Cortisol everywhere!

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Problem

On a Friday afternoon, when running the first solvent blanks on our instrument and before the System Suitability Test (SST), we observed a huge contamination of cortisol, leading to peaks with an intensity between 10^6-10^7 cps. Our field service engineer checked the proper function of the instrument and removed all contamination traces. However next Monday, contamination reappeared.

Troubleshooting Steps

Instrument decontamination

In a first approach, a thorough cleaning of the instrument was performed, which included the following steps:

1. Replacement of the rinse port cap of the injector
2. Cleaning the outside of the injector needle and needle seal with isopropanol
3. Wash settings from the autosampler were modified by increasing the Rinse dip time to 5 seconds.
4. Injection of several acetonitrile blanks in order to clean the system

→ These approaches permitted to completely clean the instrument.

Study of possible contamination sources

When we observed again the contamination we studied all the possible contamination sources (after decontamination of the system) by individual injection of the suspect solutions in LC-MS glass vials. (See table 1)

1. Ammonium acetate at 10mM and 1N used for sample and mobile phase preparation
2. Mobile phases A and B
3. Injection of water and methanol from different recipients used at the lab (glass/plastic)
4. Internal standard
5. Phosphate Bufffer Saline (PBS) with 0.1% of Bovine Serum Albumin (BSA) from different sources
6. Injection of air from empty vials using another C18 colum
7. Study of the identity of the contamination source in a contamination soluton of PBS with BSA by QTOF

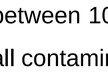


Figure 2. Library match for the contaminated sample

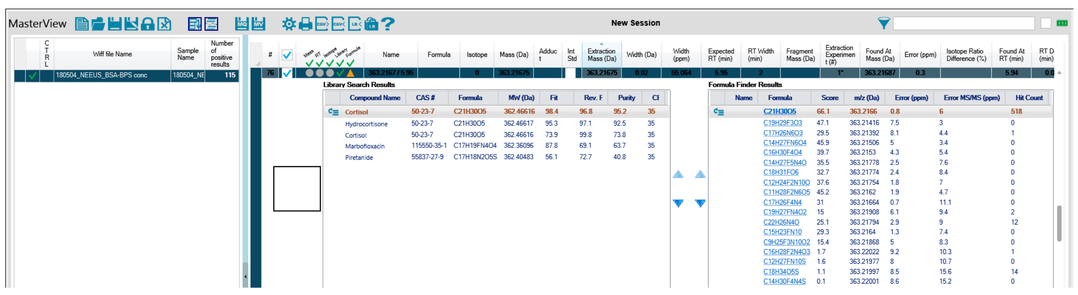
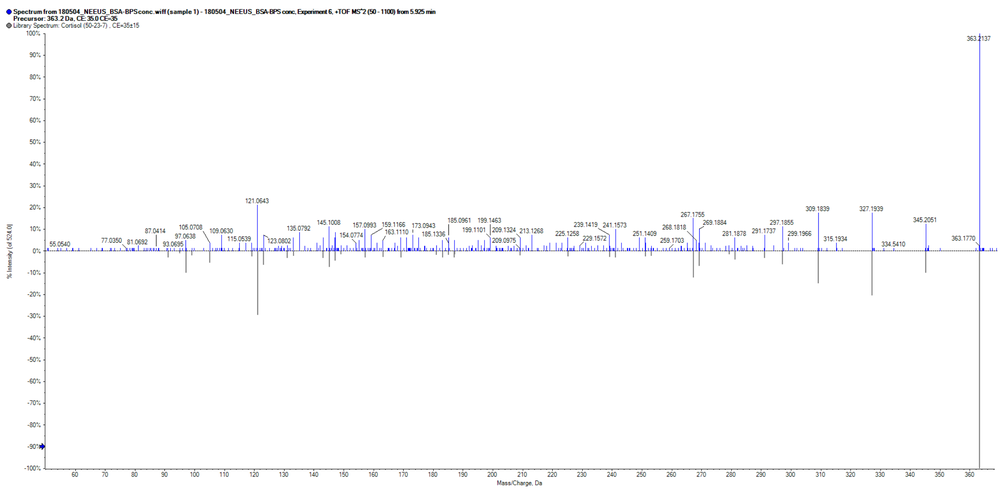


Figure 3. Comparative between the sample spectrum and the library spectrum



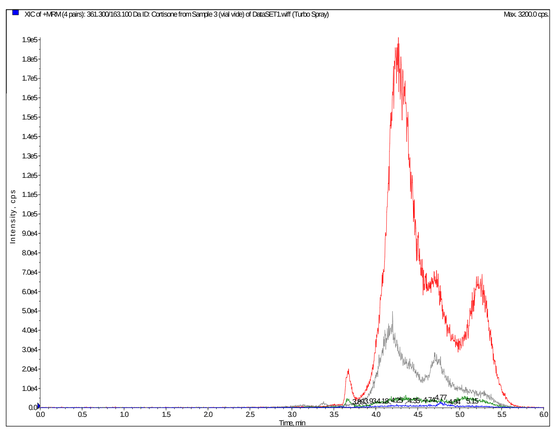


Figure 4. Chromatogram of the injection of a vial containing acetonitrile for cleaning the system.

