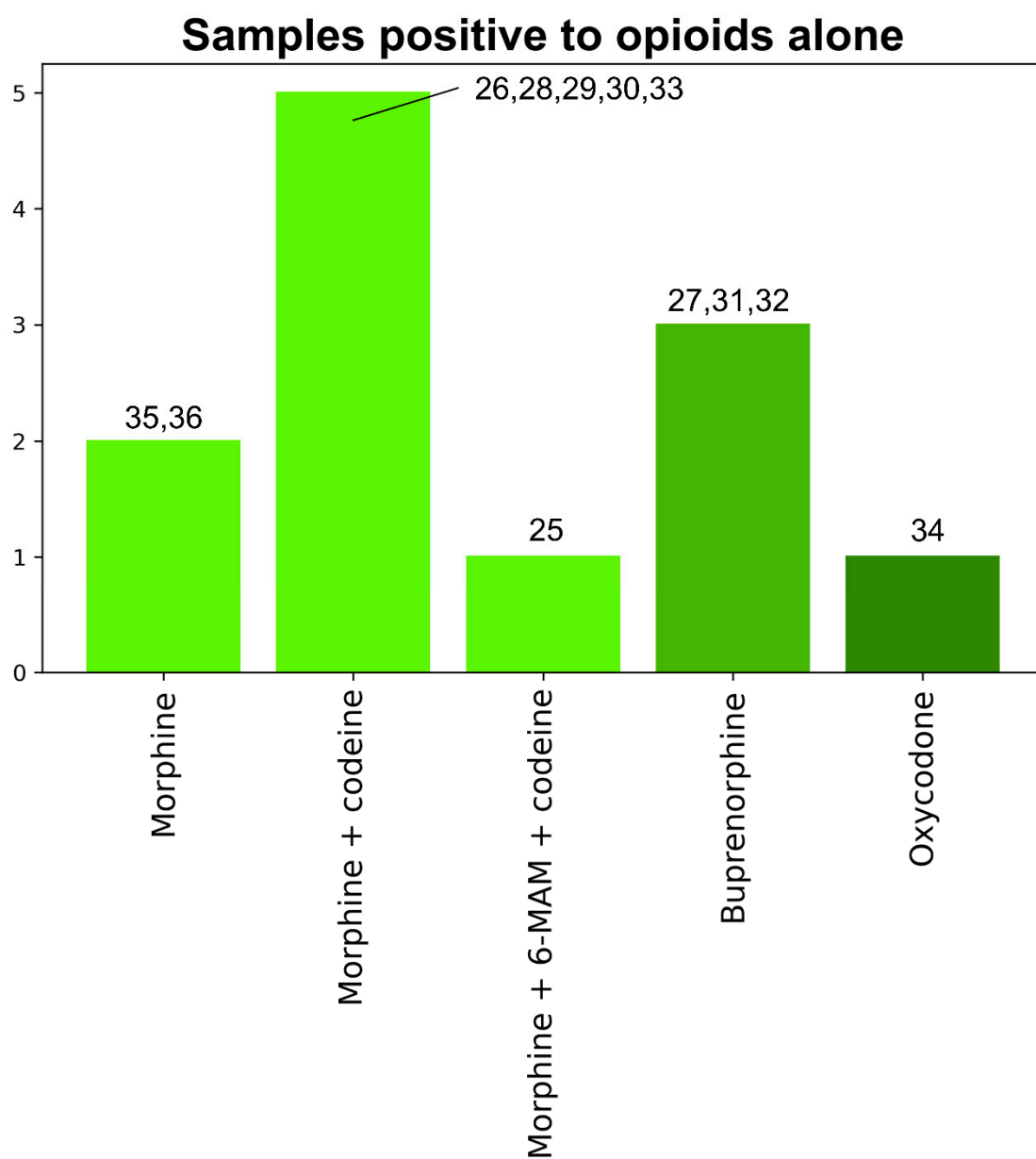
