Supplementary file

An alternative method for separation of aldosterone and a re-occurring interference observed in 3-5 % of patient samples.

As described in the original report, aldosterone eluted with a retention time of 2.7 min, with baseline separation from all other peaks normally present in the chromatogram. All compounds tested with regard to possible interference (prednisone, prednisolone, cortisone, and 18OH-corticosterone) eluted well after the aldosterone peak. However, following implementation of the method as a routine analysis, a component co-eluting with aldosterone, together with 3 other compounds, was observed in 3-5% of the samples. This co-eluting compound was detected as a result of deviating qualifier ratios, and with a distinct pattern that makes it easy to detect (figure 1 and 2). The only differences from the method described in the main article were the use of a longer, biphenyl column and a different elution gradient. Everything else was identical to the main method.

The column was a Raptor Biphenyl 2.7 µm HPLC column, 100 x 2.1 mm (Restek, cat.no 9309A12) delivered by Teknolab AS, Ski, Norway. Column temperature was set to 50 °C.

The mobile phase gradient was as shown in the table below.

Table 1. Mobile phase gradient.

The table shows the mobile phase gradient. Mobile phase A was 5 mM ammonium fluoride in water and mobile phase B was 5 mM ammonium fluoride in methanol.

Time (min)	Mobile phase A%	Mobile phase B%
0.00	55.0	45.0
0.50	55.0	45.0
5.20	42.0	58.0
5.40	42.0	58.0
5.70	5.0	95.0
6.30	5.0	95.0
6.70	55.0	45.0

Figure 1-4 show examples of chromatograms from a normal sample without interference, and a sample with interference analyzed with the original method (figure 1 and 3) and with the alternative method (figure 2 and 4). Chromatogram A shows aldosterone quantifier transition (361.2->315.1) and chromatogram B shows aldosterone qualifier transition (361.2->343.3). The green peaks are aldosterone, the yellow peaks are unknown analytes, and the blue peak is aldosterone qualifier with alarm. These figures are shown in full run-time/whole injection to msms. Thus, all peaks present in the sample are visible.

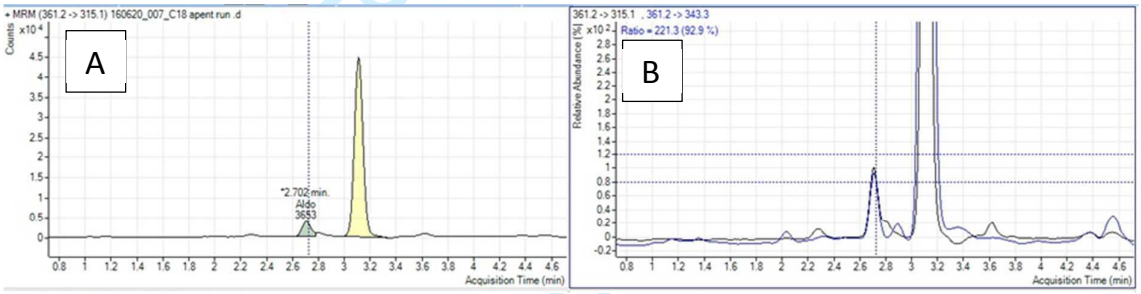

Figure 1: Normal patient sample without interference, analyzed with the original method

Panel A shows baseline separation between aldosterone eluting at 2.70 min and an unknown analyte eluting at 3.15 min, which were present in all patient samples. The edge of this peak can also be seen in Figure 1 of the main article.

Panel B shows aldosterone qualifier ion. The figure is in overlay mode and show the quantifier ion overlaid with the qualifier ion. The acceptance limits for the qualifier ratio are shown as horizontal blue lines.

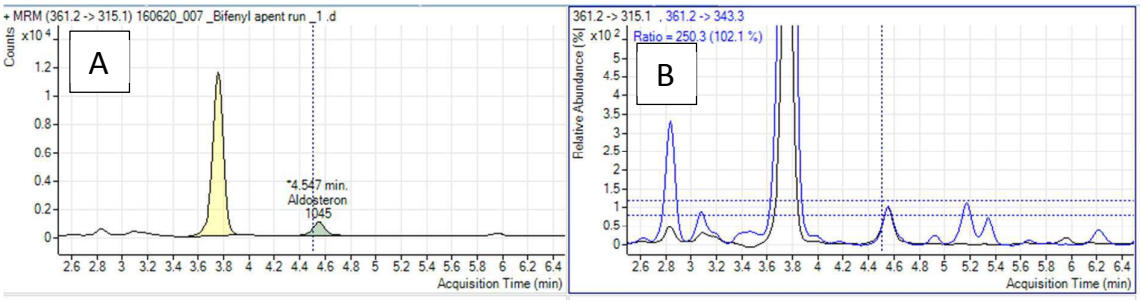

Figure 2: Normal patient sample without interference, analyzed with the alternative method

Panel A shows baseline separation between aldosterone (green) eluting at 4.55 min and the unknown analyte (yellow), present in all patient samples. Note that the unknown analyte with this method elutes well ahead (at 3.75 min) of aldosterone.

