Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for: Discriminative potential of ion mobility spectrometry for the detection of fentanyl and fentanyl analogues relative to confounding environmental interferents

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Sample and Data Collection. True positive IMS measurements were conducted using standard narcotic samples or solutions diluted in methanol to nominal concentrations of (1, 10, or 100) µg/mL depending upon desired mass deposition. Solutions were cast onto meta-aramid substrates in volumes not exceeding 2 µL to limit the total wicking area to less than 2.0 cm^2. In cases where additional volume was required to meet mass expectations, deposition was completed in increments such that the first deposit was allowed to dry before the next deposit was delivered. Wherever possible, samples were analyzed from lowest mass to highest mass to reduce peak intensity inflation due to instrument memory and residual material. In addition, cleardown cycles were conducted between true positive samples to ensure no carry over was observed.

Data Processing. A custom MATLAB-based code (MATLAB, R2017a, Mathworks, Inc., Natick, MA, USA) was developed to extract and process the raw signal data for each file. The data files provided extensive details of the instrument settings, parameters, target analyte channels, and alarm settings. Drift times and segment data for both Tube 1 and Tube 2 were provided, however for this study only Tube 1 data was considered (positive mode – narcotics). The raw IMS spectra data consisted of drift times and signal data for each "segment". These segments were comprised of the average data from a specified number of scans (across the range of drift times) and the number of scans per segment varied across two to three analysis periods per test based on the system parameters. These settings, along with the calibrant peak position and reduced mobility, reference location for baseline correction, and sample acquisition date were extracted for processing.

The extracted raw data and system settings were used to reconstruct the 3D data (signal intensity as a function of drift time/reduced mobility and segment) for each sample. Based on user-defined peak definition parameters, each segment was scanned for peaks within the specified window around each target reduced mobility value. Target reduced mobilities (and windows) were converted to drift times based on

the calibrant drift time and reduced mobility determined by the instrument firmware and specified in each raw data file.

Scheme S1. General receiver operating characteristic curve creation from background interferent signal and target analyte signal distributions. The below confusion matrix is created for varying of the alarm threshold, and at each point the true positive and false positive rates determined and plotted in ROC space.

IMS Response	**True Condition Status**		**Total**
	Positive	**Negative**	
Positive	True Positive (TP)	False Positive (FP)	TP+FP
Negative	False Negative (FN)	True Negative (TN)	FN+TN
Total	TP+FN	FP+TN	

Sensitivity = TPR = TP/(TP+FN)
Specificity = 1 – FPR = 1 – FP/(FP+TN)

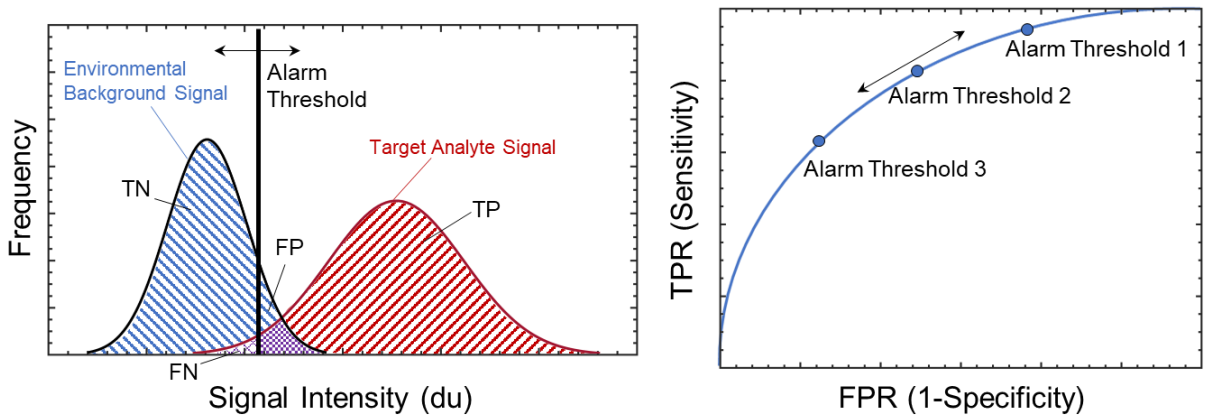

Table S1. Nominal number of replicates, nominal mass loadings, and IMS response signal intensities (du) at each mass loading (median, lower (Q1) and upper (Q3) quartiles) for each compound under laboratory conditions for the AE instrument.

AE	Analyte	Reps. at each mass	Low (ng) *Signal (du)* *Med. (Q1-Q3)*	Mid (ng) *Signal (du)* *Med. (Q1-Q3)*	High (ng) *Signal (du)* *Med. (Q1-Q3)*	Elevated (ng) *Signal (du)* *Med. (Q1-Q3)*
1	Carfentanil	5				50 *80.0 (66.0-110)*
2	Valeryl fentanyl	10	30 *20.5 (14.0-23.8)*	40 *32.0 (28.5-42.3)*	60 *66.5 (38.3-70.5)*	150 *361 (352-397.5)*
3	Furanyl fentanyl	30	30 *23.0 (15.0-32.0)*	50 *33.0 (23.3-59.3)*	60 *85.0 (28.8-135.8)*	150 *266 (229-318)*
4	*p*-Fluoroisobutyryl fentanyl (FIBF)	3				50 *219 (146-267)*
5	Crotonyl fentanyl	10	45 *23.0 (20.0-35.8)*	50 *37.5 (24.8-48.3)*	60 *71.5 (56.3-88.8)*	60 *71.5 (56.3-88.8)*
6	Butyryl fentanyl	30	20 *24.0 (14.3-29.8)*	30 *28.0 (20.0-48.8)*	40 *65.5 (50.0-99.8)*	50 *128.5 (72.0-134)*
7	*trans*-3-methylfentanyl	3				30 *90.0 (86.0-99.0)*
8	Cyclopropyl fentanyl	10	30 *12.5 (10.0-17.3)*	50 *35.5 (33.0-46.8)*	60 *58.5 (47.0-66.5)*	60 *58.5 (47.0-66.5)*
9	Heroin	30	25 *55.0 (17.3-78.0)*	50 *86.5 (51.0-133.3)*	125 *252.5 (184.5-309.0)*	150 *313 (293-340)*
10	Fentanyl	30	7.5 *13.0 (8.0-25.0)*	15 *34.0 (26.0-55.0)*	20 *62.0 (42.0-80.8)*	150 *294 (255-330)*
11	Acryl fentanyl	10	20 *31.0 (22.3-36.5)*	30 *45.5 (33.5-62.8)*	40 *76.5 (59.0-103.5)*	40 *477 (394-500)*
12	Acetyl fentanyl	10	10 *17.5 (13.8-23.5)*	25 *41.0 (33.8-47.5)*	30 *56.5 (49.8-68.5)*	50 *93.5 (84.3-117)*
13	Benzyl fentanyl	30	10 *24.0 (17.0-32.8)*	12 *42.0 (27.3-60.3)*	20 *64.0 (39.5-96.5)*	50 *154 (154-226.5)*
14	U-47700	10	5 *23.0 (11.0-49.3)*	7 *48.0 (34.0-73.5)*	10 *118.5 (73.3-167.0)*	50 *321 (201-382)*
15	Norfentanyl	30	15 *56.0 (43.5-88.0)*	25 *105.5 (68.8-146)*	35 *166.5 (94.3-217.3)*	150 *531 (523-562)*
16	Acetyl norfentanyl	10	8 *30.0 (15.5-55.0)*	10 *48.8 (39.5-69.0)*	12 *122.0 (79.5-135.5)*	50 *335 (309-340)*

Table S2. Nominal number of replicates, nominal mass loadings, and IMS response signal intensities (du) at each mass loading (median, lower (Q1) and upper (Q3) quartiles) for each compound under laboratory conditions for the N/E instrument.

