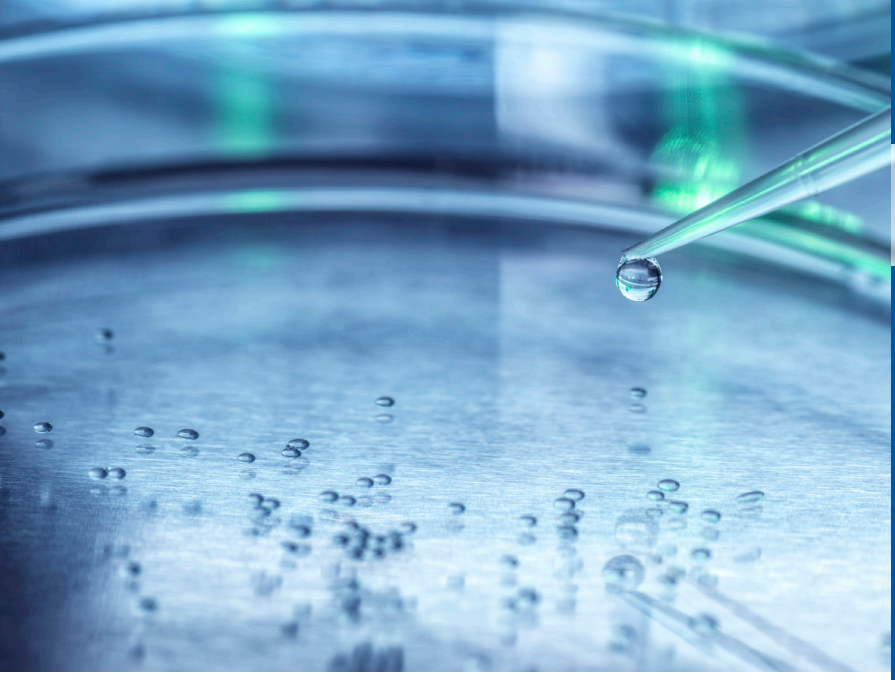

APPLICATION NOTE

Liquid Chromatography/
Mass Spectrometry

AUTHORS

Cole Strattman
Marc R. Elie
Jacob Jalali
PerkinElmer, Inc.
Shelton, CT

Fast and Targeted Analysis of Cell Culture Media Components using QSight LC/MS/MS

Introduction

Quantitative analysis of cell culture media components is a critical step in upstream bioprocess development. A comprehensive and quantitative understanding of cellular metabolic components could lead to a greater understanding of the process impacts on cell growth, productivity, and product quality during biopharmaceutical development and manufacturing. Therefore, there is a vital need to establish an efficient analytical method to monitor the concentration profiles of important cell culture media nutrients and metabolites over the course of the production process or over different lots. However, it remains challenging to simultaneously monitor multiple classes of metabolites (amino acids, vitamins, carbohydrates, fatty acids, nucleotides nucleic acids, organic acids) in cell culture media because of their diversified physical, chemical properties and large range of concentrations[1,2].

This application note describes an efficient and reproducible method developed using the PerkinElmer QSight 420® LC/MS/MS for the targeted quantification of key metabolites in a single run with high sensitivity, selectivity and is well suited for cell culture metabolite profiling experiments. The method conditions and performance data, including repeatability and linearity, are provided.

PerkinElmer®
For the Better

Experimental

Hardware/Software

Chromatographic separation was conducted utilizing a PerkinElmer QSight LX50 ultra-high-performance liquid chromatography (UHPLC) system, and detection was achieved using a PerkinElmer QSight 420 triple quadrupole mass spectrometer with a dual ionization source (ESI and APCI). All instrument control, data acquisition and data processing were performed using Simplicity™ 3Q software.

LC Conditions and MS Parameters

Commercially available compounds, that are commonly detected in culture media, were used as standards to optimize MRM transitions and LC conditions for separation. The LC method and MS source parameters are shown, respectively, in Tables 1 and 2. A 150 mm x 4.6 mm ID, particle size 2.6 µm superficially porous particle (SPP) pentafluorophenyl propyl (PFPP) phase column (USP L43) was used in this study.

The applied LC gradient program is shown in Table 1.

