Cell culture media metabolite profiling using a HRAM LC-MS approach

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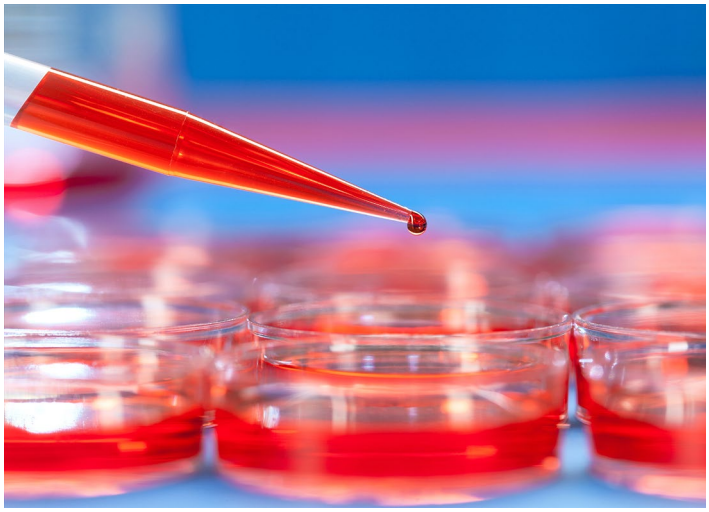
Keywords: Cell culture media, targeted metabolite profiling, high resolution accurate mass (HRAM), online polarity switching, relative quantitation



Application benefits

- Targeted quantification of diverse classes of metabolites simultaneously in a single LC-MS run
- Easy platform mass spectrometer method set up—no need for any analyte specific optimization
- Great selectivity, sensitivity, reproducibility, and robustness for the quantitative profiling of targeted metabolites in cell culture media using a high-resolution accurate mass mass spectrometer
- Single Chromatography Data System (CDS) software for streamlined instrument control and data processing
- Additional capability to quantify untargeted metabolites by retrospective HRAM MS data analysis

Goal

- Develop a targeted quantitative assay that monitors and quantifies multiple classes of cell culture media metabolites simultaneously in a single LC-MS run
- Demonstrate that the developed quantitative assay can be used to support upstream processing optimization and quality control with excellent quantitative performance, high throughput, and robustness

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Introduction

Metabolite analysis, such as cell culture media components and byproducts, is critical for understanding both the intracellular and extracellular environments in cell culture. This can lead to greater understanding of the process impacts on cell growth, productivity, and product quality during biopharmaceutical development and manufacturing. Monitoring the concentration profiles of important cell culture media nutrients and metabolites over the course of the production process or over different lots of culture media can provide valuable insights into the generation of highly productive cell lines, optimization of cell culture process conditions, and troubleshooting of a faulty bioprocess. However, it remains challenging to simultaneously monitor multiple classes of metabolites in cell culture media because of their diversified physical and chemical properties and large range of concentrations. An LC-MS method enables multiplex quantification of tens to hundreds of metabolites in a single LC-MS run with high sensitivity and selectivity and is well suited for cell culture metabolite profiling experiments. Recently, triple quadrupole mass spectrometry with multiple reaction monitoring (MRM) has been successfully applied to simultaneously analyze over 100 pre-selected cell culture metabolites.[1] Although MRM enables targeted metabolite profiling with good quantitative results, it is time consuming to optimize transitions per metabolite for MRM assay development. In addition, this method targets only the pre-selected metabolites, resulting in a loss of valuable information of untargeted metabolites.

Here we present an alternative targeted metabolite profiling approach using high resolution accurate mass (HRAM) mass spectrometry with full scan analysis. Unlike the MRM approach, the full scan HRAM approach collects all data in the cell culture media samples first, then extracts the peak areas of targeted metabolites for quantification post data acquisition. Therefore, the HRAM approach allows quantification of additional metabolites beyond the pre-selected metabolite list through retrospective HRAM MS data analysis. The Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer delivers consistent and accurate quantification data and is well suited for robust quantification of targeted cell culture media metabolites. Combining with the Thermo Scientific™ Vanquish™ Horizon UHPLC system, the Orbitrap Exploris 120 mass spectrometer enables the high sensitivity and selectivity required to support upstream process optimization and manufacturing quality control. As proof of concept, we developed a targeted metabolite quantification assay that monitors 100 cell culture metabolites simultaneously in a 20 minute LC-MS method on the Orbitrap Exploris 120 mass spectrometer. Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2.10 was used for both instrument control and data processing. The quantitative performance of the targeted 100 metabolite profiling assay was evaluated using a spiked-in amino acid standard mixture. We applied this assay to commercially available cell culture medium samples to monitor concentration changes of targeted cell culture metabolites simultaneously across various conditions. In addition, we explored the capability of this method for obtaining quantitative information on metabolites not included in the pre-selected metabolite list through retrospective HRAM MS data analysis.

Experimental

Samples

All metabolite standards were purchased from Millipore Sigma except a quantitative mixture of 18 amino acids, Thermo Scientific™ Pierce™ Amino Acid Standard H (P/N 20088). Three commercially available cell culture medium samples were used: Gibco™ MEM α, nucleosides, GlutaMAX™ Supplement; Gibco™ Advanced DMEM/F-12; Gibco™ Advanced DMEM (Thermo Fisher Scientific).

Reagents

- Thermo Scientific™ Water, UHPLC-MS (P/N W81)
- Fisher Scientific™ Acetonitrile, Optima™ LC/MS grade (P/N A955-1)
- Fisher Scientific™ Formic acid, Optima™ LC/MS grade (P/N A11710X1-AMP)

Preparation of 100 metabolite standard mixture

100 metabolite standards across different compound classes (Table 1) were mixed together at a concentration range from 1 nmol/mL to 10 nmol/mL.

Preparation of amino acid mixture dilution series

- A metabolite matrix was simulated by mixing 82 metabolite standards with the final concentration ranges of 1 nmol/mL to 10 nmol/mL per compound.
- Thermo Scientific Pierce Amino Acid Standard H (quantitative mixture of 18 amino acids, supplied at 2.5 µMol/mL) was spiked into the prepared mimic metabolite matrix at the following concentrations: 250 nmol/mL, 25 nmol/mL, 2.5 nmol/mL, 0.25 nmol/mL, 0.025 nmol/mL.