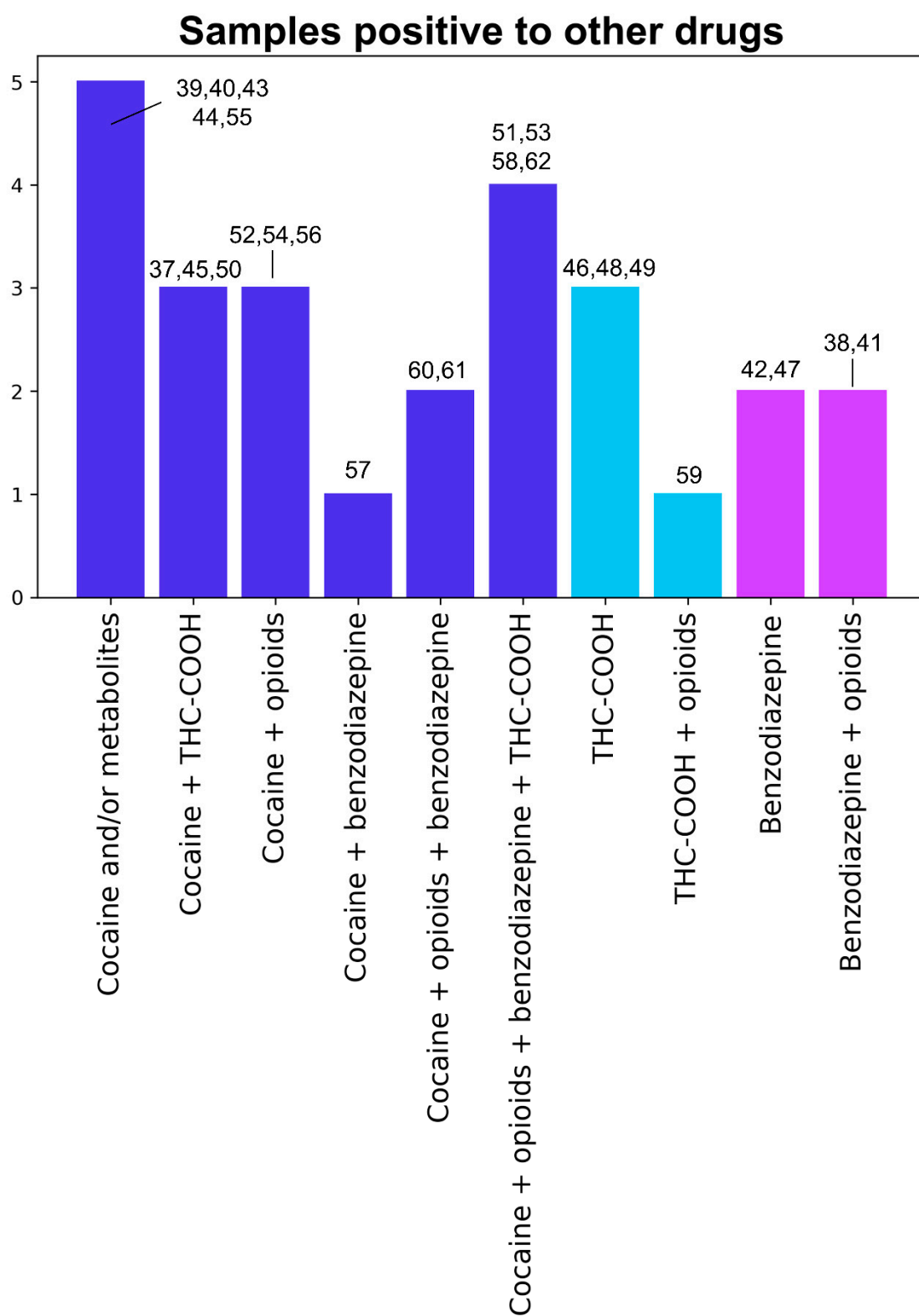