MS source parameters, including gas flows, temperatures and probe-position settings, were each optimized to achieve maximum sensitivity (Table 2). Compound-dependent parameters, such as collision energies (**CE**), entrance voltages (**EV**), and lens voltages (**CCL2**), were optimized for the target compounds, as shown in Table 3. Two MRM transitions were monitored for each analyte, with a few exceptions when only one MRM was available.

Finally, the MS acquisition method was generated using QSight Triple Quad LC/MS/MS Simplicity 3Q™ Software in the time-managed-MRM module with the retention times and corresponding retention time windows for all compounds analyzed.

Table 1: LC Parameters.

LC Column	SPP PFPP (USP 43), 150 mm x 4.6 mm, 2.6 µm		
Mobile Phase A	0.1% Formic acid in water		
Mobile Phase B	0.1% Formic acid in Acetonitrile		
Mobile Phase Gradient	Time (min)	%A	%B
	0.00	100	0
	1.40	100	0
	3.50	75	25
	7.50	65	35
	10.30	5	95
	13.70	5	95
	13.80	100	0
	17.00	100	0
Column Oven Temperature	40 °C		
Auto Sampler Temperature	20 °C		
Injection Volume	10 µL		
Flow	0.35 mL/min		
Run Time	17 min		

Table 2. MS/MS Source parameters.

Parameter	Setting
Ionization Mode	Electrospray ionization (ESI); positive and negative, depending on analyte
Drying Gas	100
HSID Temperature (°C)	300
Nebulizer Gas	250
Electrospray Voltage (V)	5000 (positive mode) -4500 (negative mode)
Source Temperature (°C)	250

Table 3. Optimized MRM Parameters for the Targeted Cell media Components.

Polarity	Name	Q1 Mass	Q2 Mass	Retention Time (min)	CE	EV	CCL2
Positive	L-Ornithine (HCl)	133	116	3.64	-11	8	-36
		133	70		-22	3	-40
Positive	Histidine	156	110	3.73	-22	5	-192
		156	83		-34	15	-56
Positive	L-Carnosine	227.8	157	3.76	-20	8	-64
		227.8	110		-33	9	-64
Positive	Leucine	132	86	4.06	-13	2	-92
		132	55		-44	11	-68
Positive	L-Isoleucine	132	90	4.12	-15	29	-32
		132	86		-11	5	-36
Positive	L-Lysine	147	130	4.04	-12	15	-36
		147	84		-19	14	-40
Positive	Cystathionine (CYS)	223	134	3.85	-18	5	-52
		223	88		-44	5	-72
Positive	L-Cystine	241	152	3.88	-50	30	-140
Positive	Glycine	76	30	3.89	-13	6	-24
Positive	Beta-Alanine	90	72	3.89	-11	11	-28
		90	45		-54	14	-80
Positive	L-Serine	106	60	3.94	-14	14	-36
		106	42		-30	10	-32
Positive	L-Asparagine	133.4	87	3.95	-13	6	-32
		133.4	74		-21	5	-32
Negative	O-phosphorylethanolamine	140	78	3.94	17	-36	40
Positive	L-Alanine	90	44	4	-13	5	-28
Positive	Taurine	126	108	3.99	-14	30	-32
		126	65		-54	31	-80
Positive	Glutamine	147	84	4.03	-14	7	-48
		147	56		-32	10	-44
Positive	Hypotaurine	110	92	4.04	-10	3	-32
		110	65		-45	15	-84
Positive	L-Threonine	120	74	4.04	-13	16	-28
		120	56		-23	11	-32
Positive	Phosphoserine	186	88	4.03	-15	13	-48
		186	70		-30	17	-44
Negative	myo-inositol	179	161	3.96	13	-36	56
		179	86		23	-36	56
Positive	Hydroxyproline (trans-4-hydroxy-L-proline)	132	86	4.1	-18	15	-40
		132	68		-27	11	-44
Positive	Choline chloride	104	60	4.18	-23	21	-32
		104	58		-24	14	-60

Table 3. Optimized MRM Parameters for the Targeted Cell media Components (continued).