N/E	Analyte	Reps. at each mass	Low (ng) *Signal (du)* *Med. (Q1-Q3)*	Mid (ng) *Signal (du)* *Med. (Q1-Q3)*	High (ng) *Signal (du)* *Med. (Q1-Q3)*	Elevated (ng) *Signal (du)* *Med. (Q1-Q3)*
1	Carfentanil	5				20 *192 (183-205)*
2	Valeryl fentanyl	10	1.5 *23.8 (16.4-28.3)*	2 *28.6 (25.4-41.2)*	4 *52.8 (46.5-62.5)*	20 *601 (466-662)*
3	Furanyl fentanyl	30	2 *18.5 (13.3-24.8)*	4 *42.7 (33.2-52.5)*	6 *79.9 (50.1-105.1)*	20 *280 (260-391)*
4	*p*-Fluoroisobutyryl fentanyl (FIBF)	10	1.5 *23.3 (17.6-31.2)*	3.5 *48.4 (37.1-61.1)*	5 *59.8 (55.6-66.3)*	20 *139 (106-227)*
5	Crotonyl fentanyl	10	2 *15.4 (2.7-21.0)*	4 *32.7 (25.5-39.1)*	6 *54.9 (6.7-82.0)*	20 *160 (139-208)*
6	Butyryl fentanyl	30	1 *12.8 (0-19.6)*	1.5 *52.5 (35.7-65.9)*	4 *75.4 (50.7-107.1)*	20 *436 (408-525)*
7	*trans*-3-methylfentanyl	5				20 *172 (172-193)*
8	Cyclopropyl fentanyl	10	3 *44.0 (36.7-56.8)*	6 *69.3 (55.6-77.9)*	20 *173.4 (143.8-201.4)*	100 *686 (646-751)*
9	Heroin	30	2 *21.2 (0-28.0)*	5 *42.7 (18.4-60.1)*	10 *71.3 (55.0-120.8)*	20 *343 (284-378)*
10	Fentanyl	30	0.05 *11.8 (0-18.8)*	0.1 *22.7 (14.7-36.1)*	0.2 *55.1 (35.1-75.9)*	20 *1062 (1053-1062)*
11	Acryl fentanyl	10	2 *10.3 (0-26.7)*	3 *27.4 (20.4-38.1)*	4 *50.5 (48.5-57.5)*	20 *353 (325-372)*
12	Acetyl fentanyl	10	1 *38.2 (25.3-53.2)*	2 *68.5 (46.4-87.4)*	5 *80.9 (61.8-132.6)*	20 *437 (379-448)*
13	Benzyl fentanyl	30	1 *23.0 (14.8-34.5)*	1.25 *36.9 (32.0-50.3)*	1.5 *55.2 (38.8-63.3)*	20 *706 (703-766)*
14	U-47700	10	0.2 *26.1 (24.1-29.3)*	0.5 *46.8 (30.5-51.4)*	1 *70.2 (68.1-87.1)*	20 *614 (577-675)*
15	Norfentanyl	30	1 *29.7 (22.7-36.4)*	1.5 *64.8 (51.6-77.5)*	2 *94.8 (76.4-119.1)*	20 *472 (330-658)*
16	Acetyl norfentanyl	10	0.75 *33.6 (23.7-40.2)*	1.5 *53.5 (49.9-67.7)*	2 *75.5 (62.2-98.2)*	20 *728 (696-744)*

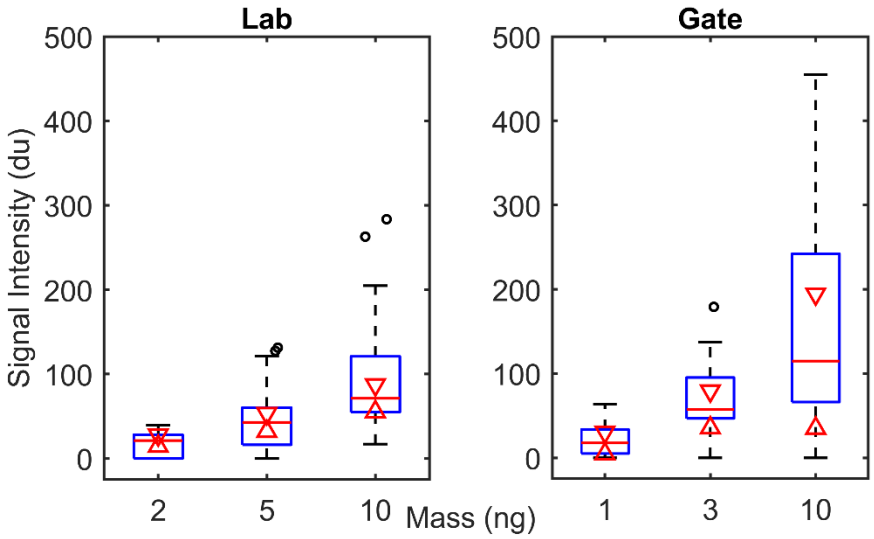

Figure S1. Comparison of IMS response to heroin in the laboratory and at the deployed gate location for a number of low mass loadings using the N/E configuration. Boxes represent the median and lower (Q1) and upper (Q3) quartiles, whiskers represent 1.5× the interquartile range (length of the box), and outliers (o) represent values out of the whisker range, and triangular markers represent 95% confidence intervals (median ± 1.57(Q3-Q1)/√(n)).

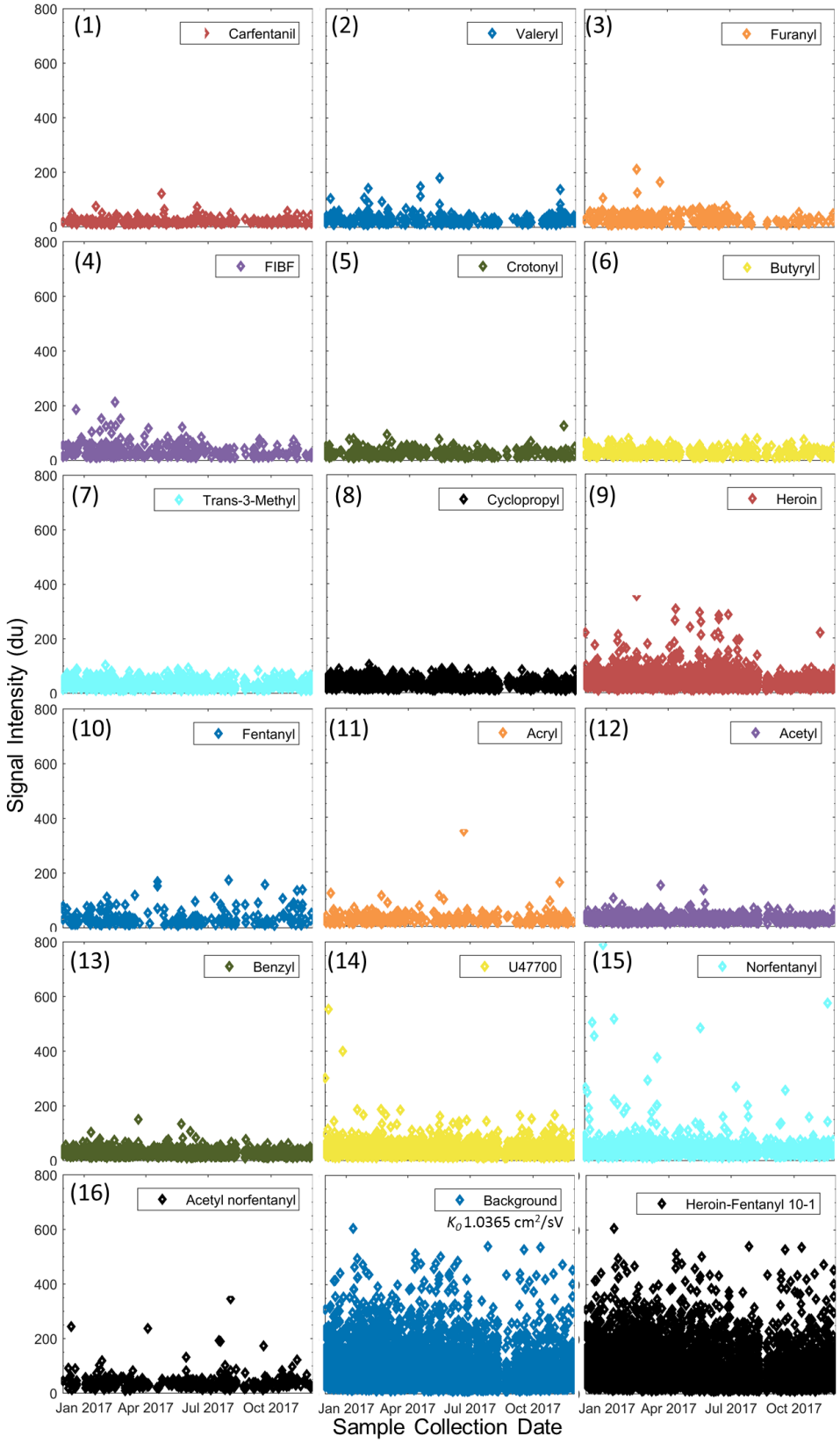

Figure S2. Environmental background intensity data within ± 0.003 cm²/sV of each target analyte reduced mobility from the AE IMS instrument during deployment across a yearlong period (9,359 samples).

