Metformin Interference in LC-MS/MS Analysis of Plasma Methoxycatecholamines

Marianne Bergmann and Anne Schmedes
Department of Clinical Immunology and Biochemistry, Lillebaelt Hospital, Vejle, Denmark

Marianne.Bergmann@rsyd.dk
Lillebaelt Hospital
Region of Southern Denmark

Problem

The LC-MS/MS analysis for plasma methoxycatecholamines has been in routine use at Lillebaelt Hospital since October 2015. During the last 9 months we have experienced an increasing problem with sample chromatograms showing lower peak height for metanephrine and d3-metanephrine, but not for normetanephrine and d3-normetanephrine. The problem only affects a few samples in each run, but these samples also show poor peak shape.

ID	nmol/l	RT	Height	IS Height	IS Area
103925053310	0.184	1.641	99195	1380271	41198.949
104015067002	0.174	1.641	90906	1331810	39968.402
104013039079	0.253	1.641	109027	1099551	33970.063
104013142902	0.219	1.645	112213	1308898	39134.094
104016704960	0.142	1.645	76800	1378553	41027.250
103925621955	0.154	1.641	67494	1119300	33431.785
104028003644	0.007	1.638	3448	1320566	39092.668
103925625179	0.089	1.645	11814	339080	13398.056
102856723339	0.084	1.641	44963	1363713	40535.309
00588	0.266	1.638	145009	1392977	40817.266
103925155687	0.254	1.641	138119	1388808	40643.723
103925521764	0.254	1.641	133601	1346045	40145.270
104028031524	0.098	1.645	50856	1325111	39736.594
103925605682	0.415	1.641	214733	1320319	38413.922
104016309280	0.177	1.641	101427	1465806	44083.316
104016685184 A	0.151	1.661	4785	80946	2553.916
103925319833 B	0.091	1.641	46716	1309007	39381.477
104008666915	0.447	1.655	26128	149287	4886.138
104027912950	0.077	1.641	45646	1506153	45535.066
102899810365	0.115	1.641	61975	1378523	40750.316
103243844483	0.136	1.641	73439	1375997	41025.898
103316381212	0.117	1.658	3703	80978	2403.075
00587	7.250	1.641	3794048	1336847	39437.715

Table 1. Several samples in the sample list display lower internal standard peak height (marked in red). Chromatograms for the two samples A and B can be seen in figure 1.

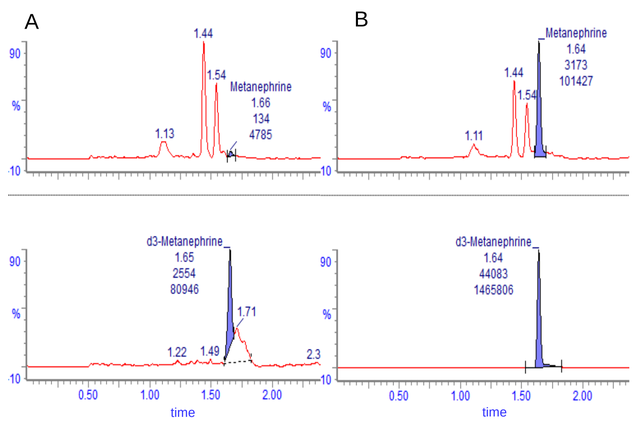

Figure 1. Chromatograms of a poor (A) and a good (B) sample. The metanephrine level in the two samples is comparable (0.151 and 0.177 nmol/L, respectively), however the peak heights and shapes are very different.

Method

Apparatus: Hamilton STARlet workstation and Waters Acquity UPLC with Xevo TQ-S tandem mass spectrometer.

Sample preparation: Plasma metanephrine and normetanephrine were extracted by using Biotage EVOLUTE EXPRESS WCX 30 mg 96-well plate with d3-metanephrine and d3-normetanephrine as internal standard. The eluate from the extraction was diluted with
250 µL acetonitrile.

