ASSESSING ACCURACY AND PRECISION USING QUALITY CONTROLS FOR NON-TARGETED ANALYSIS

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ABSTRACT

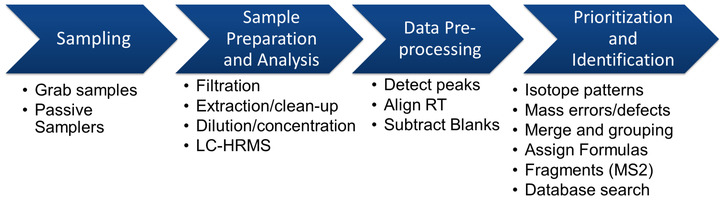
The benchmarks to assess reproducibility are not well defined for non-target analysis. Performance evaluation of analytical methodologies such as accuracy, precision and selectivity are well defined for target analysis, but remains novel but elusive topic for non-target screening analysis. In this study, quality control (QC) guidelines implemented in our laboratory are proposed with the aim to assure quality of the data in non-targeted screening methodologies using a simple set of standards. Workflow reproducibility was assessed using an in-house QC mixture containing selected compounds with a wide range of polarity that can be detected either by electrospray ionization (ESI) in positive or negative mode. The analysis was done by online solid phase extraction (SPE) liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Data processing was done by a commercially available software, Compound Discoverer. In this study, method specificity, precision, accuracy and reproducibility was evaluated in terms of peak area and retention time variability, true positive detection rate, intraday and interday variations. Accuracy was found to be consistent between intraday and interday analysis, with a detection rate of ≥ 70% for most of the QC compounds. Intraday and interday precision estimated based on peak area relative standard deviation (RSD) ranged between 30 to 50% for most of the compounds. Overall, RSDs varied largely depending on the compounds, with sulfamethoxazole, atrazine and carbamazepine exhibiting a RSD ≤ 30%, while lincomycin, gemfibrozil and mefenamic acid showed a RSD ≥ 70%. Retention time precision for both intra- and interday analysis showed great repeatability and reproducibility, with all the detected compounds having a retention time RSD ≤ 5%.

OBJECTIVES

- The main objective of this study was to introduce simple preliminary quality control guidelines to be followed in non-targeted screening methodologies.
- Workflow specificity, precision, accuracy, repeatability and reproducibility were assessed using an in-house QC mixture that could be easily implemented in a typical analytical lab and customized containing a wide range of compounds that can be detected in both electrospray ionization (ESI) positive as well as ESI negative.

MATERIALS AND METHODS

Non-target Analysis Workflow for environmental analysis adopted from Hollender *et. al.*[1]



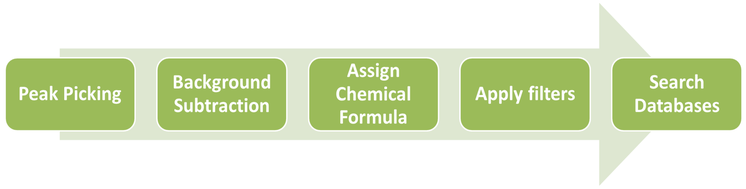
UHPLC-High Resolution Mass Spectrometry:

Figure 1. Thermo Q-Exactive Orbitrap.

- Thermo Q-Exactive Orbitrap
- ESI sources
- FS:100-800 m/z 140,000 resolution
- MS^2 for confirmation: NCE 30
- Positive and Negative modes
- 4 runs per sample (MS^1, MS^2)
- Total time per run: 15 min
- Quality control samples
- Mass tolerance: <5 ppm
- Spray Voltage (V) 5000
- Capillary Temperature (˚C) 350
- Sheath Gas (a.u) 30
- Aux Gas (a.u) 2
- S-Lens RF Level 50

RESULTS

Data Processing Workflow using Compound Discovery v. 3.0



The data analysis usually includes steps such as peak-picking, blank subtraction, componentization, molecular formula generation, isotopic pattern comparison, evaluation of adducts, and the assessment and comparison of fragmentation patterns.

Quality Control

Compound	Log K_{ow}	Molecular formula	Monoisotopic mass	Monitored ions	Retention time (mins)
Sucralose	-1.00	$C_{12}H_{19}Cl_3O_8$	396.0146	395.0073[b]	11.16
Hydrochlorothiazide	-0.10	$C_7H_8ClN_3O_4S_2$	296.9645	295.9572[b]	11.39
Caffeine	0.16	$C_8H_{10}N_4O_2$	194.0804	195.0877[a]	11.01
Lincomycin	0.29	$C_{18}H_{34}N_2O_6S$	406.2137	407.2210[a]	10.60
Sulfamethoxazole	0.48	$C_{10}H_{11}N_3O_3S$	253.0521	254.0594[a]	12.42
Trimethoprim	0.73	$C_{14}H_{18}N_4O_3$	290.1379	291.1452[a]	11.11
Norcocaine	1.96	$C_{16}H_{19}NO_4$	289.1314	290.1387[a]	12.05
Carbamazepine	2.25	$C_{15}H_{12}N_2O$	236.0950	237.1022[a]	13.11
Diltiazem	2.79	$C_{22}H_{26}N_2O_4S$	414.1613	415.1686[a]	12.80
Atrazine	2.82	$C_8H_{14}ClN_5$	215.0938	216.1010[a]	13.66
Diphenhydramine	3.11	$C_{17}H_{21}NO$	255.1623	256.1696[a]	12.86
Diclofenac	4.02	$C_{14}H_{11}Cl_2NO_2$	295.0167	294.0094[b]	14.14
Fluoxetine	4.65	$C_{17}H_{18}F_3NO$	309.1341	310.1413[a]	13.46
Gemfibrozil	4.77	$C_{15}H_{22}O_3$	250.1569	249.1496[b]	14.48
Mefenamic acid	5.28	$C_{15}H_{15}NO_2$	241.1103	240.1030[b]	14.44
Sertraline	5.29	$C_{17}H_{17}Cl_2N$	305.0738	306.0811[a]	13.57
Clotrimazole	6.26	$C_{22}H_{17}ClN_2$	344.1080	345.1153[a]	13.68