Polarity	Name	Q1 Mass	Q2 Mass	Retention Time (min)	CE	EV	CCL2
Positive	Gamma-aminobutyric acid	104	87	4.11	-14	8	-28
		104	69		-22	8	-32
Negative	Cysteic acid	168	80	4.09	25	-8	44
		168	70		24	-28	48
Positive	Phosphocholine	184	125	4.18	-22	14	-88
		184	99		-39	37	-92
Negative	D-Glucose	179	88	4.12	10	-28	44
		179	58		29	-26	44
Negative	Glutamate	146	127	4.13	14	-3	40
		146	101		18	-3	44
Negative	Alpha-ketoglutarate (AKG)	145	101	4.13	15	-30	60
		145	73		25	-25	56
Positive	Citrulline	176	113	4.18	-22	14	-52
		176	70		-34	7	-48
Negative	D-Ribose-5-phosphate (R5P)	229	96	4.16	16	-2	64
		229	78		53	-4	64
Negative	D-Mannose	179	118	4.11	11	-22	68
		179	58		24	-26	52
Positive	Proline	116	70	4.54	-22	6	-28
Positive	L-2-Aminobutyric acid	104	58	4.32	-13	11	-28
		104	43		-43	14	-40
Positive	L-Carnitine	162	103	4.46	-20	26	-48
		162	60		-21	14	-44
Positive	Thiamine	265	122	4.45	-21	10	-60
		265	81		-53	14	-76
Positive	Creatinine	114	86	4.4	-14	31	-52
		114	44		-23	26	-48
Negative	Allantoin	157	96	4.42	19	-10	56
		157	58		35	-3	52
Positive	Betaine	118	59	4.52	-23	35	-36
Negative	L-Malic acid	132	114	5.26	13	-1	40
		132	70		21	-1	44
Negative	UDP-Alpha-Glucose	565	323	4.76	31	-9	200
		565	241		28	-7	216
Positive	Cytidine-5-monophosphate (CMP)	324	112	4.8	-18	8	-72
Negative	Pyruvic acid	86	42	5.18	11	-1	28
Negative	N-Acetyl - L-lysine	187	145	5.79	19	-34	64
		187	57		26	-35	52
Negative	Orotic acid	155	110	5.84	16	-2	52
		155	67		39	-3	68

Table 3. Optimized MRM Parameters for the Targeted Cell media Components (continued).

Polarity	Name	Q1 Mass	Q2 Mass	Retention Time (min)	CE	EV	CCL2
Positive	Adenosine 5'-monophosphate (AMP)	348	136	6.33	-25	10	-88
		348	97		-46	2	-124
Positive	2-Picolinic acid	124	106	6.37	-13	15	-28
		124	78		-24	4	-28
Positive	N-alpha-Acetyl-L-glutamine	189	130	6.32	-16	14	-56
		189	84		-37	7	-60
Positive	Pyridoxal	168	150	6.53	-14	4	-64
		168	67		-40	7	-76
Positive	Nicotinamide	123	80	7.26	-25	2	-48
		123	53		-44	5	-52
Positive	Riboflavin B2	377	243	8.21	-30	38	-104
		377	172		-53	38	-136
Negative	Citric acid	191	110	7.48	17	-10	56
		191	86		25	-10	56
Positive	Adenosine	268	136	7.54	-21	9	-60
Positive	Inosine	269	137	7.54	-23	2	-64
Positive	L-Tyrosine	182	165	7.54	-12	8	-44
		182	136		-17	14	-48
Negative	Glutathione oxidized	611	306	7.52	32	-2	228
		611	272		36	-1	224
Negative	N-acetyl-L-glutamic acid	188	127	7.54	16	-3	68
		188	101		22	-3	56
Negative	Fumaric acid	114	70	7.54	12	-1	40
Negative	β-hydroxybutyric acid	102.3	58	7.55	13	-2	40
		102.3	40		32	-2	28
Negative	Xanthine	150.6	107	7.55	45	-28	92
		150.6	65		87	-20	104
Negative	Thymidine-5'-monophosphate (TMP)	321	78	7.54	50	-28	88
		321	195		23	-33	100
Positive	Nicotinamide adenine dinucleotide (NAD)	664	542	7.53	-20	33	-156
		664	136		-62	21	-184
Positive	Hypoxanthine	137	110	7.53	-26	36	-60
		137	55		-43	35	-68
Positive	Acetyl L-Carnitine	204.5	145	7.53	-15	12	-52
		204.5	85		-26	23	-52
Positive	L-Phenylalanine	166	120	7.71	-17	2	-276
		166	103		-39	14	-60
Positive	5'-Methylthioadenosine (MTA)	298	136	7.86	-24	30	-72
Positive	Folic acid	442	295	7.93	-29	18	-112
		442	176		-56	17	-116

Table 3. Optimized MRM Parameters for the Targeted Cell media Components (continued).