LC column: Waters BEH amide (50 x 2.1 mm, 1.7 µm)

Mobile phase A: 2 mM ammonium acetate, 0.1% formic acid in water

Mobile phase B: Acetonitrile

Flowrate: 4 µL diluted sample was injected at a flowrate of 0.7 mL/min.

Gradient elution: 0-1 min. 10% A, 90% B; 1- 1.5 min. linear gradient to 20% A, 80% B; 1.5-2.5 min. 90% A, 10% B; 2.5-5 min. equilibrate with 10% A, 90% B.

MRM transitions: Metanephrine 180.12>148.12; d3-metanephrine 183.12>151.12; Normetanephrine 166.08>134.09; d3-normetanephrine 169.08 >137.09.

Identification of interference

We suspected that the problem could be caused by an interfering compound in these samples that co-elutes with metanephrine, causing ions suppression. A full-scan of one of these samples displayed that a massive peak of 130 m/z co-eluted with metanephrine (Figure 2). Metformin has the molecular weight of 129 g/mol and we therefore added an MRM transition for metformin to the method. Next, the analysis of a test solution of metformin also co-eluted with metanephrine and when re-analyzing a patient sample the chromatogram displayed a massive peak for metformin (Figure 3).
Metformin is widely used in high doses (up to 2000 mg/day), in the treatment of diabetes type 2. A large patient group that often show hypertension, and therefore could have indication for methoxycatecholamine measurement.

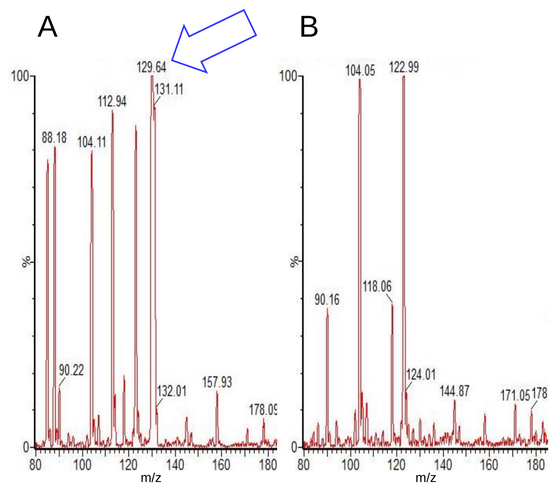

Figure 2. Mass spectrum showing peaks co-eluting with metanephrine identified in a full-scan of a poor (A) and good (B) sample. An extra peak at 130 m/z was identified in the poor sample (marked with blue arrow).

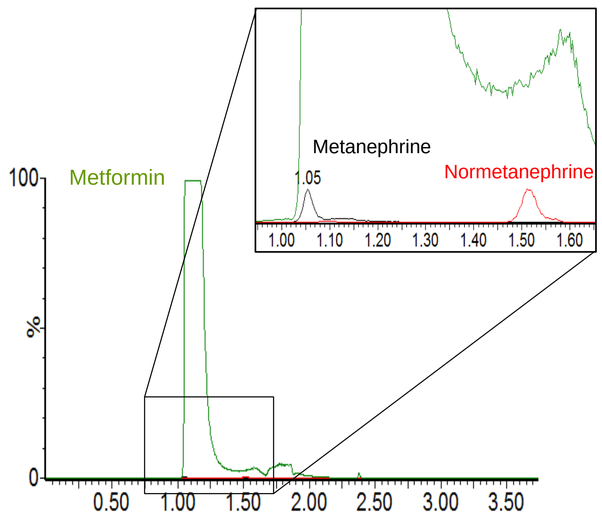

Figure 3. Chromatogram of a patient sample with an extra MRM transition for metformin (130.1>70.9) Signal is normalized to the largest peak in the chromatogram.