**Figure S1.** Bar plot reporting the samples positive to fentanyl alone and the ones involved in poly-consumptions. The numbers reported on the bars correspond to the samples' IDs.

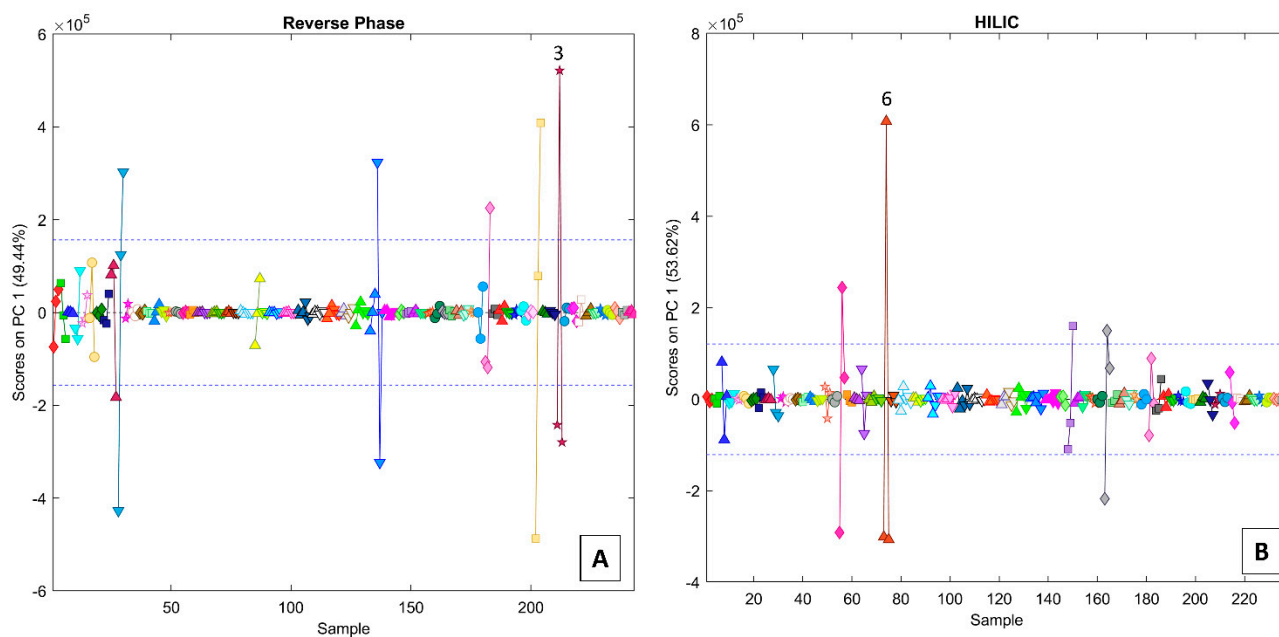


**Figure S2.** Bar plot reporting the distribution of the samples positive to traditional opioids. The numbers reported on the bars correspond to the samples' IDs.



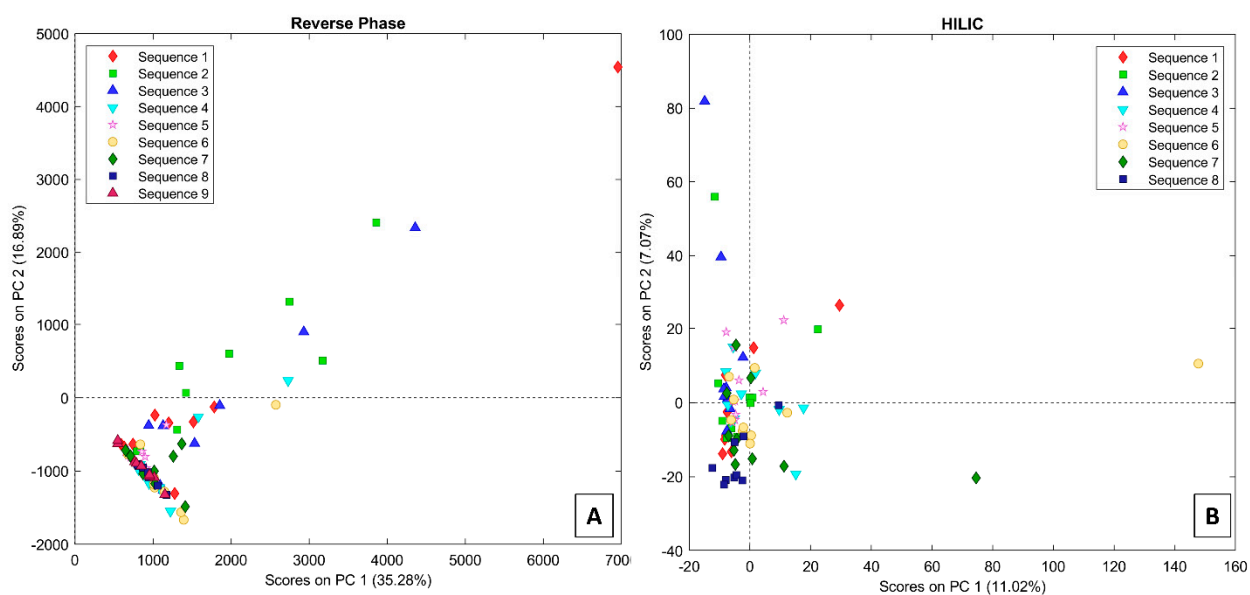
**Figure S3.** Bar plot reporting the distribution of the samples positive to traditional drugs. The numbers reported on the bars correspond to the samples' IDs.