Polarity	Name	Q1 Mass	Q2 Mass	Retention Time (min)	CE	EV	CCL2
Positive	L-Tryptophan	205	188	8.09	-15	30	-156
		205	146		-23	5	-144
Positive	Biotin	245	227	8.48	-18	5	-68
		245	97		-45	15	-84
Positive	4-Aminobenzoic acid (para-aminobenzoic acid)	138	94	8.57	-20	10	-68
		138	65		-37	13	-68
Positive	Indolelactic acid	206	188	10.67	-15	14	-64
		206	160		-16	13	-64

Materials and Methods

Solvents, Standards and Samples

Neat standards of cell culture media components, used in this study, were purchased from Sigma-Aldrich (St Louis, MO), Cayman Chemical (Ann Arbor, MI) and TCI America (Portland, OR).

Acetonitrile (LC-MS grade) and formic acid (LC-MS grade) were used in the preparation of mobile phase solutions and were purchased from Fischer scientific (Hanover Park, IL)

Water, used for both mobile phase solution and diluent, was of ultra-high purity (18.2 MΩ).

Stock Solutions and Calibration Standards

A stock standard, for each of the 71 metabolites (listed in Table 3), was prepared using water (containing 0.1% formic acid). Individual stock concentrations varied between 1 and 4 mg/L due to the signal-concentration dependence of the analyte. The linearity range, in µg/L, for each selected metabolite is presented in Table 5.

For calibration purposes, at least 10 levels were prepared via serial dilution of the stock standard, using water (containing 0.1% formic acid) as the diluent.

A 100-µg/L mixture standard was prepared using neat standards of various cell media components listed in Table 3. Water (containing 0.1% formic acid) was used as diluent to achieve desired and final concentration of the mixture.

Sample Preparation

As far as sample preparation protocol, a cell culture medium extract (Gibco, Fischer's Medium, lot #2458244) was diluted to 10-100 folds with water (containing 0.1% formic). Then, a 1-mL aliquot was added to an autosampler vial for analysis. The ideal dilution factor was determined based on the concentration of the compounds in the sample.

Results and Discussion

The increased interest in biologic drugs has resulted in significant growth in the cell culture media market, as scientists demand optimal media to ensure the viability of their work. The ability of cells to optimally grow, divide and produce recombinant protein depends on the proper control of cellular nutrients and metabolites[3]. Therefore, ensuring that the cell culture media is comprised of the optimal formulation for growth, and free of impurities, is vital to the success of biopharmaceutical development. Cell culture media contain a wide range of different metabolite classes, including amino acids, vitamins, carbohydrates, fatty acids, nucleotides, nucleic acids, and organic acids. The ability to monitor these various groups of components quickly and accurately is needed.

The LC/MS/MS methodology, described in this application note, can simultaneously measure 71 cell culture metabolites in a single 17-minute run, which makes real time in-process monitoring feasible during production. These 71 metabolites, representing various chemical classes **(Table 4)**, were detected with multiple reaction monitoring (MRM) matching using standard chemical compounds, after separation on a pentafluorophenyl propyl (PFPP) column.

Figure 1 illustrates the extracted ion chromatograms of representative components from a mixed standard solution (100 µg/L in concentration). A good separation can be seen among the various detected metabolites.

Table 4. Cell Culture Media Component Coverage (various compound classes) in Developed Method.