Table 1 (part 1). List of compounds included in the 100 metabolite standard mixture

No.	Compound name	Chemical formula	Mono isotopic mass	Class
1	2-Aminoadipic acid	$C_6H_{11}NO_4$	161.06881	Amino acid
2	Alanine	$C_3H_7NO_2$	89.04767	Amino acid
3	Alanyl-glutamine	$C_8H_{15}N_3O_4$	217.10626	Amino acid
4	Aminobutyric acid	$C_4H_9NO_2$	103.06333	Amino acid
5	Arginine	$C_6H_{14}N_4O_2$	174.11168	Amino acid
6	Asparagine	$C_4H_8N_2O_3$	132.05350	Amino acid
7	Aspartic acid	C_4H_7NO	133.03751	Amino acid
8	Citrulline	$C_6H_{13}N_3O_3$	175.09569	Amino acid
9	Cystathionine	$C_7H_{14}N_2O_4S$	222.06743	Amino acid
10	Cysteine	$C_3H_7NO_2S$	121.01974	Amino acid
11	Cystine	$C_6H_{12}N_2O_4S_2$	240.02385	Amino acid
12	Glutamic acid	$C_5H_9NO_4$	147.05316	Amino acid
13	Glutamine	$C_5H_{10}N_2O_3$	146.06914	Amino acid
14	Glutamyl-L-cysteine	$C_8H_{14}N_2O_5S$	250.06234	Amino acid
15	Glutathione	$C_{10}H_{17}N_3O_6S$	307.08380	Amino acid
16	Glutathione oxidized	$C_{20}H_{32}N_6O_{12}S_2$	612.15141	Amino acid
17	Glycine	$C_2H_5NO_2$	75.03203	Amino acid
18	Glycyl-glutamic acid	$C_7H_{12}N_2O_5$	204.07407	Amino acid
19	Histidine	$C_6H_9N_3O_2$	155.06948	Amino acid
20	Hydroxyproline	$C_5H_9NO_3$	131.05824	Amino acid
21	Isoleucine	$C_6H_{13}NO_2$	131.09463	Amino acid
22	Kynurenine	$C_{10}H_{12}N_2O_3$	208.08479	Amino acid
23	Leucine	$C_6H_{13}NO_2$	131.09463	Amino acid
24	Lysine	$C_6H_{14}N_2O_2$	146.10553	Amino acid
25	Methionine	$C_5H_{11}NO_2S$	149.05106	Amino acid
26	Methionine sulfoxide	$C_5H_{11}NO_3S$	165.04596	Amino acid
27	*N*-Acetyl-Aspartic acid	$C_6H_9NO_5$	175.04807	Amino acid
28	*N*-Acetyl-Cysteine	$C_5H_9NO_3S$	163.03031	Amino acid
29	Ornithine	$C_5H_{12}N_2O_2$	132.08988	Amino acid
30	Phenylalanine	$C_9H_{11}NO_2$	165.07898	Amino acid
31	Pipecolinic acid	$C_6H_{11}NO_2$	129.07898	Amino acid
32	Proline	$C_5H_9NO_2$	115.06333	Amino acid
33	Serine	$C_3H_7NO_3$	105.0426	Amino acid

Table 1 (part 2). List of compounds included in the 100 metabolite standard mixture

No.	Compound name	Chemical formula	Mono isotopic mass	Class
34	Threonine	$C_4H_9NO_3$	119.05824	Amino acid
35	Tyrosine	$C_9H_{11}NO_3$	181.0739	Amino acid
36	Valine	$C_5H_{11}NO_2$	117.07898	Amino acid
37	Adenine	$C_5H_5N_5$	135.05449	Nucleic acid
38	Adenosine	$C_{10}H_{13}N_5O_4$	267.09675	Nucleic acid
39	Adenosine 5′-monophosphate	$C_{10}H_{16}N_5O_8P$	347.06308	Nucleic acid
40	Cytidine	$C_9H_{13}N_3O_5$	243.08553	Nucleic acid
41	Cytidine 5′-monophosphate	$C_9H_{14}N_3O_8P$	323.05185	Nucleic acid
42	Guanine	$C_5H_5N_5O$	151.04941	Nucleic acid
43	Guanosine	$C_{10}H_{13}N_5O_5$	283.09167	Nucleic acid
44	Guanosine 5′-monophosphate	$C_{10}H_{14}N_5O_8P$	363.05800	Nucleic acid
45	Hypoxanthine	$C_5H_4N_4O$	136.03851	Nucleic acid
46	Inosine	$C_{10}H_{12}N_4O_5$	268.08077	Nucleic acid
47	Thymidine	$C_{10}H_{14}N_2O_5$	242.09027	Nucleic acid
48	Thymine	$C_5H_6N_2O_2$	126.04293	Nucleic acid
49	Uracil	$C_4H_4N_2O_2$	112.02728	Nucleic acid
50	Uric acid	$C_5H_4N^4O_3$	168.02834	Nucleic acid
51	Uridine	$C_9H_{12}N_2O_6$	244.06954	Nucleic acid
52	Xanthine	$C_5H_4N_4O_2$	152.03343	Nucleic acid
53	Deoxyadenosine	$C_{10}H_{13}N_5O_3$	251.10184	Nucleic acid
54	2′-Deoxyadenosine 5′-monophosphate	$C_{10}H_{14}N_5O_6P$	331.06817	Nucleic acid
55	Inosine	$C_{10}H_{12}N_4O_5$	268.08077	Nucleic acid
56	Inosine 5′-monophosphate	$C_{10}H_{13}N_4O_8P$	348.04665	Nucleic acid
57	Cytosine	$C_4H_5N_3O$	111.04326	Nucleic acid
58	Biotin	$C_{10}H_{16}N_2O_3S$	244.08816	Vitamin
59	Choline chloride	$C_5H_{14}ClNO$	139.07639	Vitamin
60	Cyanocobalamin	$C_{63}H_{88}CoN_{14}O_{14}P$	1354.56740	Vitamin
61	Vitamin D2	$C_{28}H_{44}O$	396.33922	Vitamin
62	Folic acid	$C_{19}H_{19}N_7O_6$	441.13968	Vitamin
63	Folinic acid	$C_{20}H_{21}CaN_7O_7$	511.11240	Vitamin
64	Lipoic acid	$C_8H_{14}O_2S_2$	206.04352	Vitamin
65	Niacinamide	$C_6H_6N_2O$	122.04801	Vitamin
66	Nicotinic acid	$C_6H_5NO_2$	123.03203	Vitamin
67	D-Pantothenic acid	$C_9H_{16}O_5N$	238.08330	Vitamin