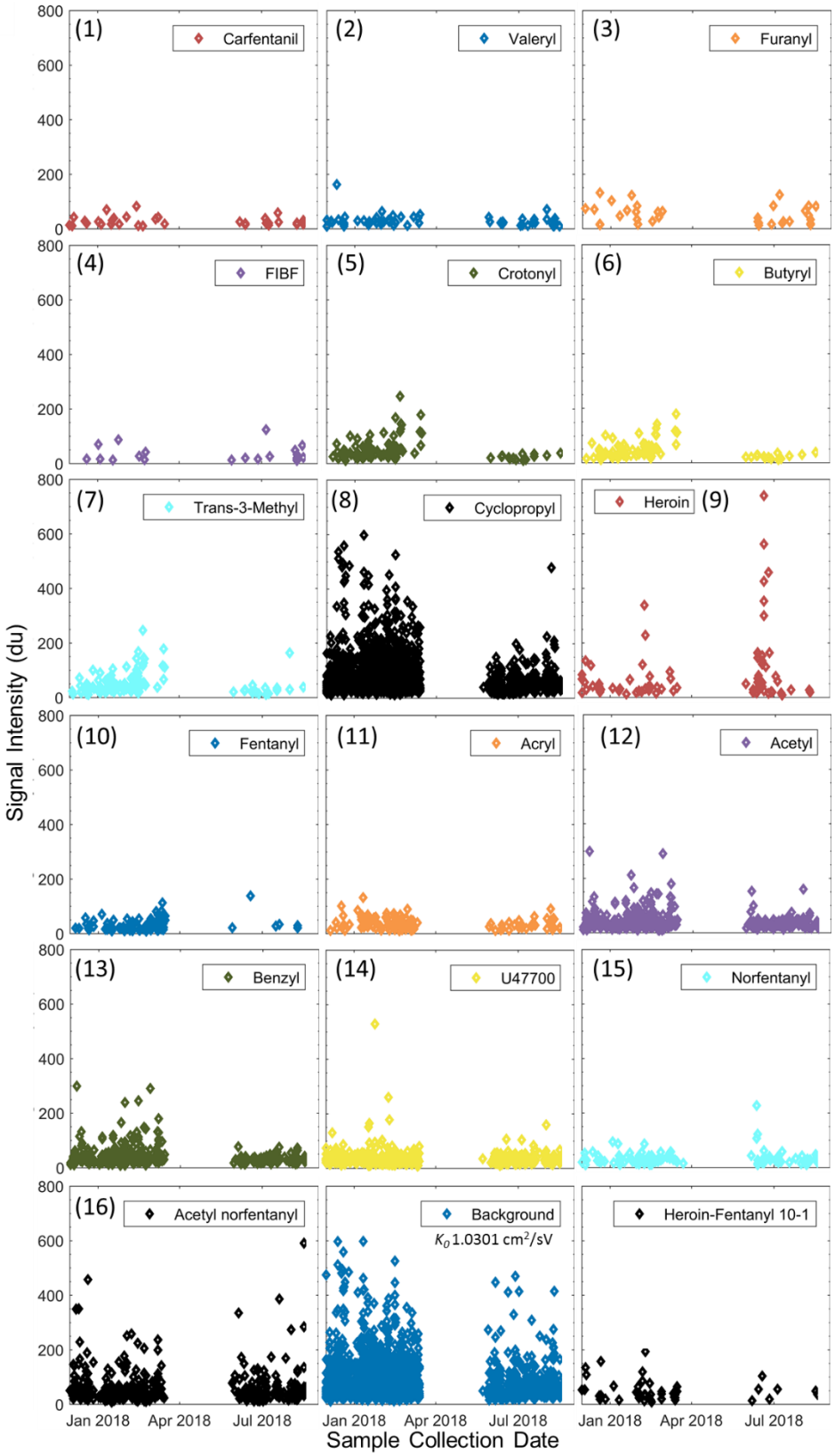

Figure S3. Environmental background intensity data within ± 0.003 cm^2/sV of each target analyte reduced mobility from the N/E IMS instrument during deployment across a multi-month period (1,996 samples).

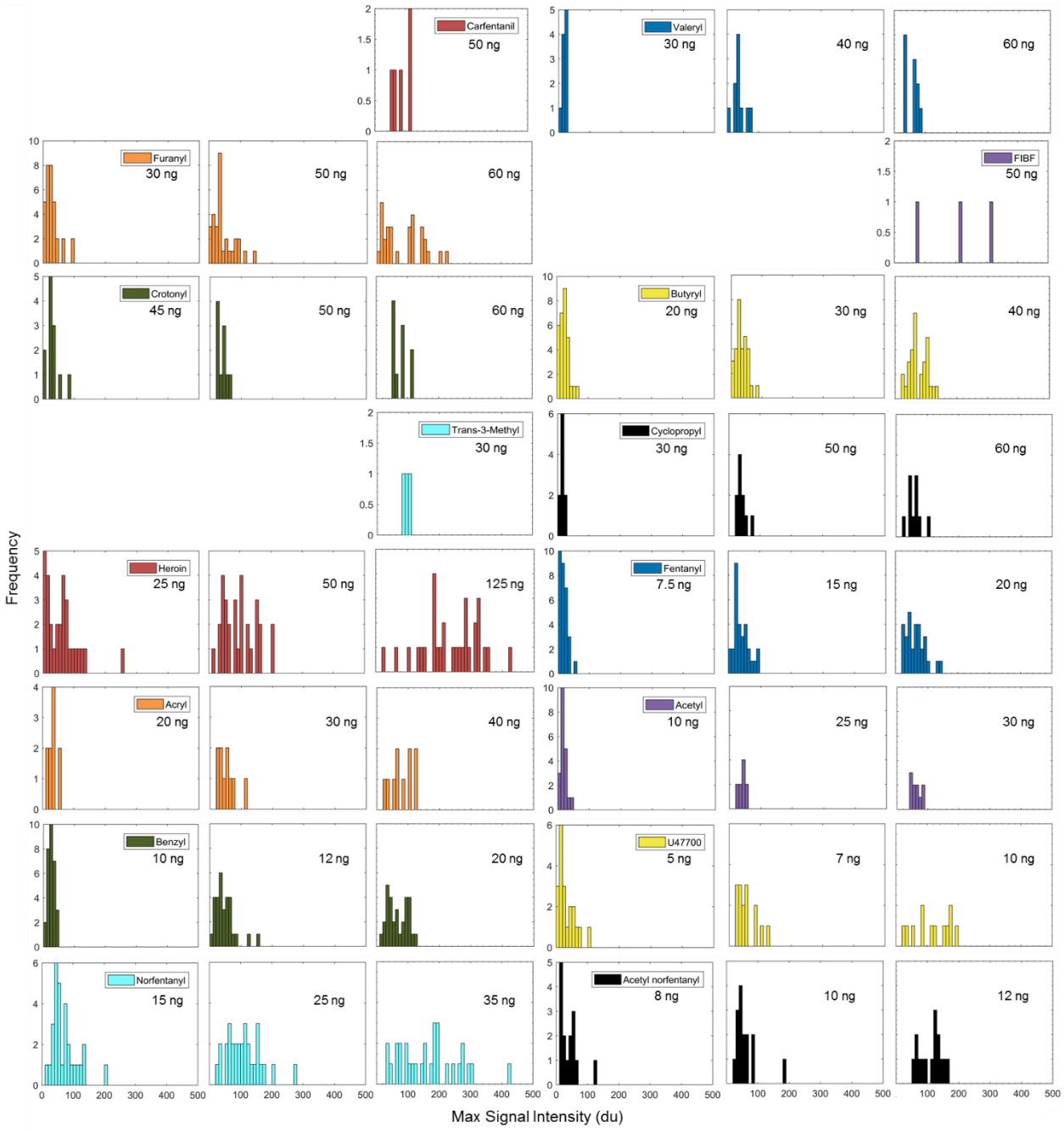

Figure S4. Frequency histograms of true positive target analyte intensity data for three mass loadings (Table 2) on the AE IMS instrument operated under laboratory conditions.