Amino Acids	Nucleobases and derivatives	Carbohydrates	Vitamins	Peptides	Others (organic acids, amino acid derivatives, etc.)
Beta-Alanine	5'-Methylthioadenosine (MTA)	D-Ribose-5-phosphate (R5P)	Biotin	L-Carnosine	2-Picolinic acid
Betaine	Adenosine	D-Glucose	Folic acid	L-Cystathionine	4-Aminobenzoic acid (para-aminobenzoic acid)
Citrulline	Adenosine 5'-monophosphate (AMP)	D-Mannose	Nicotinamide	L-Cystine	Acetyl L-Carnitine
Cysteic acid	Cytidine-5-monophosphate (CMP)	myo-inositol	Pyridoxal	Glutathione oxidized	Allantoin
Glutamate	Hypoxanthine		Riboflavin B2		Alpha-ketoglutarate (AKG)
Glutamine	Inosine		Thiamine		citric acid
Glycine	UDP-Alpha-Glucose				Creatinine
Histidine	Thymidine-5'-monophosphate (TMP)				Indolelactic acid
L-2-Aminobutyric acid	Nicotinamide adenine dinucleotide (NAD)				Fumaric acid
L-Alanine	Xanthine				β-hydroxybutyric acid
L-Asparagine					Hypotaurine
Leucine					L-Carnitine
L-Isoleucine					Hydroxyproline (trans-4-hydroxy-L-proline)
L-Ornithine					Phosphoserine
L-Threonine					Choline Chloride
Lysine					L-Malic acid
gamma-aminobutyric acid					N-Acetyl - L-lysine
L-Phenylalanine					N-acetyl-L-glutamic acid
Proline					N-alpha-Acetyl-L-glutamine
L-Serine					O-phosphorylethanolamine
L-Tryptophan					orotic acid
L-Tyrosine					Phosphocholine
L-Valine					Pyruvic acid
					Taurine