Table 1 (part 3). List of compounds included in the 100 metabolite standard mixture

No.	Compound name	Chemical formula	Mono isotopic mass	Class
68	Pyridoxal hydrochloride	$C_8H_{12}ClNO_3$	203.03492	Vitamin
69	Pyridoxine	$C_8H_{11}NO_3$	169.07389	Vitamin
70	Riboflavin	$C_{17}H_{20}N_4O_6$	376.13828	Vitamin
71	Tocopheryl acetate	$C_{31}H_{52}O_3$	472.39165	Vitamin
72	Thiamine hydrochloride	$C_{12}H_{18}C_{l2}N_4OS$	336.05784	Vitamin
73	Ergocalciferol	$C_{28}H_{44}O$	396.33922	Vitamin
74	Gluconic acid	$C_6H_{12}O_7$	196.05830	Carbohydrate
75	Glucosamine hydrochloride	$C_6H_{14}ClNO_5$	215.05605	Carbohydrate
76	Glucose	$C_6H_{12}O_6$	180.06339	Carbohydrate
77	Sucrose	$C_{12}H_{22}O_{11}$	342.11621	Carbohydrate
78	Acetyl-galactosamine	$C_8H_{15}NO_6$	221.08994	Carbohydrate
79	Threonic acid	$C_4H_8O_5$	136.03717	Carbohydrate
80	Ketoglutaric acid	$C_5H_6O_5$	146.02152	TCA Cycle
81	*cis*-Aconitic acid	$C_6H_6O_6$	174.01644	TCA Cycle
82	Citric acid	$C_6H_8O_7$	192.02700	TCA Cycle
83	Fumaric acid	$C_4H_4O_4$	116.01096	TCA Cycle
84	Lactic acid	$C_3H_6O_3$	90.03169	TCA Cycle
85	Dihydroxyacetone	$C_3H_6O_3$	90.03169	TCA Cycle
86	Pyruvic acid	$C_3H_4O_3$	88.01604	TCA Cycle
87	Succinic acid	$C_4H_6O_4$	118.02661	TCA Cycle
88	Ethanolamine	C_2H_7NO	61.05277	Other
89	Glyceric acid	$C_3H_6O_4$	106.02661	Other
90	*O*-Phospho-ethanolamine	$C_2H_8NO_4P$	141.01855	Other
91	Taurine	$C_2H_7NO_3S$	125.01412	Other
92	4-Hydroxyphenyllactic acid	$C_9H_{10}O_4$	182.05791	Other
93	Putrescine	$C_4H_{12}N_2$	88.10004	Other
94	Betaine	$C_5H_{11}NO_2$	117.07898	Other
95	2-Isopropylmalic acid	$C_7H_{12}O_5$	176.06847	Other
96	2-Aminoadipic acid	$C_6H_{11}NO_4$	161.06881	Other
97	Hydroxyphenyllactic acid	$C_9H_{10}O_4$	182.05791	Other
98	Pipecolinic acid	$C_6H_{11}NO_2$	129.07898	Other
99	Kynurenine	$C_{10}H_{12}N_2O_3$	208.08479	Other
100	Pyridoxal hydrochloride	$C_8H_{10}ClNO_3$	203.03492	Other

Preparation of dilution series of MEM samples

- Dilute each MEM sample 5-fold in 0.1% FA H_2O:ACN (50:50).
- Centrifuge at 10,000 rpm for 10 min.
- Transfer the supernatant to a new tube.
- Dilute the supernatant with 0.1% FA H_2O to make the final MEM dilution series samples in 10-fold, 50-fold, and 100-fold dilution.

HPLC

Chromatographic separations were carried out using a pentafluorophenylpropyl (PFPP) stationary phase column (150 × 2.1 mm, 3 µm) on a Thermo Scientific™ Vanquish™ Horizon UHPLC system consisting of the following modules:

- Thermo Scientific™ System Base Vanquish™ Horizon/Flex (P/N VF-S01-A-02)
- Thermo Scientific™ Vanquish™ Binary Pump H (P/N VH-P10-A)
- Thermo Scientific™ Vanquish™ Split Sampler FT (P/N VH-A10-A)
- Thermo Scientific™ Vanquish™ Column Compartment (P/N VH-C10-A)

Solvent A was water with 0.1% formic acid and solvent B was acetonitrile with 0.1% formic acid. The gradient and flow rate used are shown in Table 2. The column temperature was 40 ^{0}C. The injection volume was 2 µL.

Table 2. HPLC gradient conditions

Time	Flow (mL/min)	%A	%B
0	0.3	100	0
1.4	0.3	100	0
3.5	0.3	75	25
7.5	0.3	65	35
10.3	0.3	5	95
14	0.3	5	95
14.1	0.4	100	0
19.5	0.4	100	0
20	0.3	100	0

Mass spectrometry

The Orbitrap Exploris 120 mass spectrometer was used for data collection. The MS method consisted of Full MS only scans applying polarity switching, resulting in alternating scans in positive and negative ionization mode with all scans acquired at a resolution setting of 60,000 (@*m/z* 200). The Easy-IC was enabled. The mass range was set to *m/z* 600–700 for positive ionization mode and *m/z* 70–480 for negative ionization mode (Table 3).

Table 3. Mass spectrometer parameters

ESI source	Orbitrap Exploris 120 MS
Sheath gas: 45 Aux gas: 8 Sweep gas: 1	RF lens: 70
Spray voltage positive ion (V): 3,400	Easy IC: On
Spray voltage negative ion (V): 3,000	Resolution: 60K (FWHM at *m/z* 200)
Ion transfer tube temp. (°C): 300	AGC target: Standard
Vaporizer temp. (°C): 300	Positive ion scan range (*m/z*): 60–700
Mild trapping: true	Negative ion scan range (*m/z*): 70–480

HPLC-MS instrument control and data processing

Chromeleon CDS was used for LC-MS instrument method setup, sequence generation, data acquisition, and data processing including visualization and reporting.