### Verification of the technical replicates reproducibility

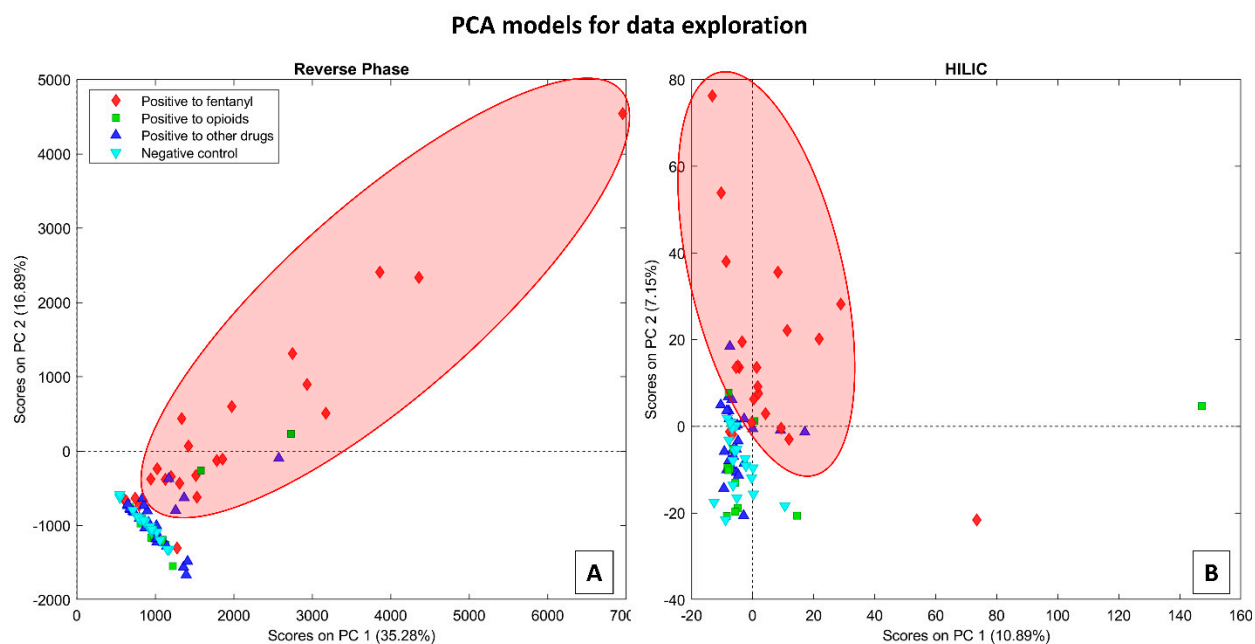


**Figure S4.** PCA models calculated using the class-centering pre-treatment to investigate the reproducibility of the technical replicates. Each sample is differently colored and the technical replicates are linked by a line. (A) RP experiments and (B) HILIC experiments.

