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Innovation Development of Multidimensional Holographic Analysis Technology of the Multi-Mycotoxin in Pu’er Tea Sample Analysis

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Introduction

Mycotoxins are biological toxins that are produced by fungi. To date, more than 200 types of mycotoxins have been discovered. Analysis of mycotoxins and sample cleanup in Pu'er tea sample is challenging because of the high cost and complex operation. A simple, fast, and fully automated multi-dimensional LC-MS/MS method was developed for the determination of 11 mycotoxins (using an innovative technology called: Multidimentional Holographic Analysis Technology (MHAT). This next generation fully automated analysis platform delivers sensitivity, versatility, robustness, and intelligence.

11 mycotoxins include: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), Aflatoxin G2 (AFG2), Aflatoxin M1 (AFM1), Aflatoxin M2 (AFM2), Ochartoxin A (OTA), Ochartoxin B (OTB), Deoxynivalenol (DON), Fumonisin B1 (FB1) and Fumonisin B2 (FB2).

Experimental

1290 UHPLC Loading & Trap System

1D Column: Agilent Poroshell HILIC-OH5 column, 2.1X100mm, 2.7 µm

Trap column: Agilent SB-C8 cartridge column, 4.6X12.5mm, 5 µm

Mobile phase A: 10mM NH_4CO_2H and 0.1% HCOOH in water
Mobile phase B: 10mM NH_4CO_2H and 0.1% HCOOH in acetonitrile/water=90/10
Injection volume: 10 µL
Column Oven: 40 °C
Gradient program: (Flow rate: 0.3 mL min^{-1})

Time (min)	0	2.5	3.0	3.8	10	10.5	30.0
A (%)	2	2	10	80	80	2	2
B (%)	98	98	90	20	20	98	98

Experimental

1290 UHPLC Analytical System

series connection columns:
Agilent Poroshell PFP, 2.1X50mm, 2.7 µm and Agilent Poroshell Phenyl-Hexyl, 2.1X50mm, 2.7 µm

Mobile phase A: 5mM NH_4CO_2H and 0.001% formic acid in water
Mobile phase B: 5mM NH_4CO_2H and 0.001% formic acid in methanol.
Column Oven: 40 °C
Gradient program: (flow rate: 0.3 mL min^{-1}; Add Water with 1.5 mL min^{-1} from 1.0 to 2.5 min)

Time (min)	0	0.9	1.0	2.5	2.55	3.5	5.0	5.5	7.5	11.0
A (%)	95	95	100	100	100	100	100	95	85	50
B (%)	5	5	0	0	0	0	0	5	15	50

Time (min)	18.0	21.0	24.0	26.0	26.1	30.0
A (%)	30	15	0	0	95	95
B (%)	70	85	100	100	5	5