Results and discussion

High efficiency separation method for metabolites

Although the HRAM approach is highly specific for detecting individual metabolites through extracting the accurate mass of each metabolite using a narrow mass window (3 ppm), good chromatographic separation is still essential to reduce interference for accurate quantification. The prepared 100 metabolite standard mixture was used for the evaluation of metabolite separation efficiency of the adopted PFPP phase column. The PFPP column was used in reversed-phase mode and provided good peak shape and separation of all targeted metabolites. The baseline resolved separation of isomeric compounds, such as leucine and isoleucine, was observed (Figure 1).

Targeted 100 metabolite quantification assay development and quantitative performance evaluation

The 100-metabolite standard mixture was analyzed in triplicate using full scan MS operated with positive/negative polarity switching mode.

Although a high resolution setting of 60,000 (FWHM) at *m/z* 200 was used for both positive and negative MS scan events, the fast scan speed and fast positive and negative scan switching offered by the Orbitrap Exploris 120 mass spectrometer generated sufficient data points across each targeted metabolite peak for reproducible peak area calculation. The extracted ion chromatogram of histidine collected in the positive ion scan using a 3 ppm mass tolerance window (Figure 2a) displays 12 scans across the eluted peak. The extracted ion chromatogram of uridine collected in the negative ion mode using 3 ppm mass window (Figure 2b) displays 20 scan points across the eluted peak.

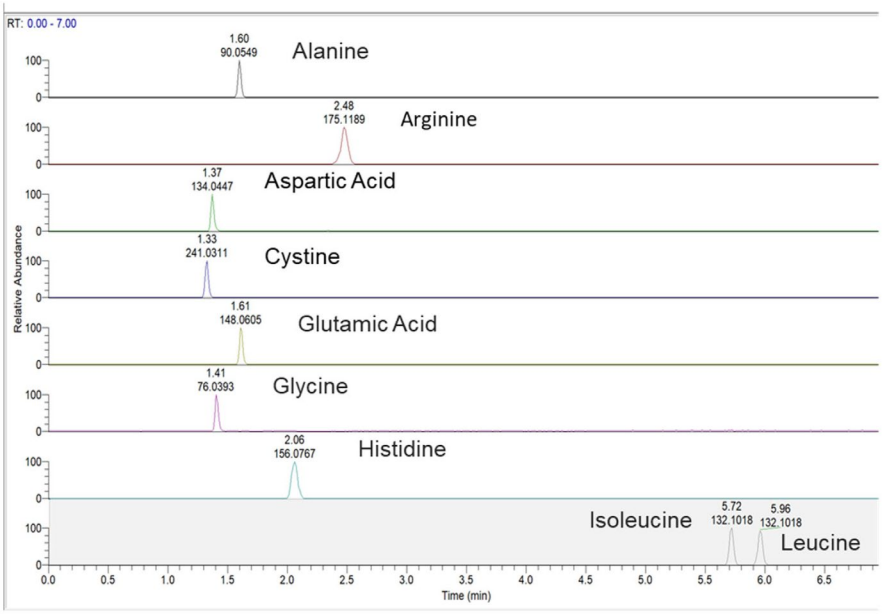


Figure 1. Elution profiles of representative targeted amino acid standards

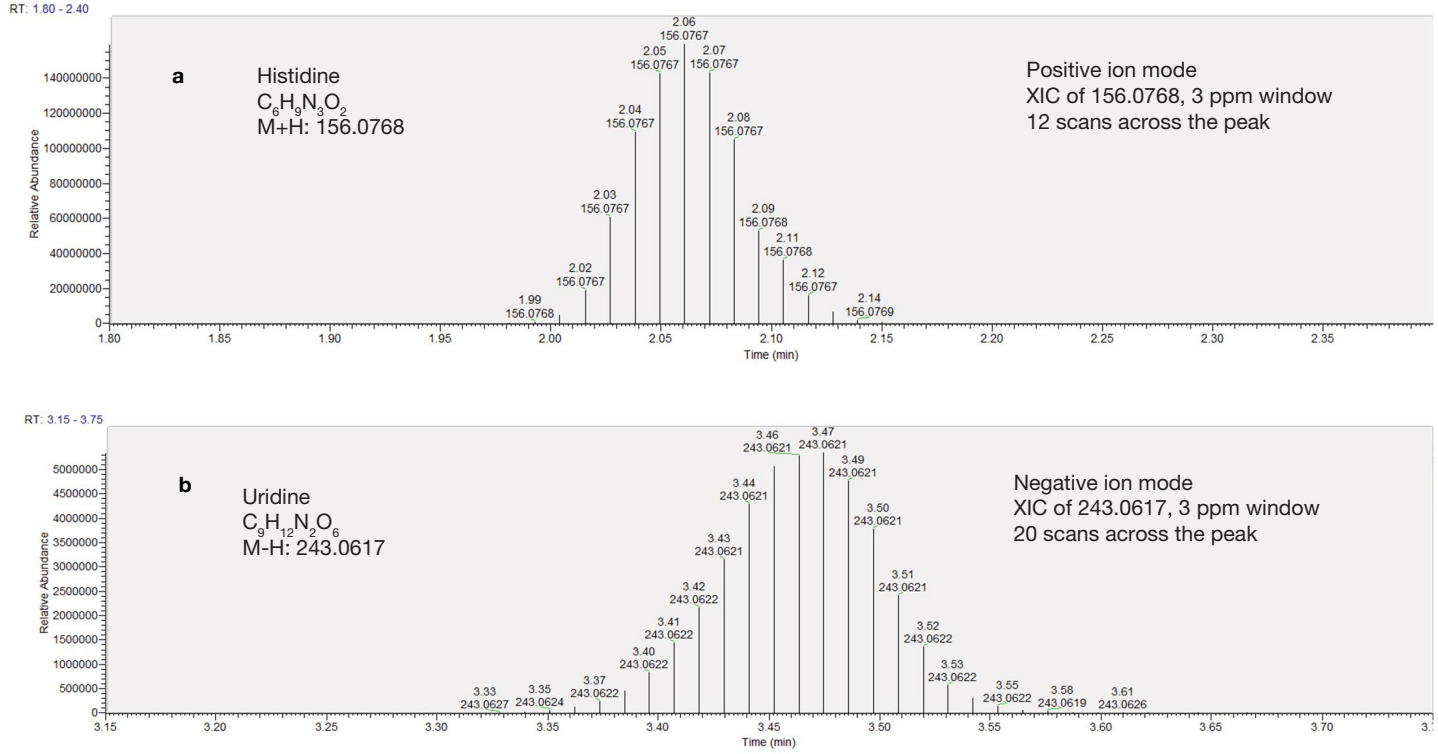


Figure 2. Extracted ion chromatograms of histidine (a) and uridine (b). Sufficient scans across each metabolite peak allow reproducible quantification results.