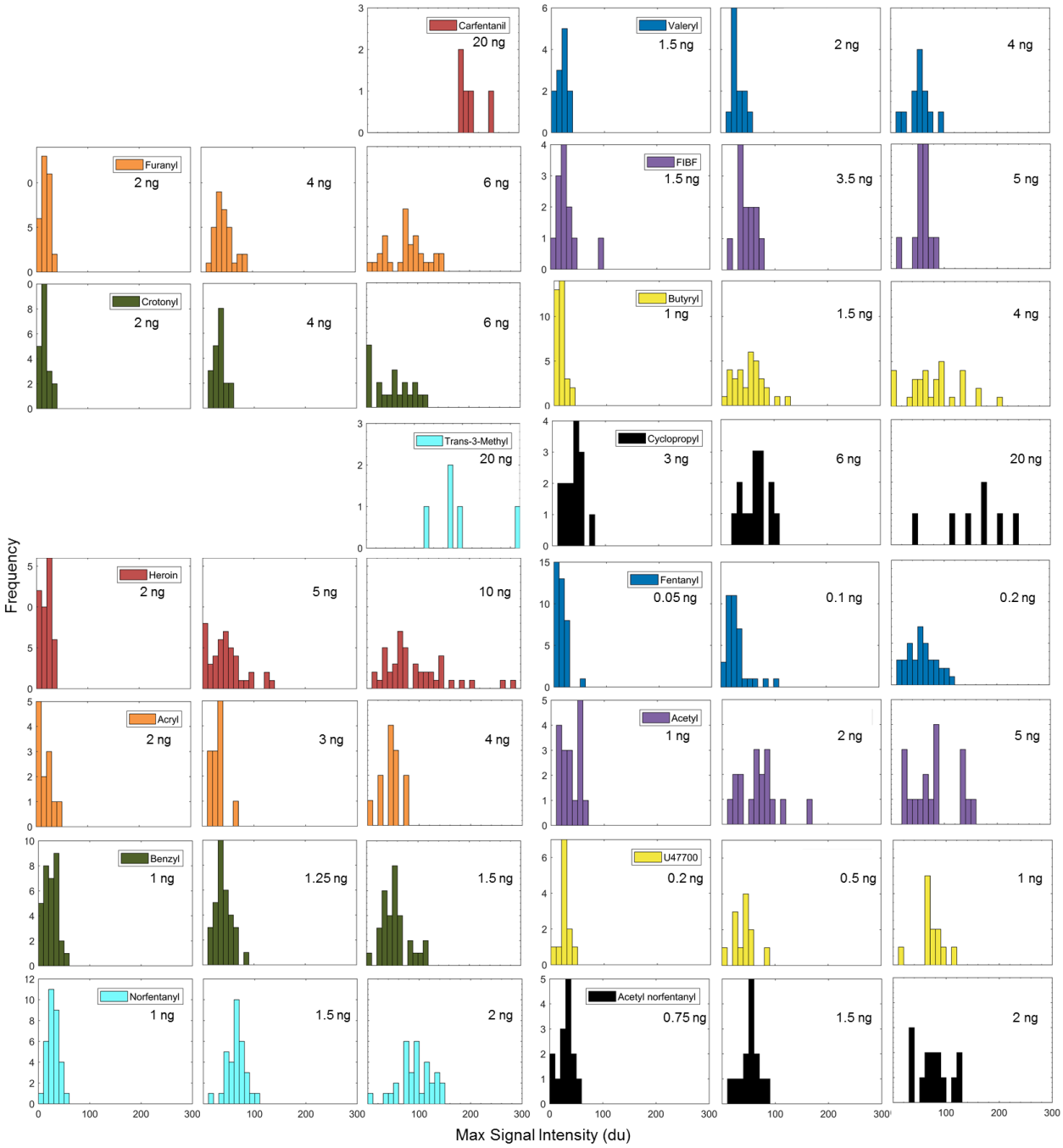

Figure S5. Frequency histograms of true positive target analyte intensity data for three mass loadings (Table 3) on the N/E IMS instrument operated under laboratory conditions.

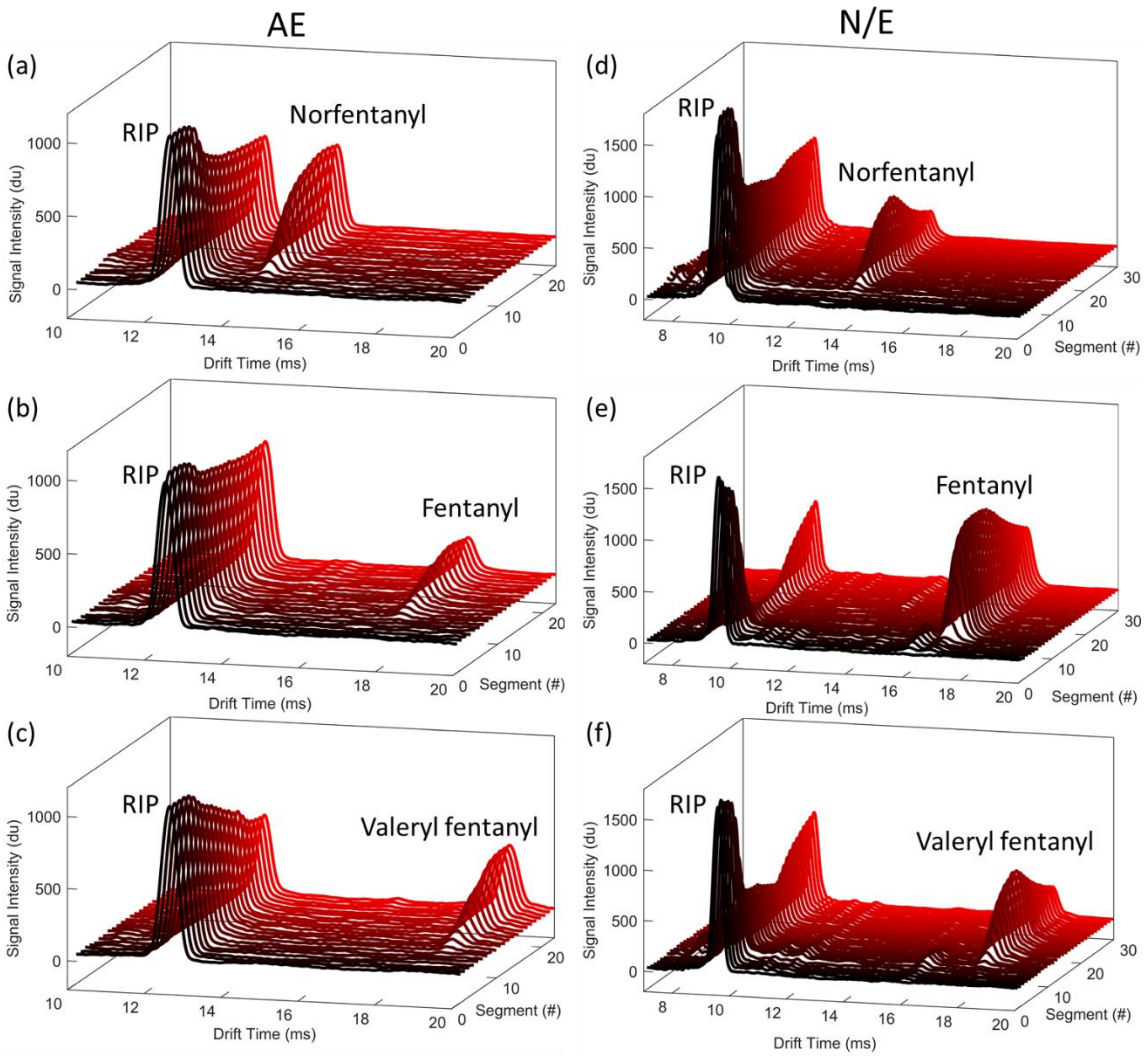

Figure S6. Representative IMS spectra as a function of drift time and segment number for norfentanyl ((a) and (d)), fentanyl ((b) and (e)), and valeryl fentanyl ((c) and (f)). (a) – (c) and (d) – (f) represent IMS spectra from the AE instrument at 150 ng loading and N/E instrument at 20 ng loading for each, respectively. (RIP: reactant ion peak).

