

Supplemental Information

Chromatographia

Development of an improved LC-MS/MS assay for the quantification of oximes in KIKO mouse plasma

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Phenomenex Luna Omega Sugar Inter-Lot Variability Testing

MATERIALS & METHODS

Oxime standards were prepared in 0.1% formic acid in methanol. Standards were prepared at the following concentrations: 100 ng/mL 2-PAM with 50 ng/mL of 2-PAM-*d*3, 10,000 ng/mL HI-6 with 1,000 ng/mL of HI-6-*d*4, 1,500 ng/mL HLö-7 with 700 ng/mL of HLö-7-*d*4, and 1,000 ng/mL MMB-4 with 19.2 ng/mL of 4-PAM.

Standards were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS analysis utilizing separation performed on a Phenomenex Luna Omega Sugar column (3 μ m, 2.1 x 150 mm; Phenomenex, Torrance, CA) was conducted as previously published [18]. All standards were first analyzed by column lot H22-204524. Immediately after, all standards were analyzed by column lot H23-034542.

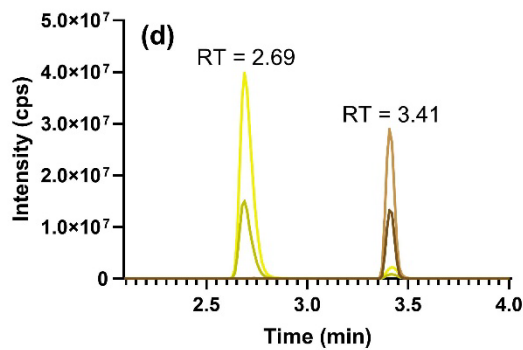
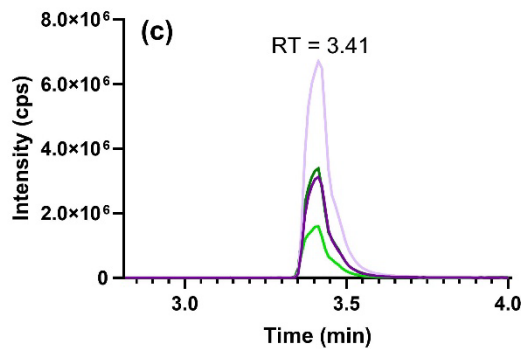
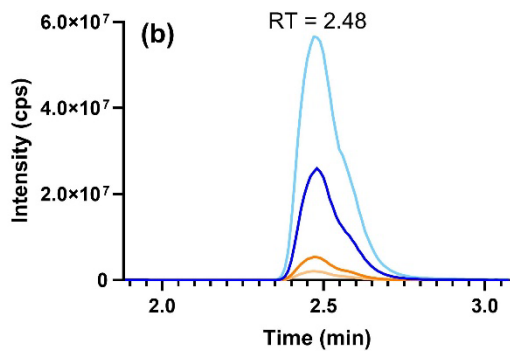
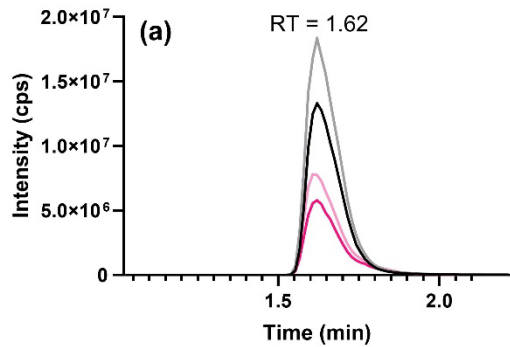
RESULTS & DISCUSSION

The major advancement of our previous study was the Phenomenex Luna Omega Sugar HILIC column which achieved retention of all four oxime compounds [18]. However, after completion of the study, we started receiving column lots that, under validated assay conditions, behaved differently to the column lots used for method development and validation. Instead of a narrower, more gaussian peak shape, on these new column lots, some analyte peaks became very wide, and some analyte peaks split with the split peaks occasionally being baseline resolved. In addition, there were very large retention time shifts with new column lots, and both peak shape and retention time issues would not resolve even after multiple hours of equilibration.

To demonstrate the inter-lot variability, oxime standards were prepared and analyzed by validated LC-MS/MS methods utilizing separation performed with the Phenomenex Luna Omega Sugar column. Standards were first analyzed with column lot H22-204524 and then analyzed with column lot H23-034542. Fig. S1 depicts the chromatogram for each oxime analyzed by each Phenomenex column lot. For 2-PAM, even though both columns produced peaks with a standard gaussian shape, 2-PAM had two distinct peaks when analyzed with column lot H23-034542 (Fig. S1e), compared to the single peak with column lot H22-204524 (Fig. S1a). For HI-6, the peak width increased from 0.45 minutes with lot H22-204524 to 1.3 minutes with lot H23-034542. Additionally, while the HI-6 peak had a slight shoulder with lot H22-204524

(Fig. S1b), the HI-6 peak has two major shoulders with lot H23-034542 (Fig. S1f). MMB-4 had an acceptable peak shape and width with both columns (Fig. S1d and S1h), but 4-PAM split into three distinct peaks when analyzed with lot H23-034542 (Fig. S1h), versus the single peak with lot H22-204524 (Fig. S1d). Also, the width of 4-PAM's major peak increased from 0.5 minutes with lot H22-204524 to 1.2 minutes with lot H23-034542. Like MMB-4, HLö-7 had acceptable peak shape and width with both column lots (Fig. S1c and S1g). However, regardless of peak shape, all analytes eluted earlier with lot H23-034542 than with lot H22-204524. The major peak for 2-PAM eluted 0.58 minutes earlier, HI-6 eluted 1.04 minutes earlier, HLö-7 eluted 0.59 minutes earlier, and MMB-4 eluted 0.27 minutes earlier. This lack of reproducibly filled Phenomenex Luna Omega Sugar LC columns hindered the future utility of the oxime analytical methods.