The prepared amino acid mixture dilution series was used for evaluating the quantitative performances of the developed 100 targeted metabolite assay. Each sample was analyzed in triplicate. Great sensitivity, reproducibility, and wide dynamic range were observed for the spiked-in amino acid compounds. Figure 3 shows the quantification results of the leucine. Even at the lowest concentration level of 0.025 nmol/mL, the spiked-in leucine was detected clearly with high signal to noise ratio (S/N 34) (Figure 3a). The R^2 of the calibration curve over four orders of concentration ranges was 0.9986 (Figure 3b). The coefficient of variations (%CV) were less than 5% across all concentration levels (Table 4).

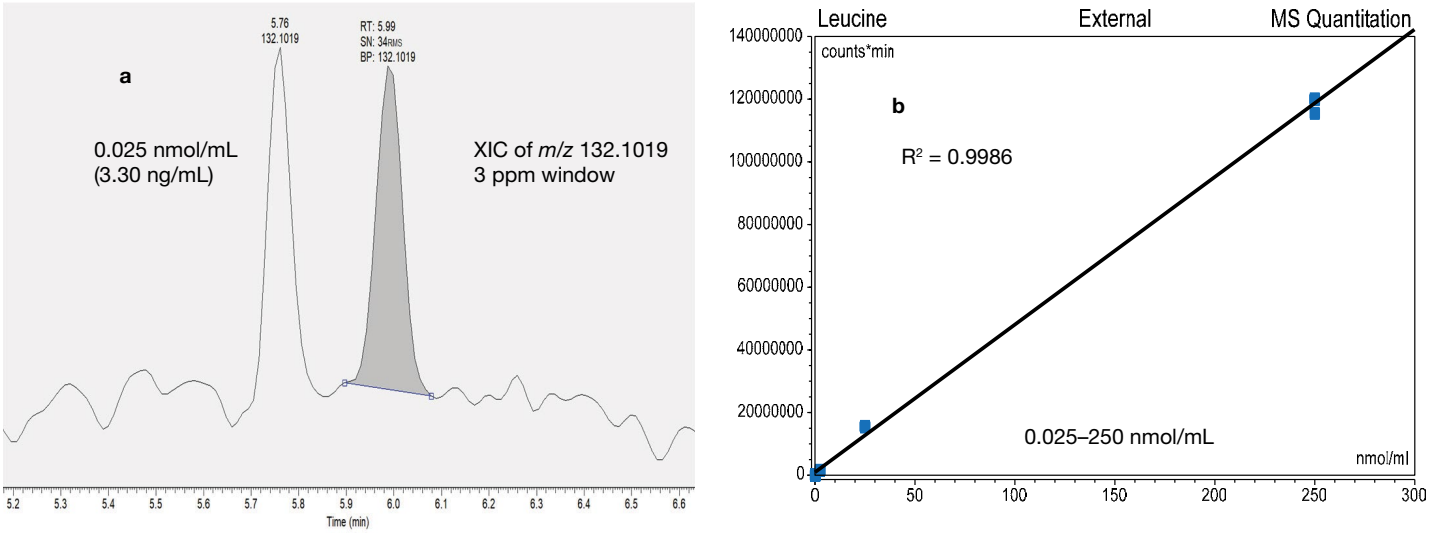


Figure 3. Quantitative results of leucine spiked in the metabolite matrix. (a) Detected leucine peak at the lowest 0.025 nmol/mL concentration level with the signal to noise ratio of 34. (b) Observed calibration curve of leucine over 4 orders of dynamic ranges.

Table 4. The coefficient of variations observed from the spiked-in leucine

Concentration	0.025 (nmol/mL)	0.25 (nmol/mL)	2.5 (nmol/mL)	25 (nmol/mL)	250 (nmol/mL)
Peak area of run1	13,012	142,808	1,513,088	15,569,619	119,870,080
Peak area of run 2	12,253	144,677	1,574,285	15,373,291	120,126,499
Peak area of run 3	12,978	155,830	1,575,751	15,609,516	115,467,328
Average peak area	12,747	147,772	1,554,375	15,517,475	118,487,969
CV %	3.4	4.8	2.3	0.8	2.2

Applying the developed 100 metabolites quantification assay to commercially available cell culture media

The developed 100 targeted metabolite quantification assay was applied to the quantitative profiling of three commercially available cell culture medium samples from Thermo Fisher Scientific as a case study. The metabolite components included in each medium sample are summarized in Table 5. The vitamin components have the lowest concentration ranges.

Table 5A. The list of metabolite components and their concentrations in the three media samples (amino acids)

Metabolite	Raw medium sample		
	MEM alpha, GlutaMAX(TM), nucleosides: Concentration (mg/L)	Advanced D-MEM/F-12: Concentration (mg/L)	Advanced DMEM: Concentration (mg/L)
Amino acids			
Glycine	50	18.75	37.5
L-Alanine	25	4.45	8.9
L-Arginine	105	147.5	84
L-Asparagine	50	7.5	13.2
L-Aspartic acid	30	6.65	13.3
L-Cysteine	100	17.56	–
L-Cystine	31	31.29	63
L-Glutamic acid	75	7.35	14.7
L-Glutamin	292	–	–
L-Histidine	31	31.48	42
L-Isoleucine	52.4	54.47	105
L-Leucine	52	59.05	105
L-Lysine	73	91.25	146
L-Methionine	15	17.24	30
L-Phenylalanine	32	35.48	66
L-Proline	40	17.25	11.5
L-Serine	25	26.25	52.5
L-Threonine	48	53.45	95
L-Tryptophan	10	9.02	16
L-Tyrosine	52	55.79	104
L-Valine	46	52.85	94

Table 5B. The list of metabolite components and their concentrations in the three media samples (vitamins, ribonucleosides, deoxtribonucleosides, and other components)