Troubleshooting steps and outcome

The extraction procedure

Extration solvents: The Biotage EVOLUTE EXPRESS WCX 30 mg plate and another weak cation exchange plate (Phenomenex Strata X-CW 30 mg) was tried with different wash steps (water, 10 mM ammonium acetate, methanol) and elution solutions (acidic, basic, different compositions of methanol, acetonitrile and water). However, this did not affect recovery of metanephrine, normetanephrine and metformin markedly. Only the basic elution differed so that both metanephrine and normetanephrine was lost in the extraction.

Extraction plates: A strong cation exchange plate (Phenomenex Strata-X-C 30 mg), was tried. The rationale being that metanephrine (and normetanephrine) and metformin has different pka values and theoretically, at a pH of 10, metanephrine/normetanephrine should be neutral or negatively charged, while metformin should be positively charged (and therefore be retained by the negatively charged plate).
The recovery from the Phenomenex Strata-X-C extraction, compared with the original Biotage EVOLUTE EXPRESS WCX, was 72% and 61% for d3-metanephrine and d3-normetanephrine. At the same time the metformin peak decreased from being in total overload to a peak size comparable to metanephrine (Figure 3 and 5).

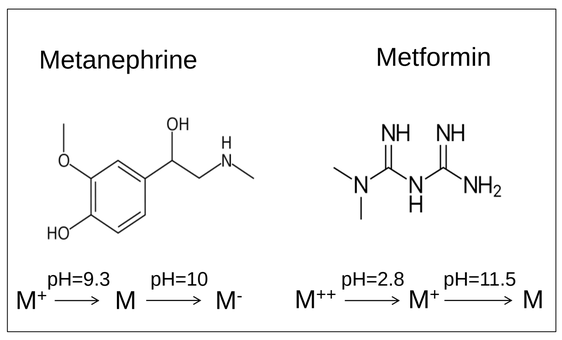

Figure 4. Molecular structure of metanephrine and metformin and their pka values.

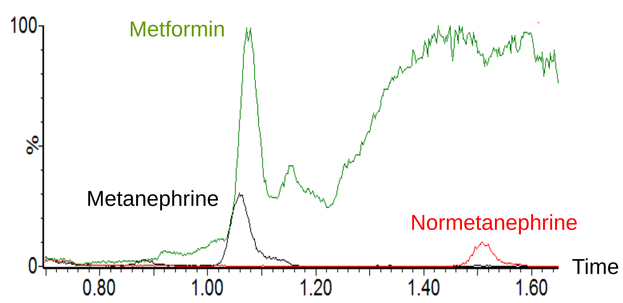

Figure 5. Chromatogram of a patient sample extracted with Strata-X-C extraction plate. *Signal is normalized to the largest peak in the chromatogram.*

Chromatographic separation

A solution containing metanephrine, normetanephrine and d6-metformin/metformin was tested on several columns (some can be seen in Figure 6). The gradient elution and flow rate were optimized for each column to give the best separation of metformin and metanephrine. The different columns mostly did not display markedly different selectivity toward the compounds of interest. However, the Restek Raptor HILIC-Si column displayed the best separation of metformin and metanephrine. Furthermore, the metformin peak elutes after both metanephrine and normetanephrine. This is an advantage when running patient samples were the metformin peak is very large and prolonged. On the down side, this column does not show good separation of metanephrine and norepinephrine.