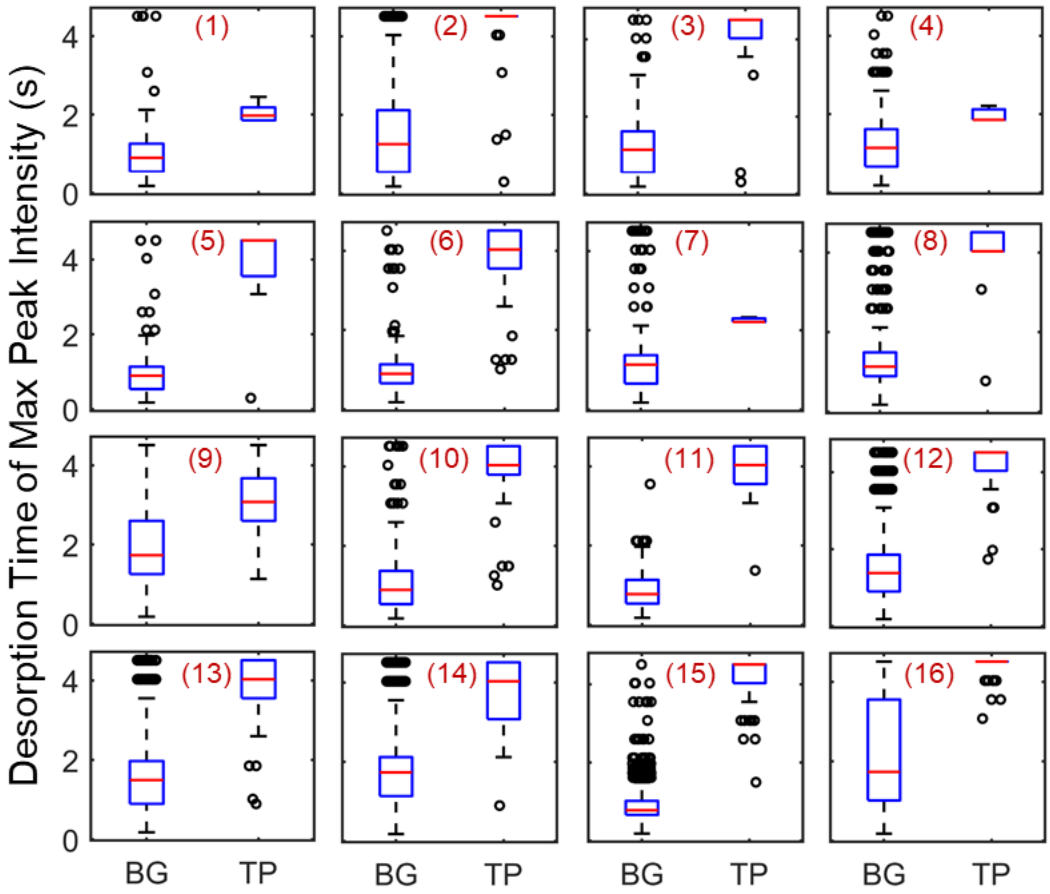

Figure S7. Desorption time of the maximum peak intensity of environmental background (BG) observed at each target compound's reduced mobility window, compared to the true positive (TP) target compound maximum peak intensity time (across all mass loadings investigated) on the AE configured instrument (5 s total sampling time). Boxes represent the median and lower and upper quartiles, whiskers represent 1.5× the interquartile range (length of the box), and outliers (o) represent values out of the whisker range. Analyte labels (#) correspond to identifications in Table 2.

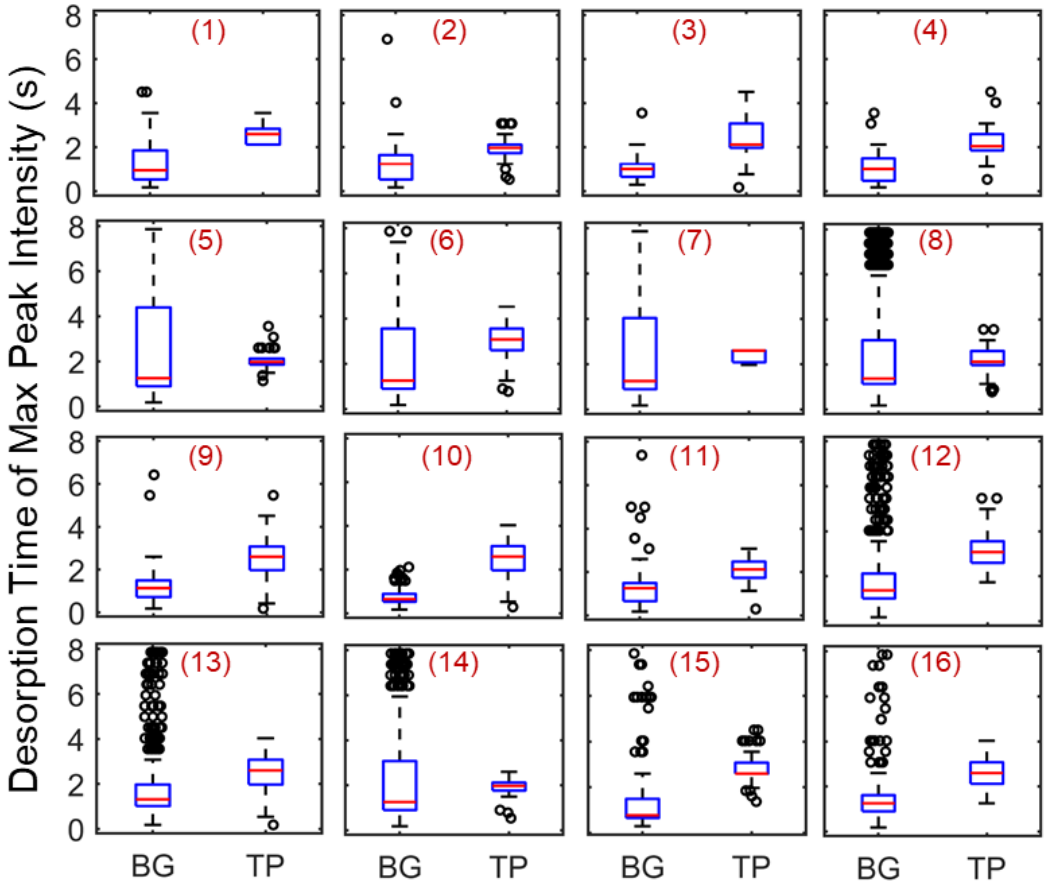

Figure S8. Desorption time of the maximum peak intensity of environmental background (BG) observed at each target compound's reduced mobility window, compared to the true positive (TP) target compound maximum peak intensity time (across all mass loadings investigated) on the N/E configured instrument (8 s total sampling time). Boxes represent the median and lower and upper quartiles, whiskers represent 1.5× the interquartile range (length of the box), and outliers (o) represent values out of the whisker range. Analyte labels (#) correspond to identifications in Table 2.