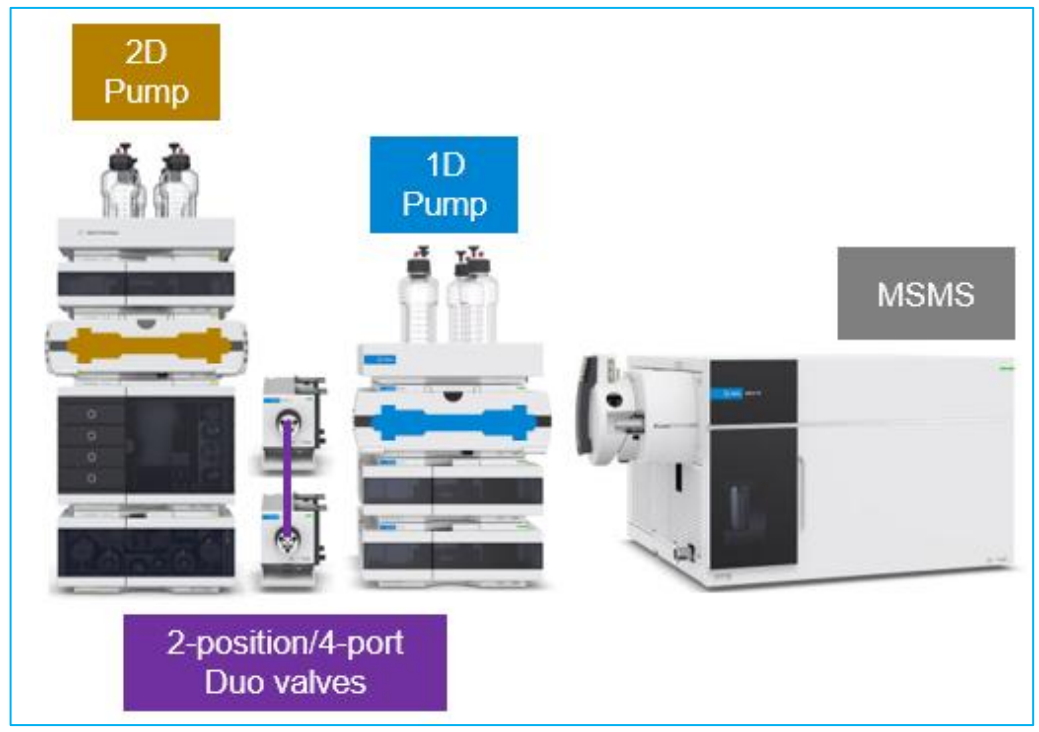

Figure 1. Agilent CDLPT 6495 LC/TQ System

Results and Discussion

The MHAT LC/TQ technology for mycotoxins analysis of complex Pu'er tea sample is composed of three parts: 1) automatic multi-dimensional (MD) extraction, 2) MassHunter Study Manager software and 3) MassHunter automatic quantitation workflow (Figure 2). In a 1D system the mycotoxins are separated with high interferences. The mycotoxins were separated by heart-cutting into column trapping and retention using C8 material with two 2position/4-port Duo valve systems. After the system flush (2.5 minutes), the mycotoxins were analyzed with the 2D system. (Figure 3). The MassHunter Study Manager software and MassHunter automatic quantitation workflow were configured to inject and report a qualified result automatically. The sample preparation of this system is simple and faster than traditional techniques like liquid-liquid extraction (LLE), Immunoaffinity preparation, filter extraction or QuEChERS.

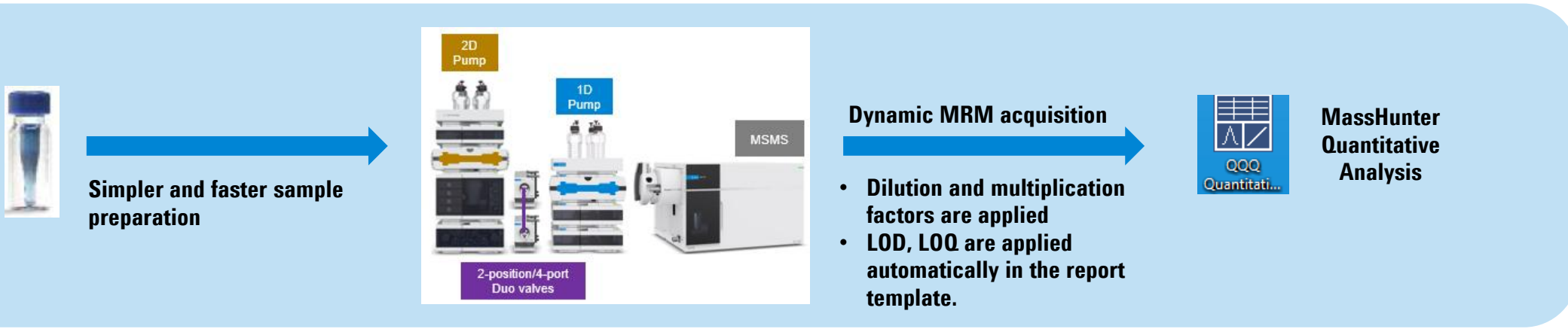

Figure 2. Automatic Workflow of MHAT LC/TQ Analysis and Data Processing with Masshunter

The MHAT LC/TQ technology is a compound lossless system. All 11 mycotoxins are eluted and analyzed including HILIC separation of FB1 and FB2 (2.5-5.5 min), PFP and Phenyl-Hexyl series connection columns separation (5.5-30.0 min) or no separation (0-2.5 min). In this study, besides FB1 and FB2, other mycotoxins could be captured by trap column and analyzed in the second dimensional separation. (Figure 3).