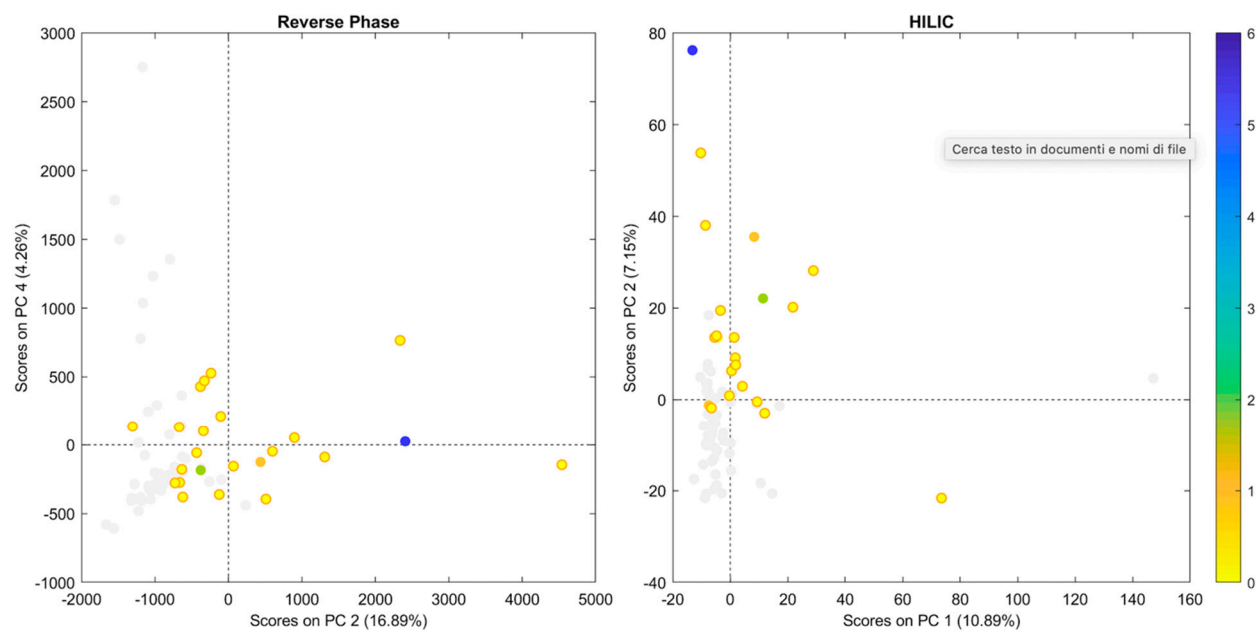
### Verification of the batch effect correction



**Figure S5.** PCA model calculated on the Pareto scaled RP dataset (A) and of the autoscaled HILIC dataset (B). The data-points are coloured on the basis of the analytical sequence.