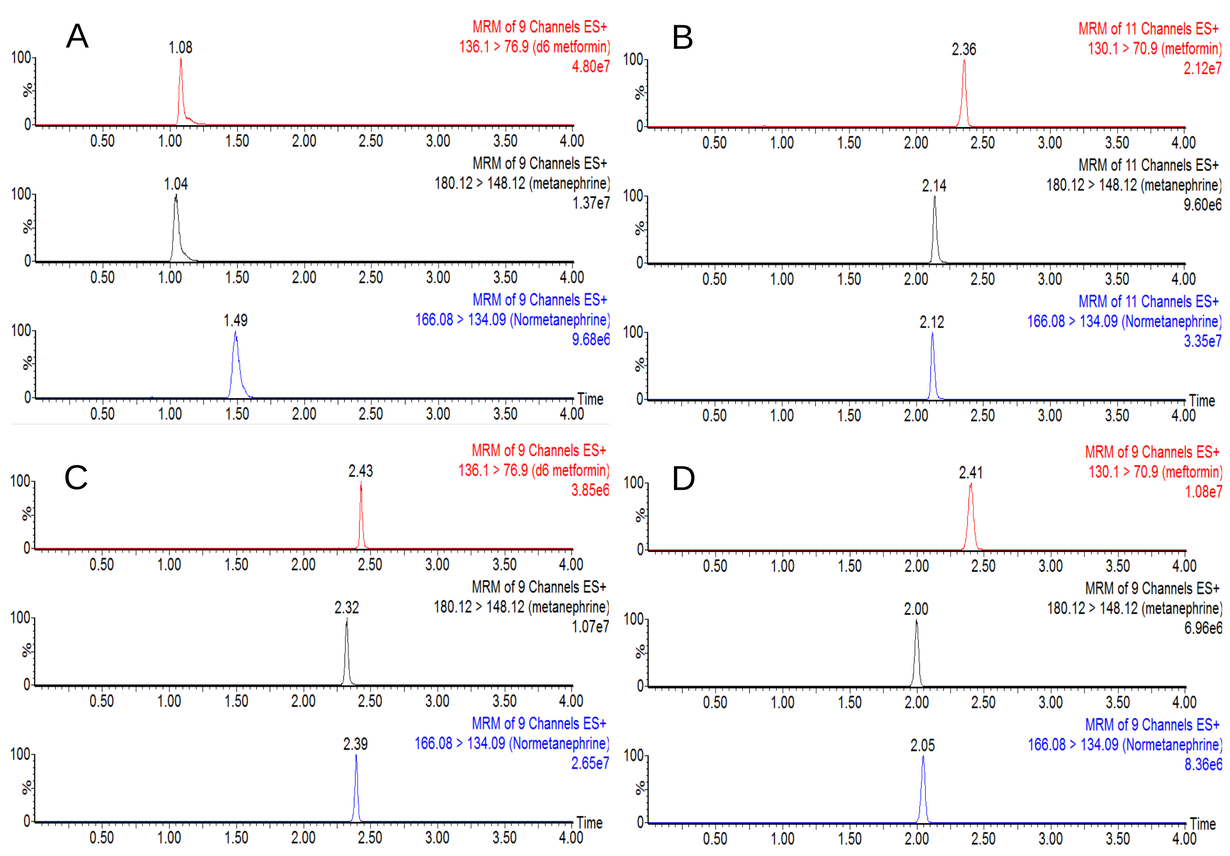

Figure 6. Chromatograms showing our best separation of a test solution containing metanephrine, normetanephrine and d6-metformin/metformin on different columns; (A)Waters BEH amide (50 x 2.1 mm, 1.7 µm), (B) Phenomenex Kinetex HILIC (100 x 2.1 mm, 1.7µm), (C) Merck SeQuant ZIC-HILIC (100 x 2.1mm, 3.5µm), (D) Restek Raptor HILIC-Si (50 x 2.1mm, 2.7µm).

Conclusion

We were able to identify metformin as an interfering compound in our plasma methoxycatecholamines analysis. We were not able to find an extraction procedure that could differentiate metformin from the methoxycatecholamines. However, using a Restek Raptor HILIC-Si column we could separate the compounds during liquid chromatography and this column is now being used in the analysis. The method is modified from Liang et al*, and have been in routine use since the beginning of January 2018.

*Shun-Hsin Liang, Justin Stemling, Landon Wiest, Sharon Lupo, Frances Carroll, Ty Kahler, Sue Steinike, Paul Connolly. Analysis of Plasma Free Metanephrine, Normetanephrine and 3-Methoxytyramine by Hydrophilic Interaction Liquid Chromatography. Poster presentation, MSACL EU 2017.