Method Information

Instrumental conditions

UHPLC: Shimadzu Prominence UFLCXR

- Column A: Nucleoshell RP C18 (Macherey-Nagel) (50 x 2mm, 2.7µm)
- Column B: Gemini NX C18 110A (Phenomenex)(100x2mm, 3µm)
- Gradient LC program, Flow: 0.375 mL/min
- Injection volume: 5 µL
- Column oven 40 °C
- Mobile phases:
  - A: H_2O 0.1% HCOOH + ammonium acetate 2mM
  - B: MeOH

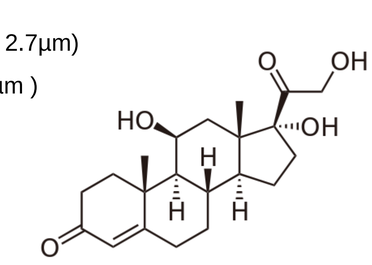


Figure 1. LC-MS(QqQ) used for the measurements

MS: Sciex QTRAP 5500

- Quadrupole-linear ion trap working triple quadrupole mode.
- Electrospray ionization in positive mode (ESI+)
- Multiple Reaction Monitoring (MRM) (363 >121 and 363 > 91).

Sample preparation

- 100 µL Urine with 10 µL Internal Standard (IS) and 400 µL ammonium acetate extraction with ethyl acetate
- or 50 µL of serum with 20µL of IS and 1600 µL of MTBE for the extraction

Table 1. Comparative table with the different reagents and conditions tested for the contamination study

Sample Name	mobile phase composition	Column	Results
Empty vial	with modifiers (new preparation)	A	yes (Rt 3.68)
Acetonitrile LCMS	with modifiers (new preparation)	A	yes (Rt 3.59) (see Fig. 3)
Acetonitrile LCMS	with modifiers (new preparation)	A	yes (Rt 3.59) decreased peak area
Acetonitrile LCMS	with modifiers (new preparation)	A	yes (Rt 3.59)contamination peak is almost gone
Acetonitrile LCMS	without modifiers	A	no
Empty vial	without modifiers	A	no
Empty vial	without modifiers	B	no
Ammonium acetate 10 mM (old preparation)	without modifiers	B	yes (Rt 5.96)
Ammonium acetate 1 N (old preparation)	without modifiers	B	yes (Rt 5.95)
Mobile phase A (theoretically contaminated)	without modifiers	B	yes (Rt 5.95)
Mobile phase B (theoretically contaminated)	without modifiers	B	yes (Rt 5.93)
H2O LCMS directly from a new bottle	without modifiers	B	no
Rinsing plastic bottle	without modifiers	B	yes (Rt 5.95)
Rinsing plastic bottle (after being cleaned)	without modifiers	B	no
H2O LCMS from shared Narrow Mouth bottles, amber	without modifiers	B	yes (Rt 5.95)
Methanol LCMS directly from a new bottle	without modifiers	B	no
New ammonium acetate soliton 10 mM 18/04/20	without modifiers	B	no
Methanol LCMS from shared narrow mouth bottles, amber	without modifiers	B	yes (Rt 5.93)
PBS with BSA 13/04/18	without modifiers	B	yes (Rt 5.95)
Ethyl acetate	without modifiers	B	no
Methanol LCMS from used for redissolution (aliquote)	without modifiers	B	no
H2O LCMS used for redissolution (aliquote)	without modifiers	B	no
Internal standard d4-cortisol 1 µg/ml	without modifiers	B	yes (Rt 5.95)
Ammonium acetate 1 N new preparation	without modifiers	B	yes (Rt 5.94)
H2O LCMS used for redissolution (aliquote)	without modifiers	B	no
Ammonium acetate 10 mM (new preparation)	without modifiers	B	no
PBS with BSA (old preparation)	without modifiers	B	yes (Rt 5.96)
Mobile phase A (new preparation)	without modifiers	B	yes, but very small (Rt 5.92)
Mobile phase B (new preparation)	without modifiers	B	yes, but very small(Rt 5.92)
PBS solution (new preparation)	without modifiers	B	no
PBS solution with BSA (new preparation)	without modifiers	B	yes (Rt 5.94)
PBS solution (new preparation)	without modifiers	B	no
PBS 0.1% BSA (new bottle of BSA) 26/04/18	without modifiers	B	no
Empty vial	without modifiers	B'	no
Empty vial	with modifiers (contaminated phases)	B'	yes (5.93)

Outcome

- Contamination coming from the instrument was solved by increasing the rinse dip time from 0 to 5 seconds and by changing the rinse port cap diameter
- Two main contamination sources were found, being the ammonium acetate solutions and the BSA
- The identity of the contaminant on the BSA was confirmed by QTOF as cortisol even though we suspected from a plasticizer. We still do not know how cortisol (or hydrocortisone) reached these materials

Acknowledgements : Christophe Bries