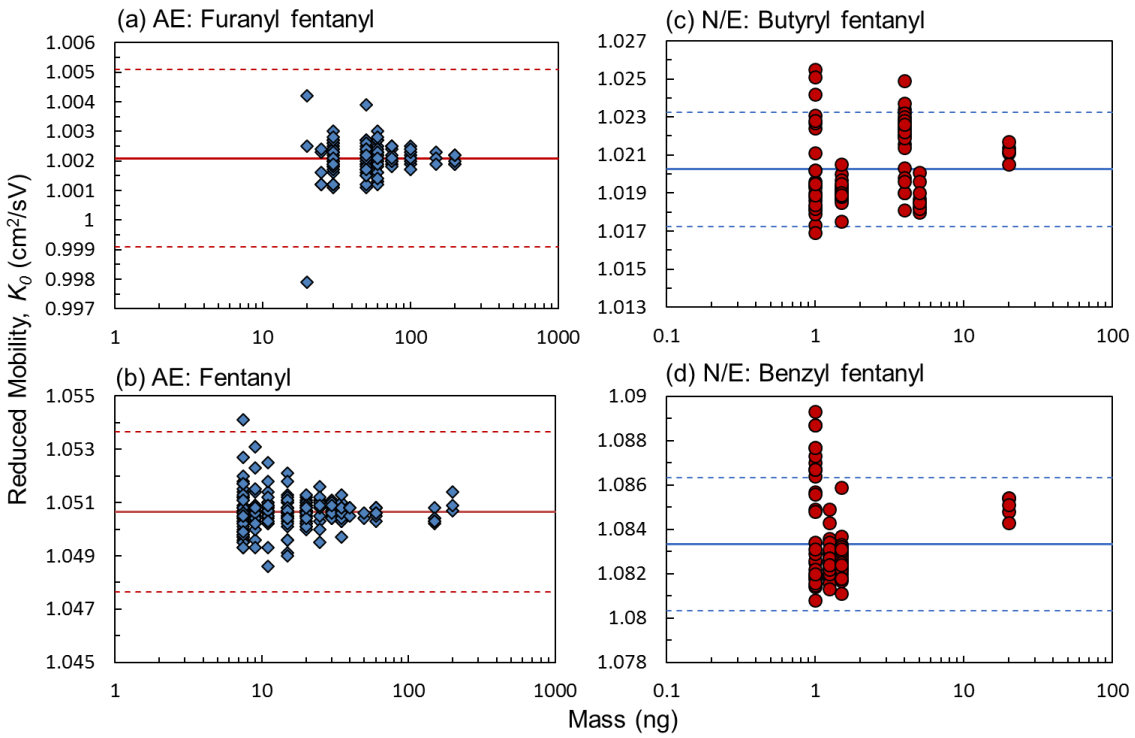

Figure S9. Experimentally measured reduced mobilities, K_0, values as a function of decreasing mass loading for (a) furanyl fentanyl and (b) fentanyl on the AE configured instrument and (c) butyryl fentanyl and (d) benzyl fentanyl on the N/E configured instrument. Average K_0 values (solid horizontal lines) and ± 0.003 cm²/sV windows (dashed horizontal lines) displayed for each.

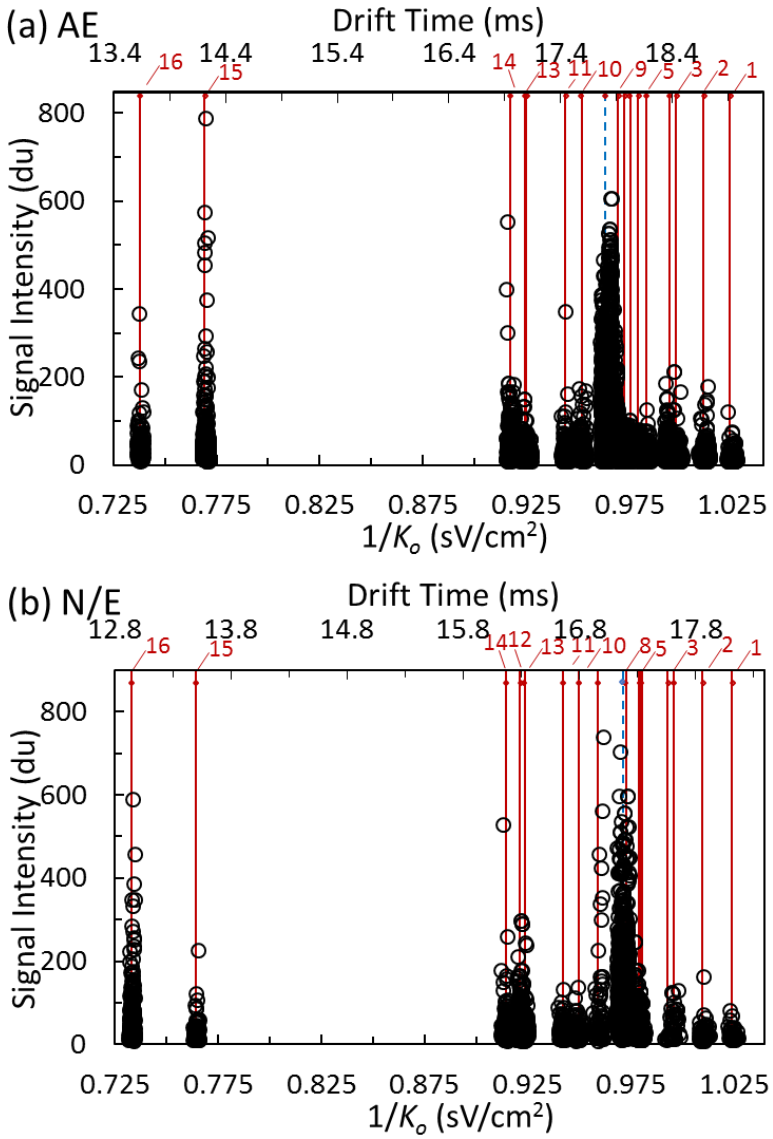

Figure S10. Environmental background interferent peak intensity levels for target analyte drift time (top axis) and inverse of reduced mobility (bottom axis) windows from (a) AE and (b) N/E instruments during their deployments. Solid red vertical lines correspond to the reduced mobility values in Table 3 and dashed blue line corresponds to observed deployed location specific background contamination peak. Analyte labels (#) correspond to identifications in Table 2.