Lot: H22-204524



Lot: H23-034542

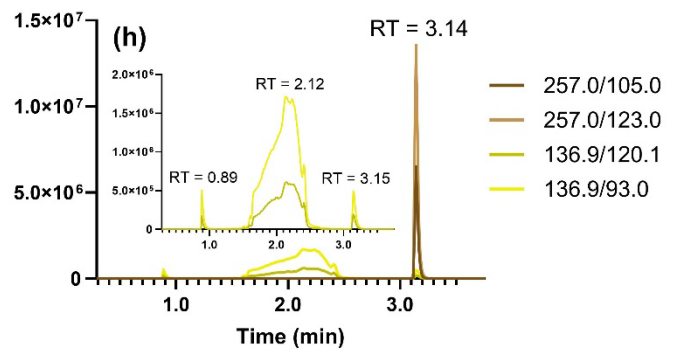
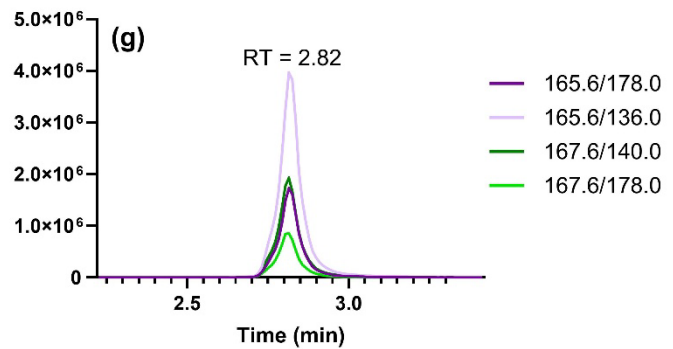
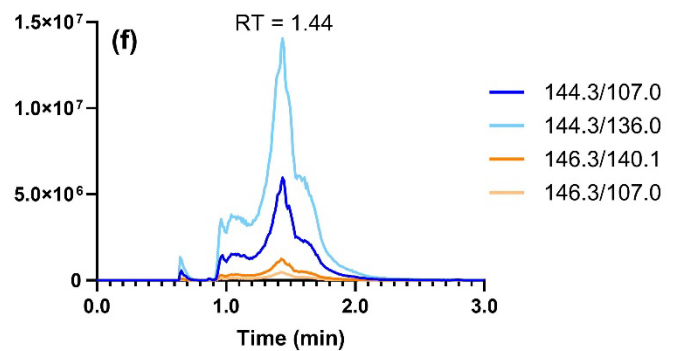
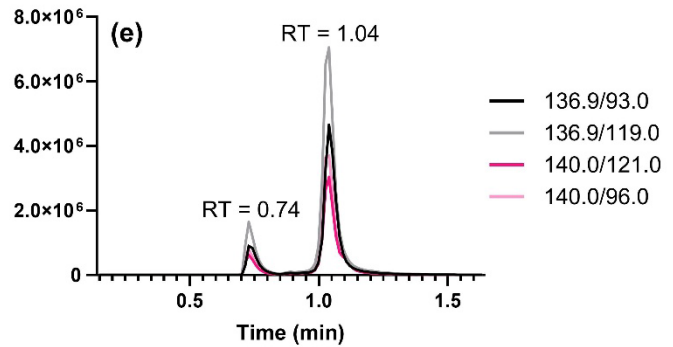


Fig. S1 Reconstructed ion chromatograms. A-D: A representative reconstructed ion chromatogram of 100 ng/mL 2-PAM with 50 ng/mL 2-PAM-*d*3 (a), 10,000 ng/mL HI-6 with 1,000 ng/mL HI-6-*d*4 (b), 1,500 ng/mL HLö-7 with 700 ng/mL HLö-7-*d*4 (c), and 1,000 ng/mL MMB-4 with 19.2 ng/mL 4-PAM (d) by Phenomenex Luna Omega Sugar column lot H22-204524. E-H: A representative reconstructed ion chromatogram of 100 ng/mL 2-PAM with 50 ng/mL 2-PAM-*d*3 (e), 10,000 ng/mL HI-6 with 1,000 ng/mL HI-6-*d*4 (f), 1,500 ng/mL HLö-7 with 700 ng/mL HLö-7-*d*4 (g), and 1,000 ng/mL MMB-4 with 19.2 ng/mL 4-PAM (h) by Phenomenex Luna Omega Sugar column lot H23-034542. Both quantitative and qualitative ion transitions are presented for each compound and IS