[a]Ions were monitored in ESI positive (70.6%), [b]Ions were monitored in ESI negative (29.4%)

Table 1. List of quality control compounds and their respective log K_{ow} , molecular formula, monoisotopic mass and monitored ions.

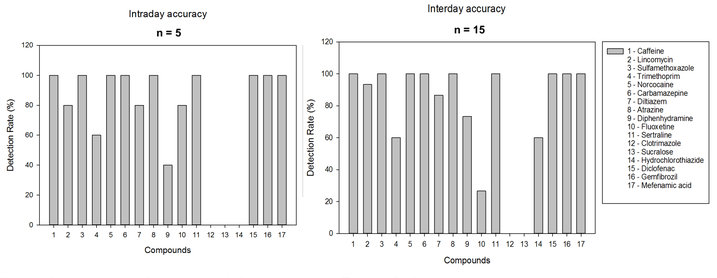


Figure 2. Accuracy of compound discoverer in detecting and identifying QC compounds within the same day (n=5).

Figure 3. Accuracy of compound discoverer in detecting and identifying QC compounds over 3 consecutive days (n=15).

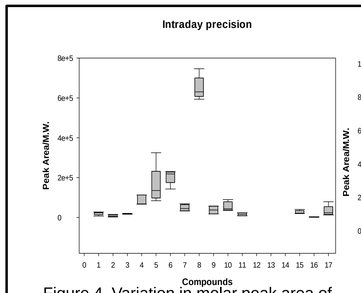


Figure 4. Variation in molar peak area of the detected QC compounds within the same day (n=5).

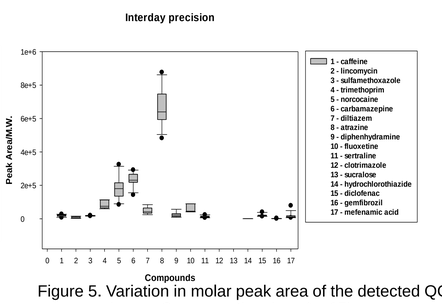


Figure 5. Variation in molar peak area of the detected QC compounds over 3 consecutive days (n=15).

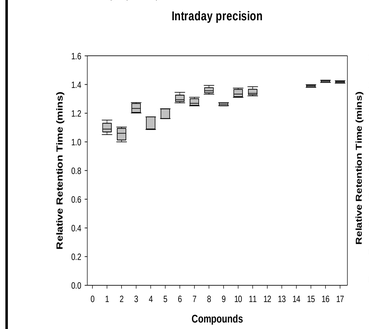


Figure 6. Variation in relative retention time of the detected QC compounds within the same day (n=5).

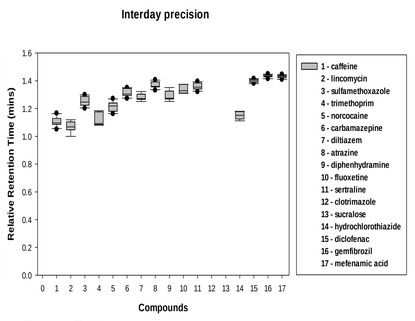


Figure 7. Variation in relative retention time of the detected QC compounds over 3 consecutive days (n=15).

CONCLUSIONS

- The intraday accuracy of the NTA workflow was greater than 75% detection rate for majority of the detected QC compounds except for trimethoprim and diphenhydramine which were detected 60% and 40% respectively; and 3 compounds that were not detected or correctly identified (clotrimazole, sucralose and hydrochlorothiazide).
- The interday accuracy of the NTA workflow was consistent with that of the intraday study, in which the majority of the detected QC compounds had a detection rate greater than 75%..
- Intraday precision in terms of peak area for the detected compounds varied by compound, ranging from a RSD of 8.2% for sulfamethoxazole to 106.5% for gemfibrozil. Sulfamethoxazole, atrazine and carbamazepine exhibiting a RSD less than 30%, four compounds, diphenhydramine, lincomycin, gemfibrozil and mefenamic acid showing a RSD greater than 70% and the other compounds having a RSD between 30 to 50%.
- Interday precision in terms of peak area for the detected compounds were consistent with that of the intraday. Intraday and interday precision in terms of RT for all the detected compounds were ≤ 5%, showing a very good reproducibility and repeatability in terms of retention time.

REFERENCES

1. Hollender, J.; Schymanski, E. L.; Singer, H. P.; Ferguson, P. L., Nontarget screening with high resolution mass spectrometry in the environment: ready to go? *Environ. Sci. Technol.* **2017**, 51, 11505-11512.

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