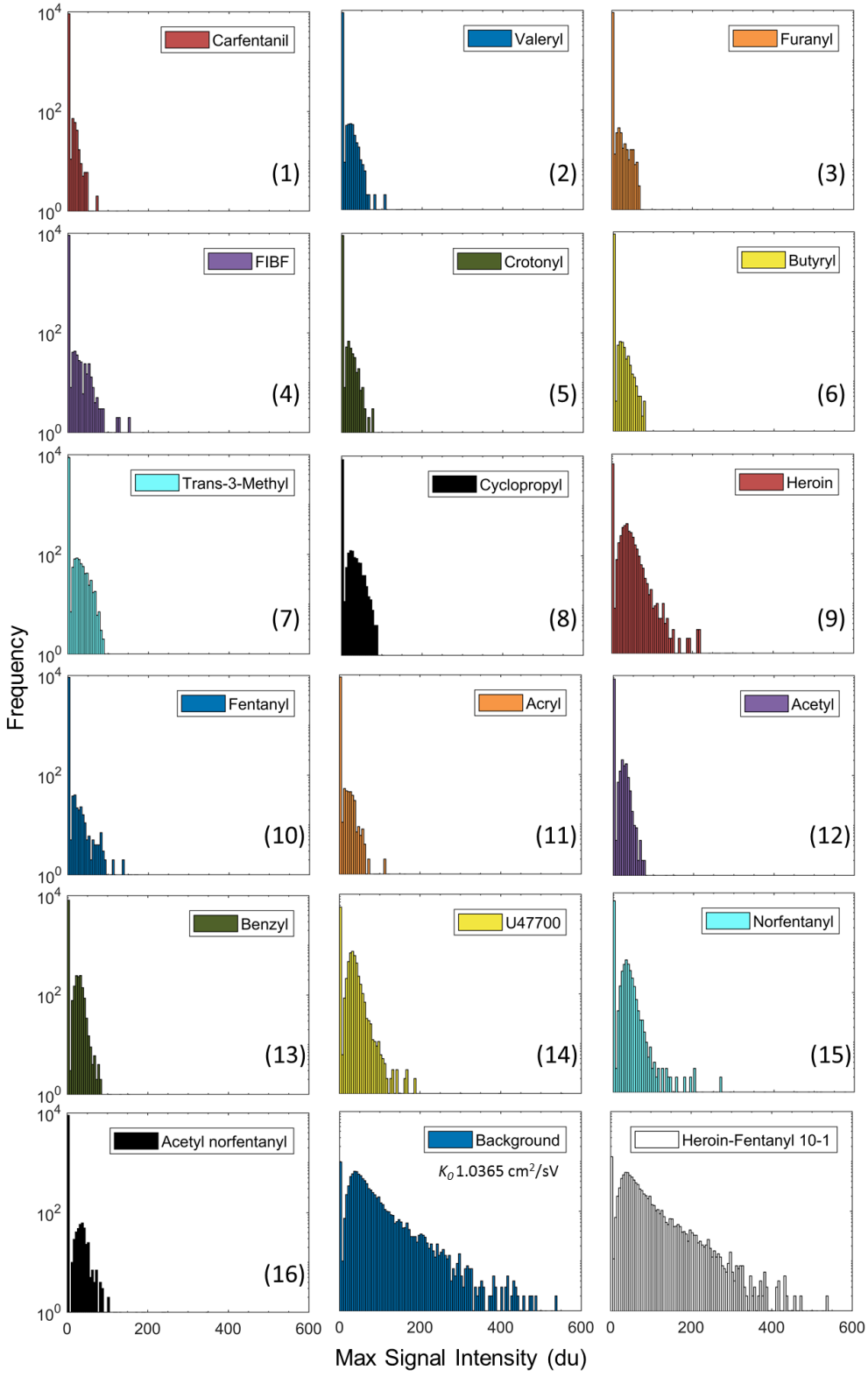

Figure S11. Frequency histograms of background intensity data from the deployed AE IMS instrument across a year period (9,359 total files/samples)

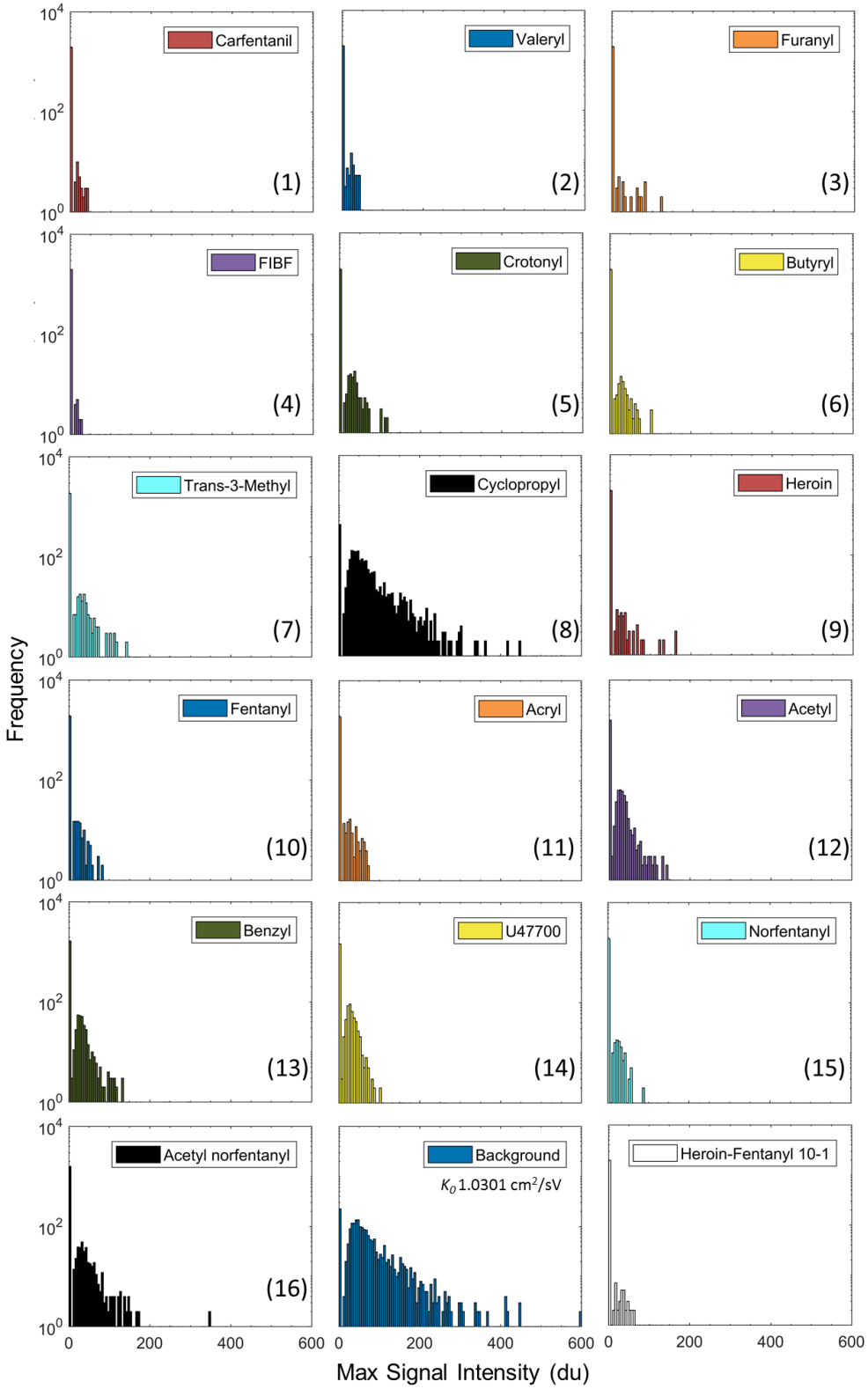

Figure S12. Frequency histograms of background intensity data from the deployed N/E IMS instrument across a multi-month period (1,996 total files/samples)

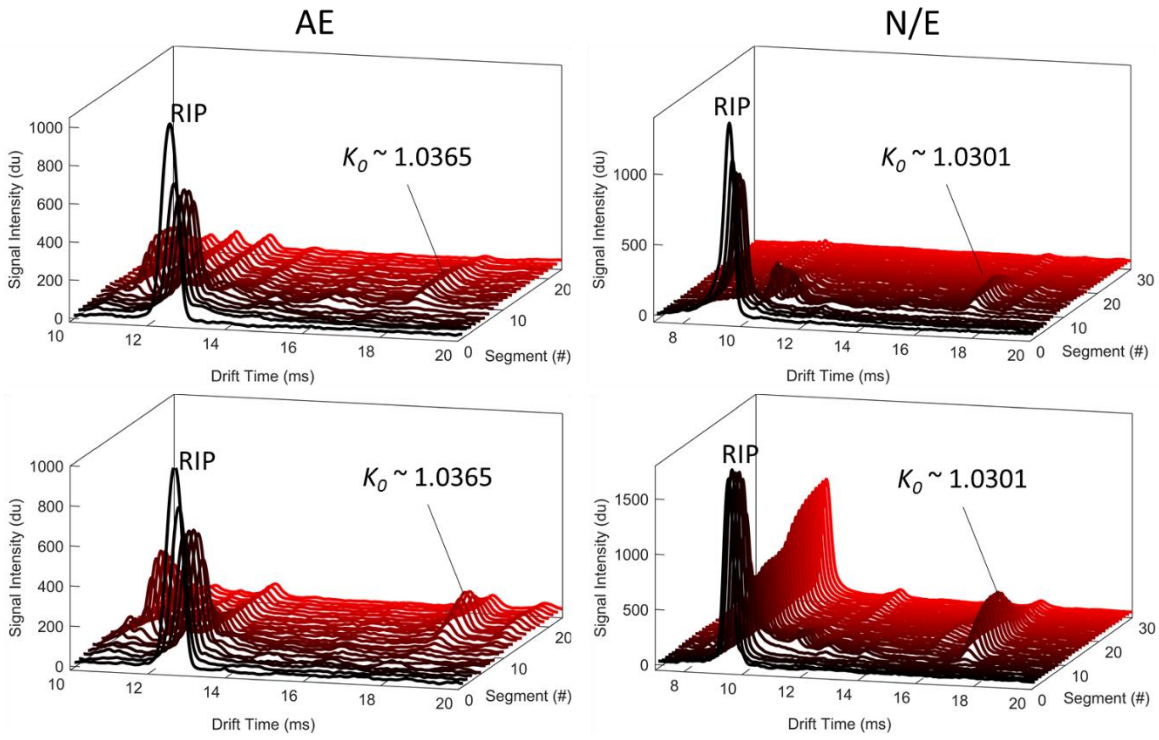

Figure S13. Representative IMS spectra as a function of drift time and segment number for vehicle screening background peaks observed in both AE (left) and N/E (right) instruments.