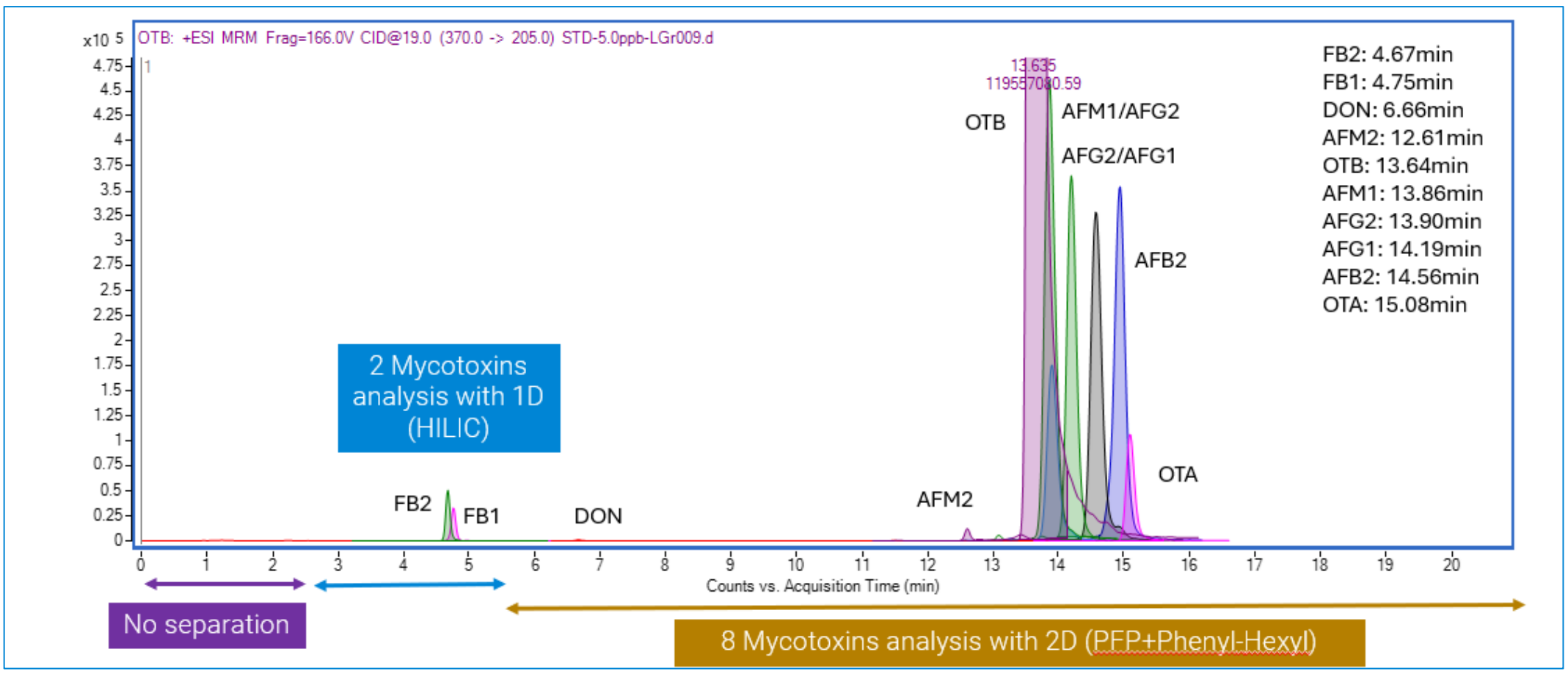

Figure 3. The 11 Mycotoxin standards at a concentration of 5.0 ng/mL were analyzed with the MHAT LC/TQ system.

Results and Discussion

11 mycotoxins were measured in Pu'er tea matrices with 10 times sample dilution by injecting a matrix-matched calibration curve at 7 concentrations ranging from 1 to 100 ng g^{-1} in sample. As an example. good precision and linearity of three representative mycotoxins is shown for quantitative determination. (Figure 4). Precision was <20% and the R^2 value was better than 0.99 in linear range.