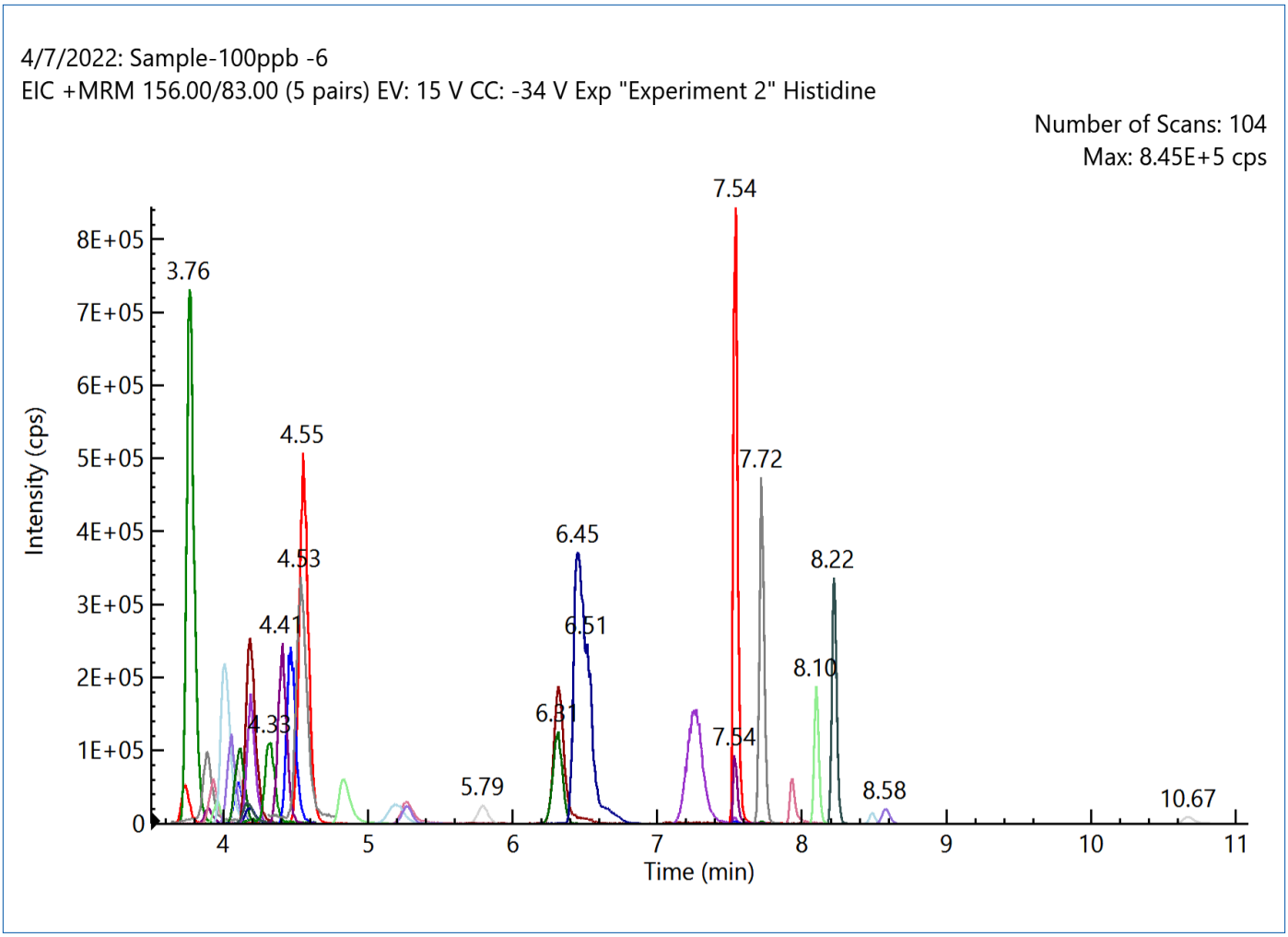

Figure 1. Extracted Ion Chromatograms of selected components from mixed compound standard (100 µg/L in concentration). Only one MRM transition, for each selected component, is shown.

Additionally, an extract (1:100 dilution) of the Fisher's Medium cell culture medium (lot #2458244) was analyzed and evaluated using this developed method. As shown in **Figure 2**, several components were found in the medium that align with the mixed standard solution components in Figure 1.