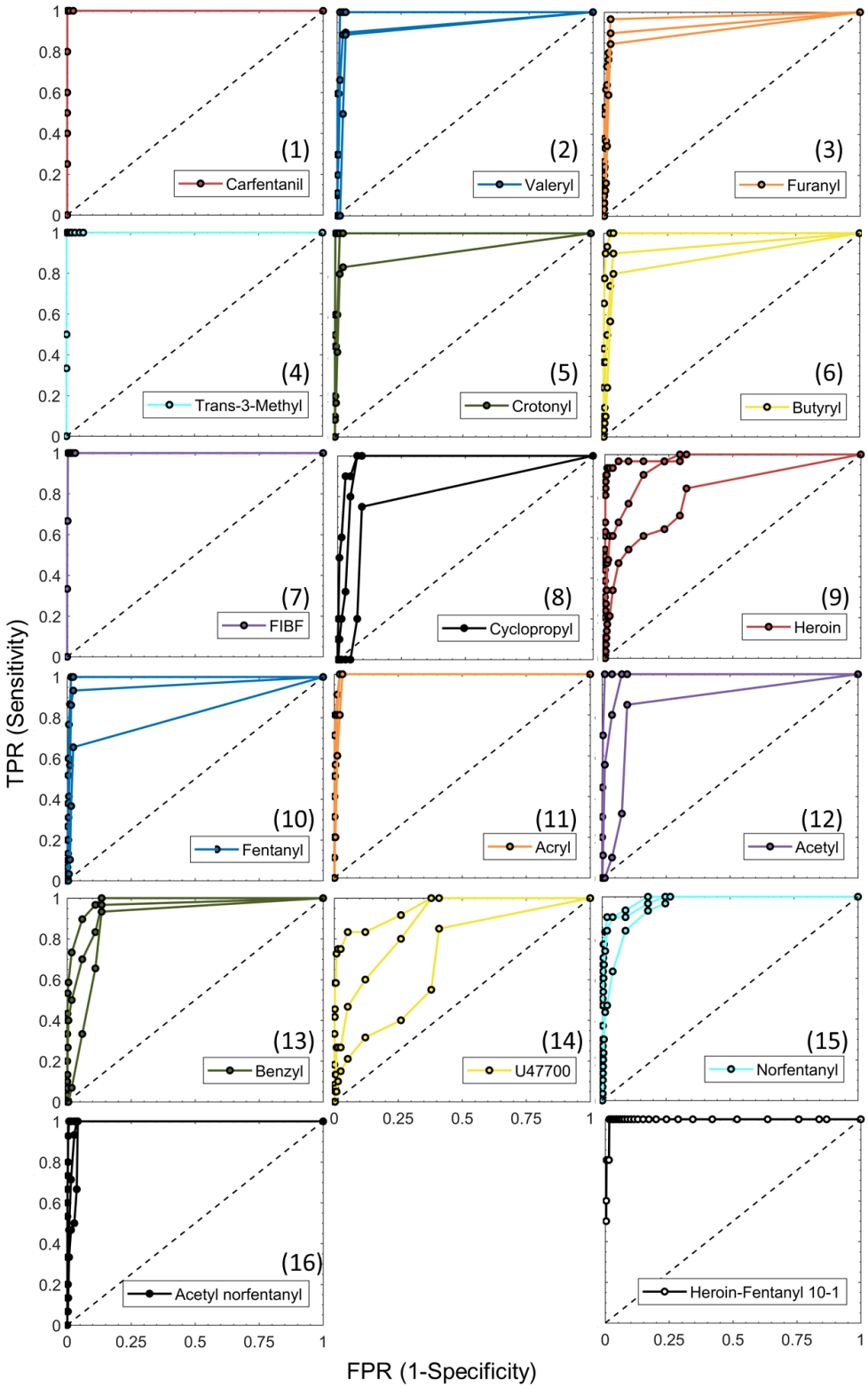

Figure S14. ROC Curves for each target analyte at three mass loading levels (see Table 2) for the AE IMS with a ± 0.003 cm^2/sV reduced mobility window. Alarm thresholds were varied from 0 du to 600 du in 10 du increments.

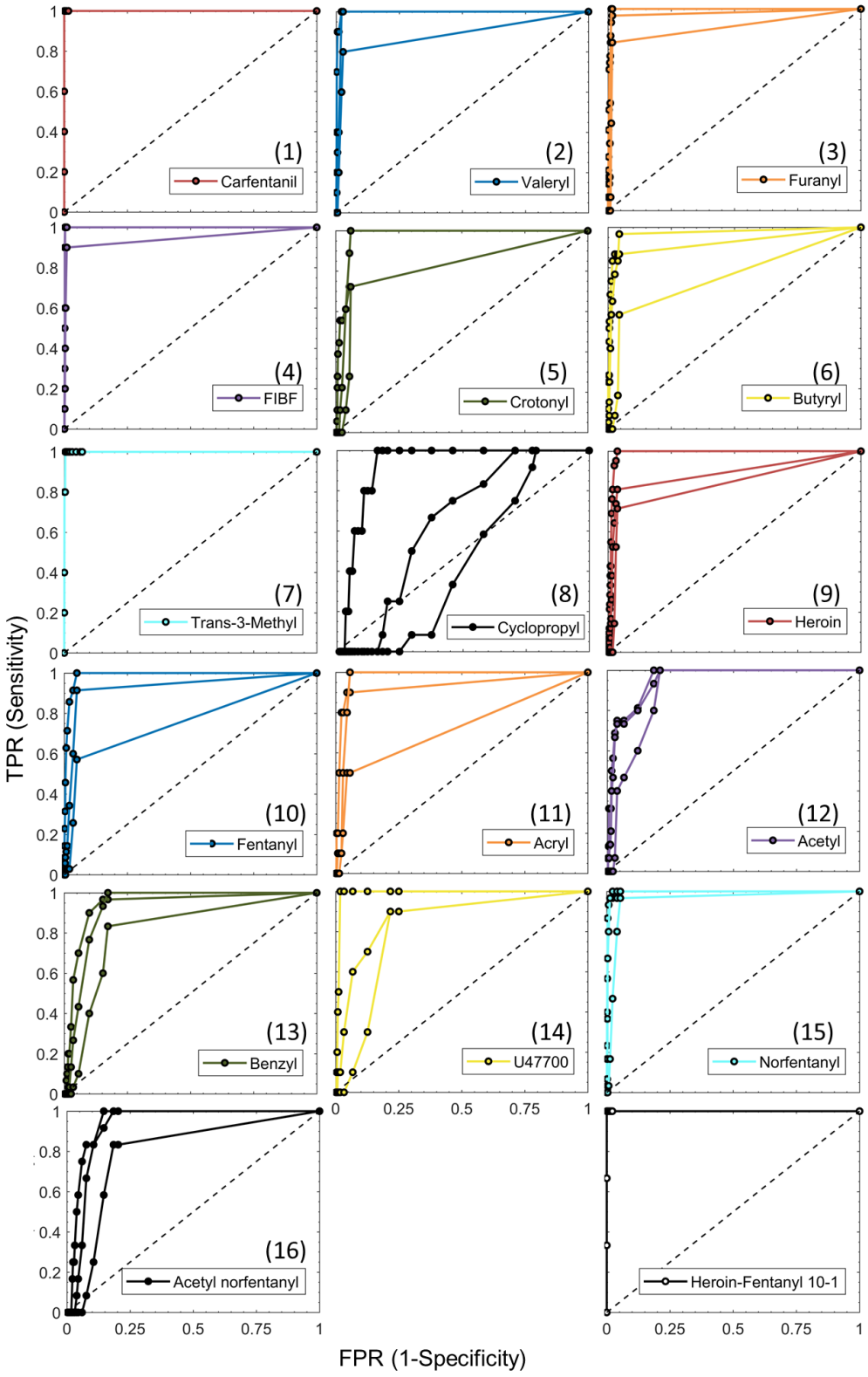

Figure S15. ROC Curves for each target analyte at three mass loading levels (see Table 2) for the N/E IMS with a ± 0.003 cm^2/sV reduced mobility window. Alarm thresholds were varied from 0 du to 600 du in 10 du increments.