Metabolite	Raw medium sample		
	MEM alpha, GlutaMAX(TM), nucleosides: Concentration (mg/L)	Advanced D-MEM/F-12: Concentration (mg/L)	Advanced DMEM: Concentration (mg/L)
Vitamins			
Ascorbic acid*	50	2.5	2.5
Biotin	0.1	0.0035	–
Choline chloride	1	8.98	4
D-Calcium pantothenate	1	2.24	4
Folic acid	1	2.65	4
Niacinamide	1	2.02	4
Pyridoxal hydrochloride	1	2	4
Riboflavin	0.1	0.219	0.4
Thiamine hydrochloride	1	2.17	4
Vitamin B12	1.36	0.68	–
i-Inositol*	2	12.6	7.2
Ribonucleosides			
Adenosine	10	–	–
Cytidine	10	–	–
Guanosine	10	–	–
Uridine	10	–	–
Deoxtribonucleosides			
2'Deoxyadenosine	10	–	–
2'Deoxycytidine HCl*	11	–	–
2'Deoxyguanosine*	10	–	–
Thymidine	10	–	–
Other components			
D-Glucose	1000	3151	4500
Ethanolamine	–	1.9	1.9
Glutathione (reduced)*	–	–	1
Hypoxanthine*	–	2.39	–
Linoleic acid*	–	0.042	–
Lipoic acid	0.2	0.105	–
Phenol Red*	10	8.1	15
Putrescine	–	0.081	–
Sodium pyruvate	110	110	110
Thymidine	–	0.365	–

* Component not included in the 96 targeted metabolite quantitative assay.

In total, there are 50 metabolite components included in the three medium samples. In order to demonstrate the sensitivity and reproducibility of the quantitative assay, each medium sample was diluted 10 times, 50 times, and 100 times, respectively, as described in the sample preparation session. Each sample from the dilution MEM series was analyzed in triplicate.

Chromeleon CDS was used for data processing. Among the 50 metabolites, 43 of them were included in the 100-metabolite quantitative assay and were detected and quantified with excellent analytical precision. To demonstrate excellent reproducibility of quantification, peak areas of choline chloride from the diluted series of Gibco MEM α, nucleosides, GlutaMAX Supplement are shown in Figure 4. Great peak area reproducibility was observed even at 100 times diluted level. The Orbitrap Exploris 120 mass spectrometer was able to detect the lowest abundant vitamin analytes, biotin and riboflavin, at 1 ng/mL concentration level (Figure 5).

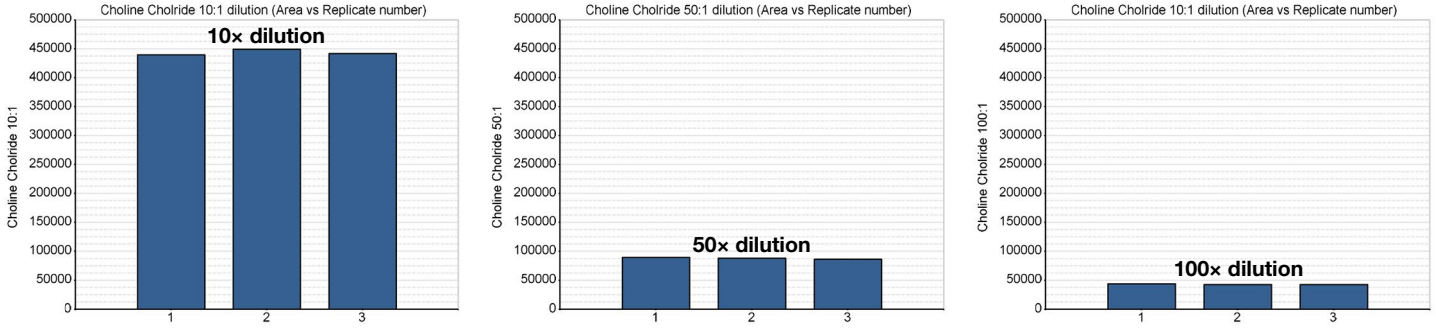


Figure 4. The plot of observed peak areas for choline chloride in the medium sample of MEM α, nucleosides, GlutaMAX Supplement. The x-axis shows the number of replicate runs. The y-axis shows the observed peak area.

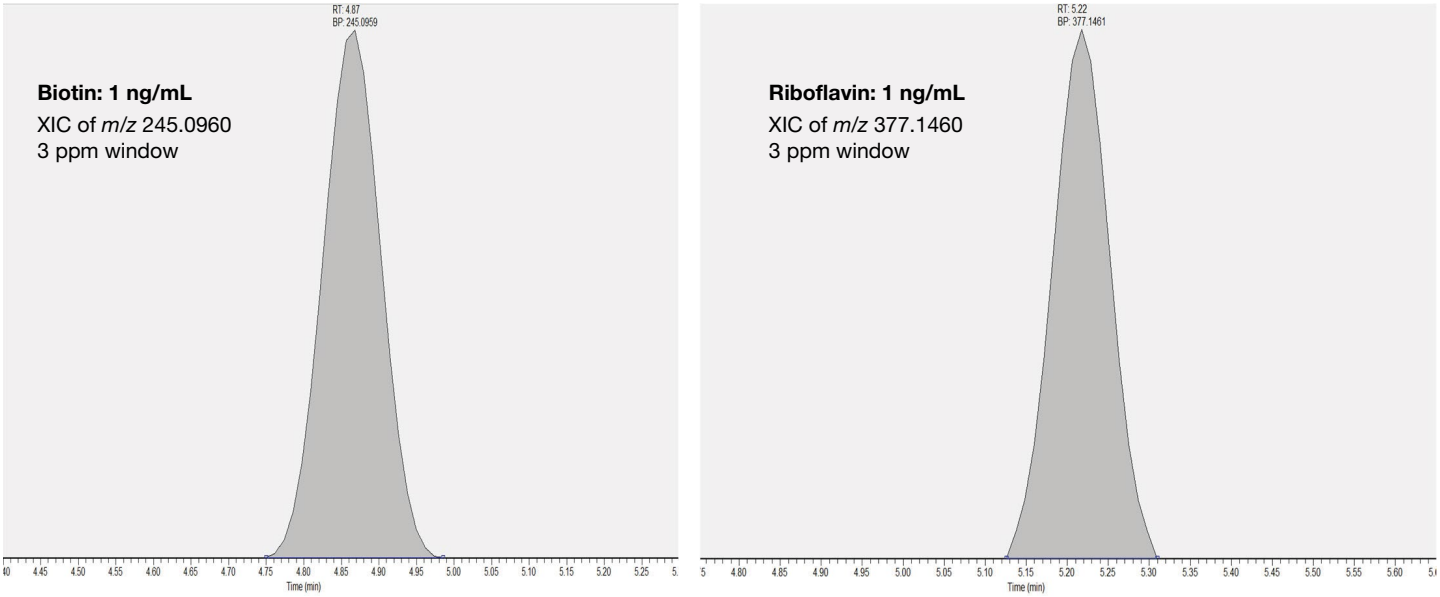


Figure 5. The extracted ion chromatograms of biotin and riboflavin from 100 times diluted medium sample of MEM α, nucleosides, GlutaMAX Supplement