Panel B shows aldosterone qualifier ion. The figure is in overlay mode and show the quantifier ion overlaid with the qualifier ion. The acceptance limits for the qualifier ratio are shown as horizontal blue lines.

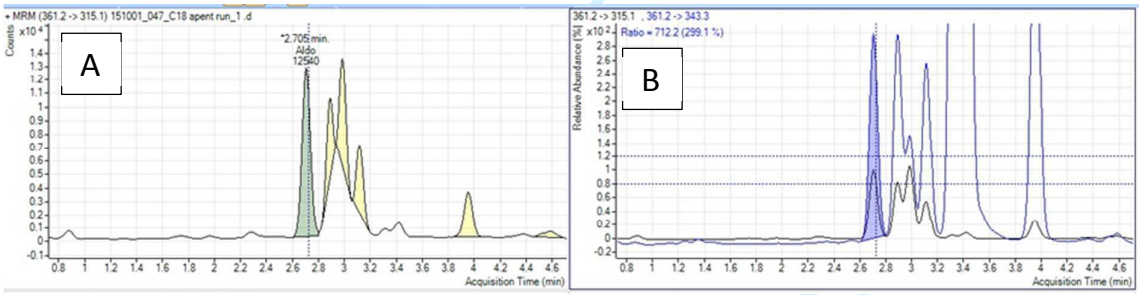

Figure 3: Patient sample with interference, analyzed with the original method

Panel A shows that there is not baseline separation between aldosterone and the unknown analytes. The unknown analyte present in all samples at 3.15 min is now accompanied by four additional peaks.

Panel B shows aldosterone qualifier ion (blue) in overlay mode similarly to Figure 1B. The qualifier ratio is clearly outside the limits of acceptance, shown as horizontal, blue lines.

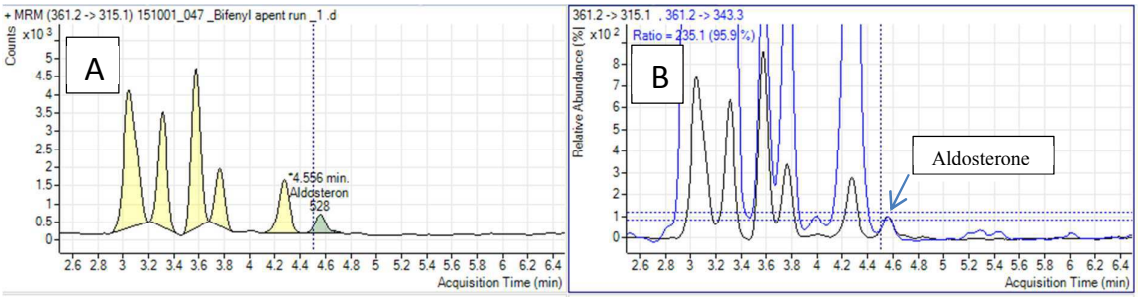

Figure 4: Patient sample with interference, analyzed with the alternative method

Panel A shows baseline separation between aldosterone and all unknown analytes. Note that all the unknown analytes now elute as 5 peaks well ahead of aldosterone.

Panel B shows aldosterone qualifier ion. The figure is in overlay mode and show the quantifier ion overlaid with the qualifier ion. The acceptance limits for the qualifier ratio are shown as horizontal blue lines.

Since the original method works perfectly well for more than 95 % of patient samples, and have a shorter runtime than this alternative method, we kept the original method for routine use. Any sample which presents with this problem is simply reinjected on the instrument, using this alternative method which is time efficient, easy and works well in the routine lab.

Supplementary Table 1. Recovery of aldosterone.

The table shows all figures for aldosterone recovery which is basis for Table 3 in the main article.

Five serum samples with aldosterone concentrations from 57.0 to 1000 pmol/L were each divided into 5 aliquots of 600 µL which then were spiked with 0, 0.20, 0.50, 1.00 and 2.50 pmol aldosterone. After spiking the 5 aliquots represents Level 0-4 where "Measured concentration before spiking" equals Level 0.

	Level 1 spiked with 0.20 pmol					Level 2 spiked with 0.50 pmol					Level 3 spiked with 1.00 pmol					Level 4 spiked with 2.50 pmol				
Measured concentration before spiking (pmol/L) (Level 0)	57.0	104	237	666	1000	57.0	104	237	666	1000	57.0	104	237	666	1000	57.0	104	237	666	1000
Theoretic concentration after spiking (pmol/L)	378	423	552	967	1290	822	865	988	1380	1690	1670	1710	1840	2260	2580	3900	3940	4070	4460	4770
Measured concentration after spiking (pmol/L)	377	435	558	989	1380	814	830	1060	1430	1750	1610	1720	1880	2220	2550	3670	3600	4030	4350	4680
Recovery %	99.8	102.8	101.1	102.3	107.0	99.0	95.9	107.1	103.6	103.6	96.4	100.6	102.2	98.2	98.8	94.1	91.4	99.0	97.5	98.1
Mean recovery, % ±SD	102.6 ± 2.7					101.8 ± 4.4					99.2 ± 2.2					96.0 ± 3.2				