamination) via sweat and/or washout, which are known to be common mechanisms in cases of structurally similar compounds, e.g. anabolic steroids.

6. References

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