Chromeleon CDS enables instant data processing and accurate peak detection, with flexible spreadsheet-based reporting and data visualization tools. The quantitative changes of targeted metabolites can be visualized across different samples, over different concentration levels, and over different time ranges easily using either analyte peak areas directly or analyte/internal standard peak area ratios. Figure 6 shows the quantitative value of representative compounds across three medium samples over different concentration ranges.

Quantification of untargeted metabolites by retrospective HRAM MS data analysis

Beyond targeted quantification of pre-selected metabolites, it is valuable to quantify and confirm additional analytes not included in the pre-selected metabolite list. The additional quantitative information of untargeted metabolites can provide additional insights for process development, troubleshooting a faulty bioprocess, and raw material qualification. This HRAM LC-MS approach simultaneously collects full positive MS and negative MS data and allows for quantification of every detectable metabolite in the sample independent of pre-selection.

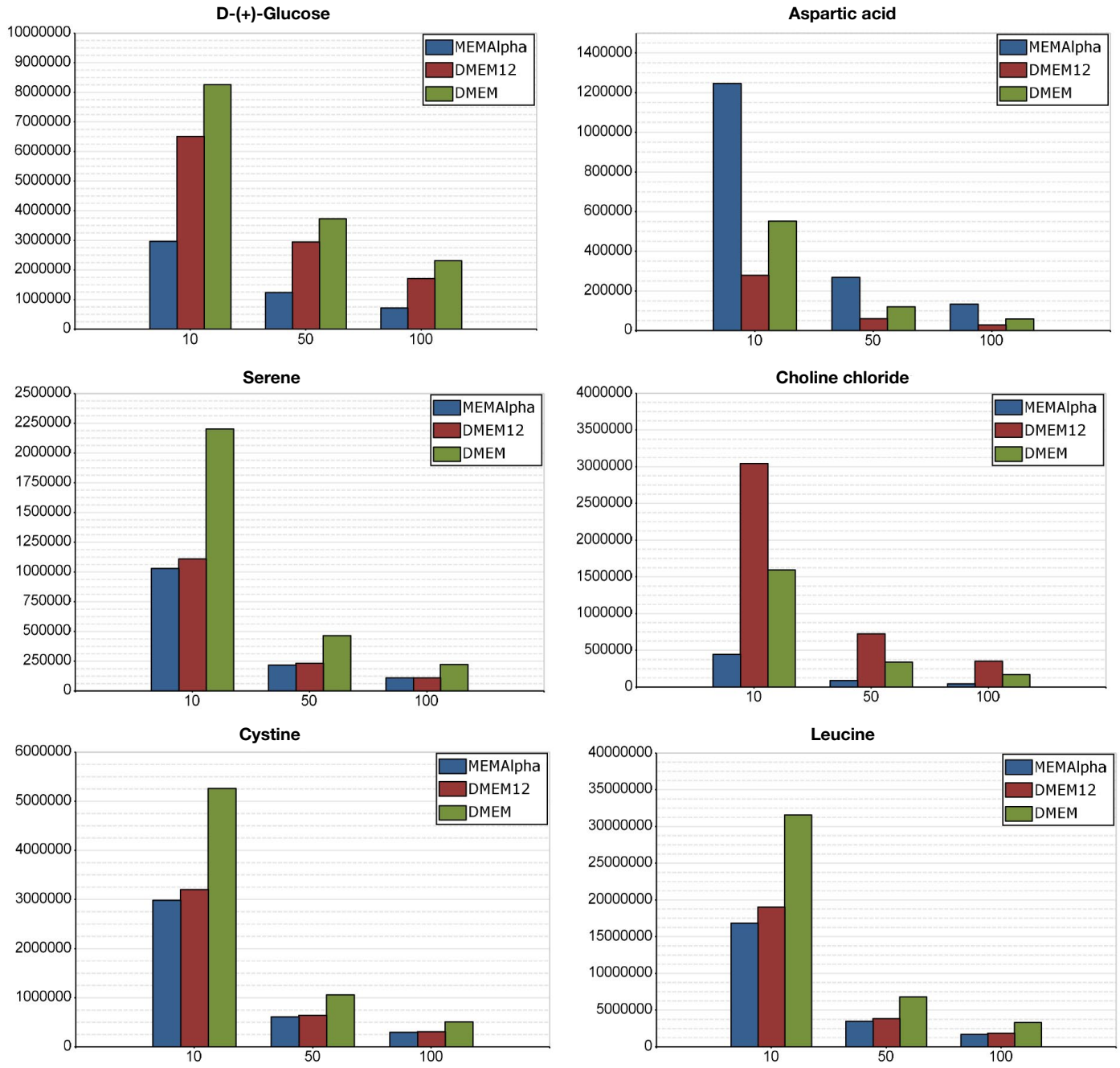
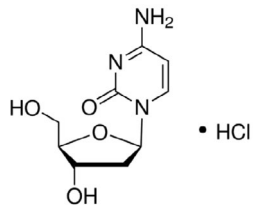


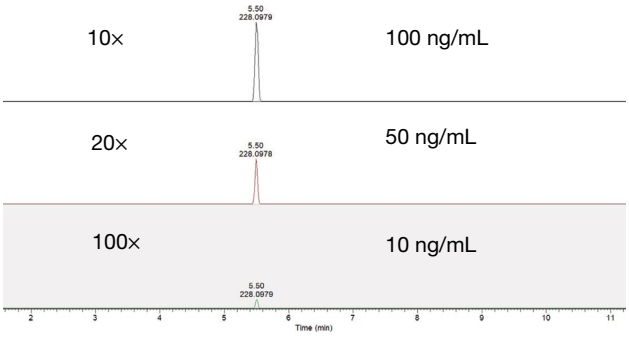
Figure 6. Representative metabolite profiling of three medium samples. In each case, analyte peak area is presented vs. different dilution factors.