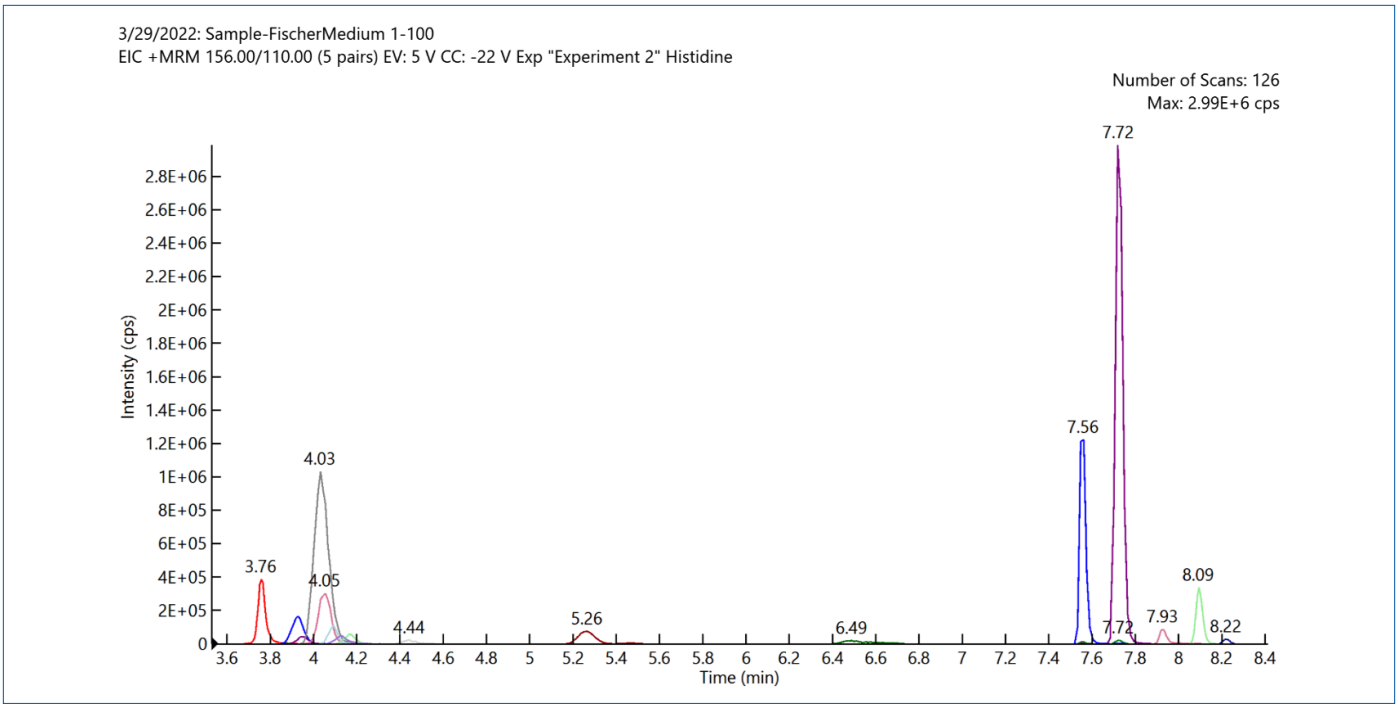

Figure 2. Extracted Ion Chromatograms of detected components from Fischer cell culture medium (1:100 diluted extract). Only one MRM transition, for each component, is shown.

Linearity and reproducibility results

With the optimized LC method and MRM transitions, single compound solutions were made to generate individual calibration curves for metabolite quantification. The dynamic linear range was estimated by first determining the lower limit at S/N = 10; then the upper limit was determined where the signals remain in a linear relationship with analyte concentration of standard curves.

Figure 3 Illustrates overlaid extracted Ion chromatograms for a selected cell culture media component (Threonine). The measured quantity from the highest to lowest calibration point is plotted against retention time. A Savitzky-Golay derivative filter was applied to the calibration curve data.

Additionally, **Table 5** shows the following parameters for each cell culture media component: retention time, quantitative linear range, and coefficient of determination (R^2) for calibrating the quantitative linear range, which was determined using an external calibration method. For each metabolite, the linear range, reproducibility of retention time and peak area were obtained from 6 consecutive injections of a specific standard solution.

Good linearity was obtained for each analyte, from 1 µg/L up to 1000 µg/L, with regression coefficients (R^2) greater or equal to 0.99. Moreover, these metabolites could be quantified within a wide dynamic range. Therefore, the presented methodology enables simultaneous detection and quantitation of various metabolites with great sensitivity.

As an additional performance benchmark, the reproducibility of each peak area was evaluated. The median CV was 2.5%, and all the compounds could be detected with very high reproducibility (%RSD below 5.5%).

Together, these results demonstrate that the presented method is sensitive and reproducible for the monitoring and profiling of cell culture media components.