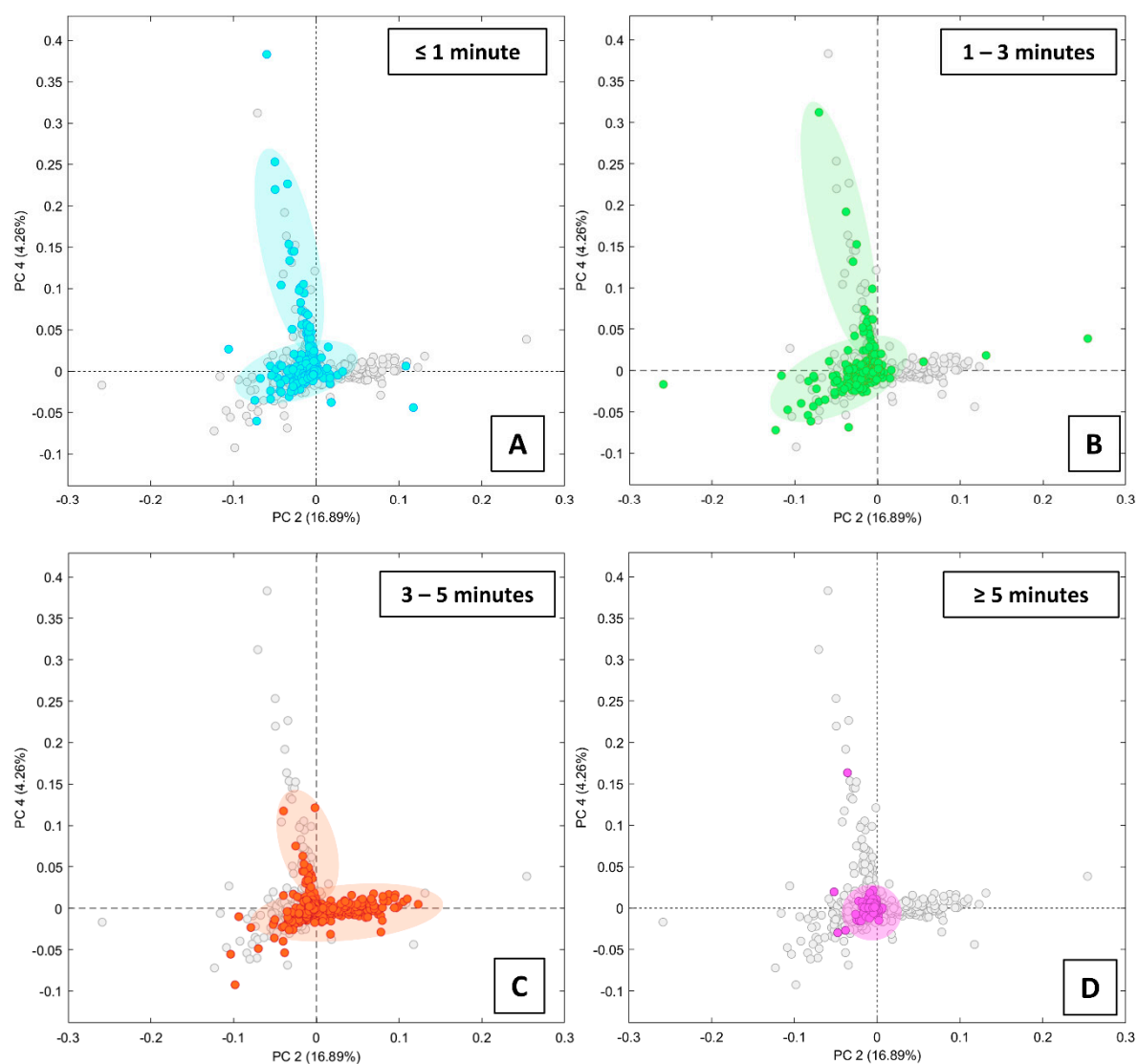


**Figure S6.** PCA model calculated on the Pareto scaled RP dataset (A) and of the autoscaled HILIC dataset (B). The data-points are coloured on the basis of the four groups identified (see Materials and Methods).



**Figure S7.** Investigation of a trend related to the fentanyl urinary dosage in the RP (A) and HILIC (B) dataset, respectively.

### RP dataset PCA – Loadings plot



**Figure S8.** PC2 vs PC4 loadings plot of the PCA model built with the RP dataset. The loadings are coloured on the basis of the retention time: (A) lower than (or equal to) 1 minute, (B) between 1 minute and 3 minutes, (C) between 3 minutes and 5 minutes, and (D) higher than 5 minutes.



**Table S1.** Fentanyl and norfentanyl dosage. The method's limit of detection is equal to 0.2 ng/mL

#ID	Fentanyl (ng/mL)	Norfentanyl (ng/mL)
1	120	220
2	100	210
3	70	64
4	25	6.0
5	25	68
6	21	59
7	14	6.9
8	14	44
9	14	15
10	12	3.7
11	4.6	2.3
12	4.2	1.9
13	3.0	2.0
14	2.3	1.4
15	2.3	9.7
16	2.1	1.9
17	1.9	20
18	1.0	1.1
19	0.9	3.1
20	0.9	0.5
21	0.9	10
22	0.7	0.5
23	0.4	1.2
24	0.2	<LOD