For example, 2′-deoxycytidine hydrochloride is present in the MEM α, nucleosides GlutaMAX sample. It was not included in the 100 targeted metabolite list and not processed by Chromeleon CDS. However, 2′-deoxycytidine hydrochloride could easily be quantified by retrospective data analysis (Figure 7).

Sometimes, accurate mass information obtained from full scan mass analysis alone is not specific enough to identify an untargeted metabolite. MS^2 data is further needed for confident identification. By running an additional MS^2 analysis for the MEM α, nucleosides GlutaMAX sample on the Orbitrap Exploris 120 mass spectrometer, 2′-deoxycytidine hydrochloride was identified confidently (Figure 8) with the additional fragment ion information.

2'-Deoxycytidine hydrochloride
$C_9H_{13}N_3O_4 \cdot HCl$; MW: 263.68





	10 times diluted	20 times diluted	100 times diluted
Area of run 1	38514320	18003011	3297314
Area of run 2	38291377	18382446	3484585
Area of run 3	38087252	17967885	3468555
Ave	38297650	18117781	3416818
RSD %	0.5	1.0	2.5

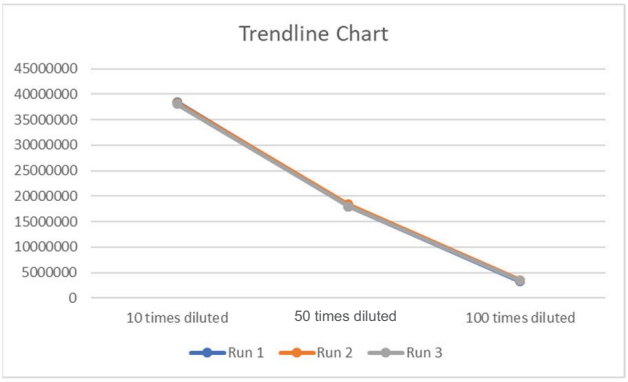


Figure 7. Quantitative results of 2′-deoxycytidine hydrochloride with retrospective data analysis

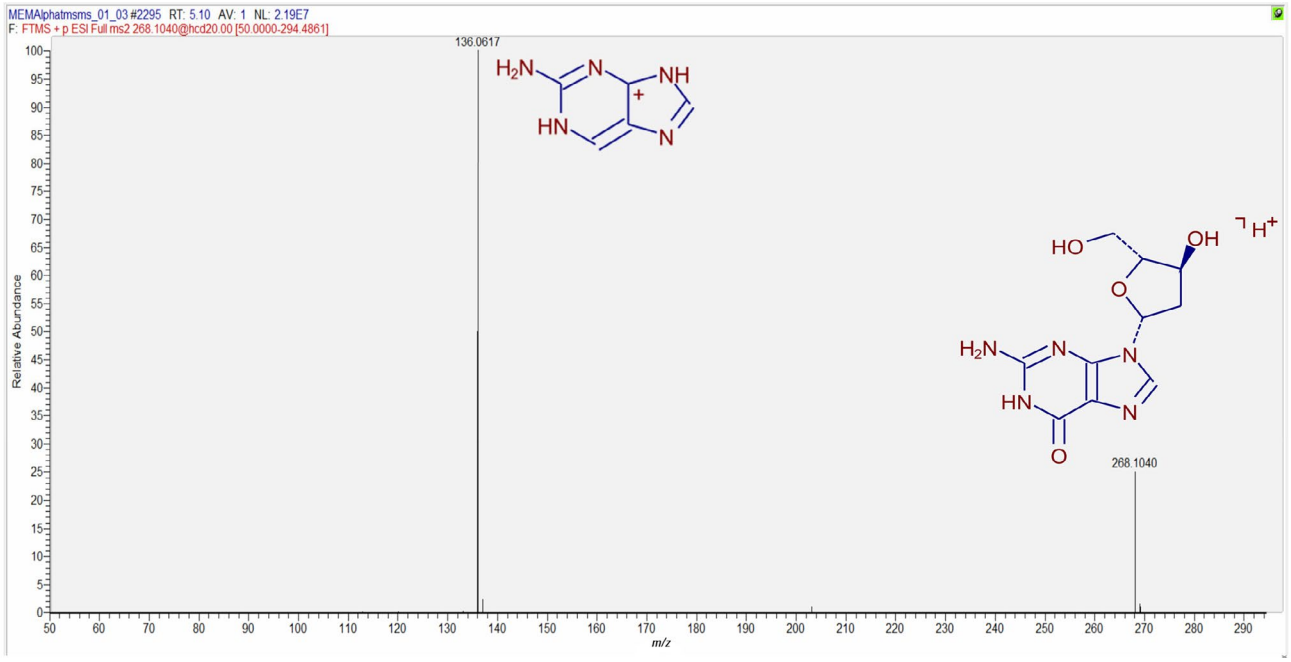


Figure 8. Identity confirmation of 2′-deoxycytidine hydrochloride with MS^2 data

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Conclusions

In summary, an Orbitrap HRAM full scan MS method has been developed on the Orbitrap Exploris 120 mass spectrometer for high throughput cell culture media metabolite profiling.

- The method uses online switching full scan positive MS and negative MS approach and is very easy to set up.
- The fast scan speed and high resolving power of the Orbitrap Exploris 120 mass spectrometer allows for sufficient data points and excellent mass accuracy, resulting in precise and accurate quantification.
- Excellent sensitivity and greater than four orders of linear dynamic range were observed with the spiked-in amino acid standard mixture.
- The developed 100 targeted metabolite assay was successfully applied to quantitatively analyze raw media samples. The low abundant vitamin components were detected even at 1 ng/mL concentration level.
- Quantitative analysis of untargeted compounds beyond the initial targeted metabolites was possible by retrospective HRAM MS data analysis.
- Chromeleon CDS 7.2.10 managed and streamlined data acquisition, data processing, and reporting.

Reference

1. Sun, Z. et al. High-throughput LC-MS quantitation of cell culture metabolites. *Biologicals,* **2019,** *61,* 44–51.

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