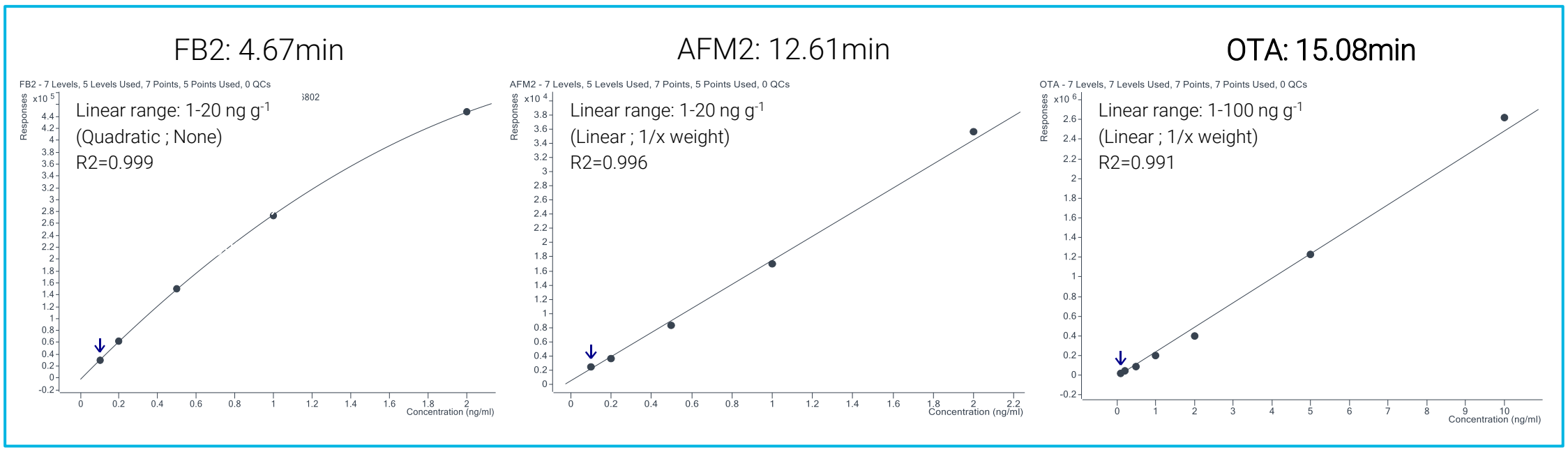

Figure 4. The Excellent Matrix-Match Calibration for Three Representative Mycotoxins.

The recovery testing used different sample dilutions to evaluate the system with 10 µL after the sample preparation with solvent and filter extraction. The total recovery of 11 mycotoxins meet food regulation and were found to be in the range of 60-120% with 10 times sample dilution and the detection limit (DL) was between 1.0-2.0 ng g^{-1} for quantitation with matrix-match calibration. (Figure 5).

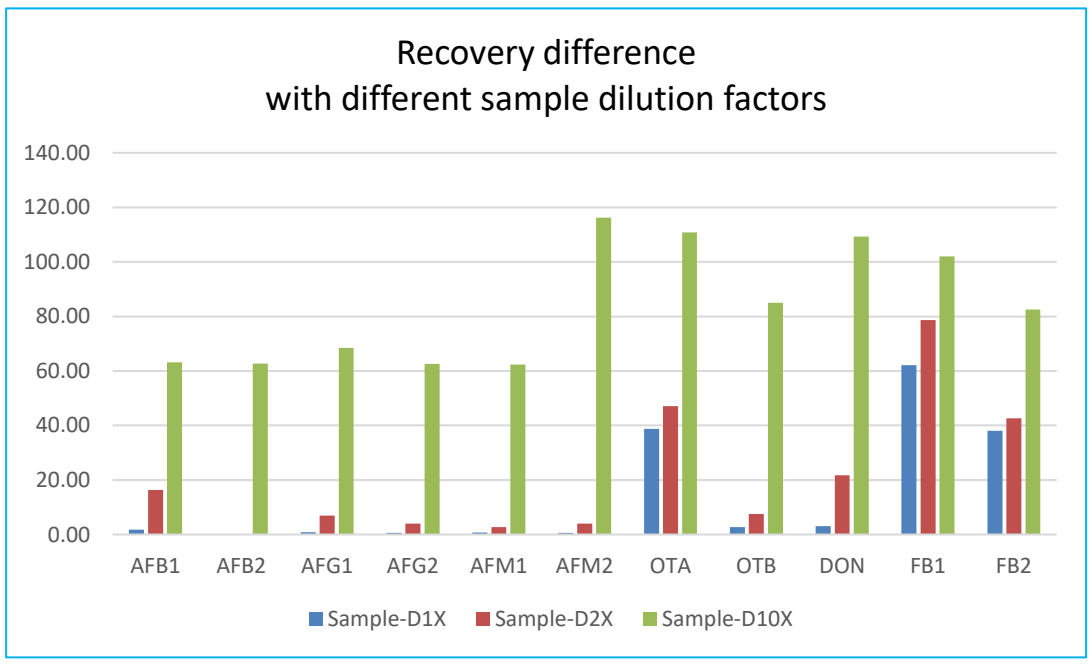

Figure 5. The distribution of recovery of 11 mycotoxins. Spiked final concentration of 50ng g^{-1} .

Conclusions

Gradient elution and Dynamic MRM with the innovative MHAT LC/TQ technology were employed for simultaneous identification of 11 mycotoxins in Pu'er tea sample.

The benefits were:

- Simple sample preparation with solvent extraction and 10 times dilution, then injection into LC/TQ.
- Although the result shows only 11 mycotoxins, we are planning to do more 50 mycotoxins in the future.
- >50% time saving with automatic data processing.

References

[1] Analysis of Mycotoxins in Food Matrices Using the Agilent Ultivo Triple Quadrupole LC/MS. Agilent Application Note: 5991-8962EN

[2] Development, validation, and application of a multi-method for the determination of mycotoxins, plant growth regulators, tropane alkaloids, and pesticides in cereals by two-dimensional liquid chromatography tandem mass spectrometry. Ann-Kristin Rausch, Robert Brockmeyer, Tanja Schwerdtle. Analytical and Bioanalytical Chemistry, (2021) 413:3041-3054.

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