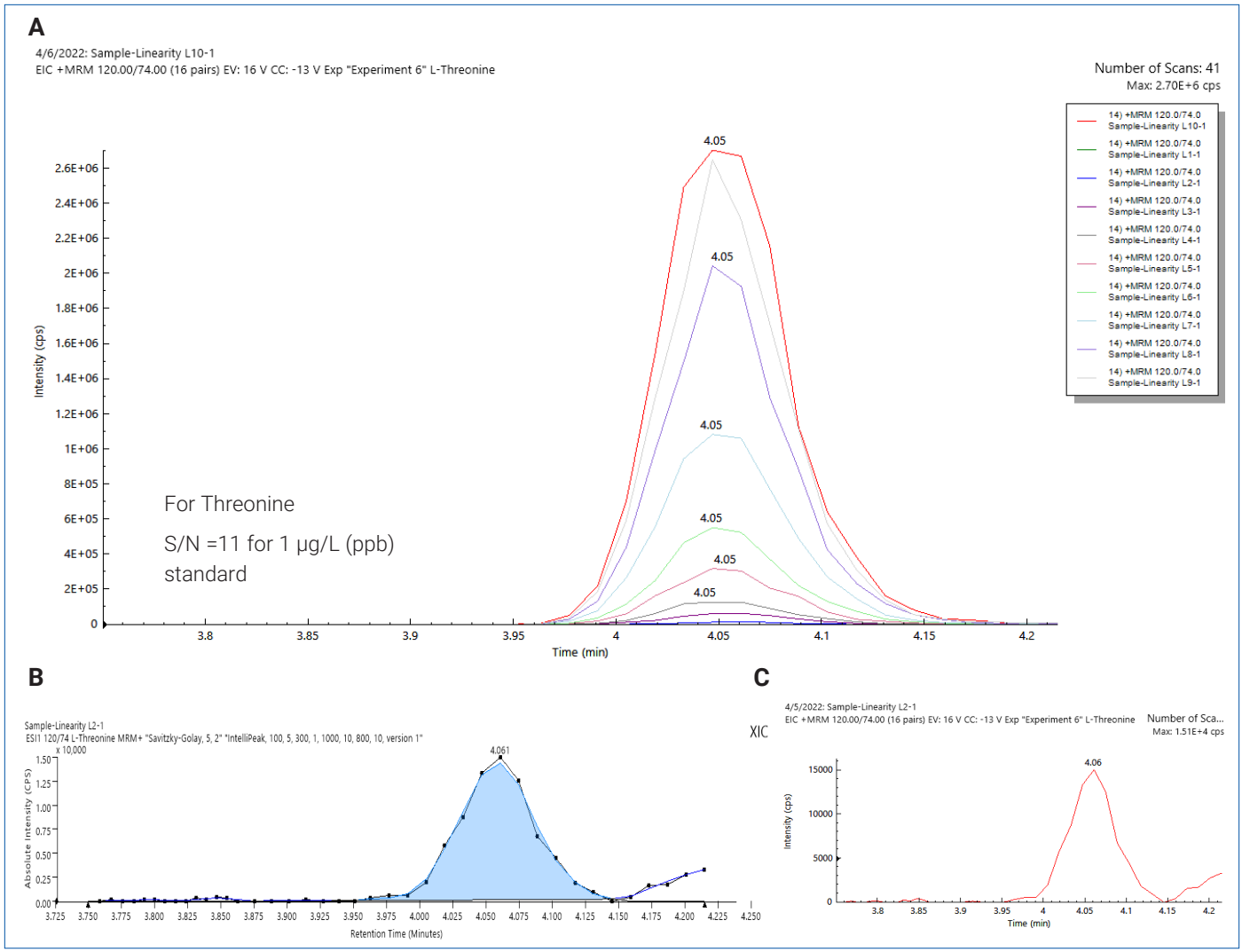

*Figure 3. Overlay of Extracted Ion Chromatograms for Various Calibration Levels of Threonine **(A)**. One MRM transition (quantifier Ion) is shown **(B)**. The linear dynamic range varies several orders of magnitude. The Signal to Noise (S/N) ratio value indicating the lowest point on the calibration curve of compound. Savitzky-Golay smoothing was applied to the calibration curve data **(C)**.*

Table 5. Linearity, Accuracy and Reproducibility Results for the Targeted Cell Media Components (n=6).

Analyte	Retention Time	% Accuracy (Area counts)	Peak Area (%RSD)	Retention Time Deviation (%RSD)	R^2	Linear Range (µg/L)
L-Ornithine (HCl)	3.648	99.521932	2.404823	0.05	0.9995	1-1000
Histidine	3.737	101.616802	1.005451	0.138	0.9987	1-1000
L-Carnosine	3.764	102.9	3.405677	0.411	0.9911	1-1000
Leucine	4.1	108.118424	3.403416	0.038	0.9933	1-1000
L-Isoleucine	4.1	104.665066	2.520949	0.011	0.9948	1-1000
Lysine	4.041	109.843521	1.329002	0.159	0.9907	1-1000
Cystathionine (CYS)	3.855	101.070973	1.984852	0.062	0.9978	1-1000
L-Cystine	3.884	105.871	1.9	0.294	0.99	250-4000
Glycine	3.894	109.236812	3.607174	0.067	0.9931	1-1000
Beta-Alanine	3.894	111.796143	2.531906	0.161	0.9915	1-1000

Table 5. Linearity, Accuracy and Reproducibility Results for the Targeted Cell Media Components (n=6) (Continued).

Analyte	Retention Time	% Accuracy (Area counts)	Peak Area (%RSD)	Retention Time Deviation (%RSD)	R^2	Linear Range (µg/L)
Serine	3.938	109.807426	3.180752	0.226	0.9932	1-1000
L-Asparagine	3.957	109.527371	2.565541	0.015	0.9927	1-1000
O-phosphorylethanolamine	3.94	98.840266	4.849209	0.134	0.9915	1-1000
L-Alanine	4.008	111.324086	2.508927	0.016	0.9937	1-1000
Taurine	4.008	111.041428	3.298163	0.073	0.9907	1-1000
Glutamine	4.031	111.144526	2.390514	0.009	0.9913	1-1000
Hypotaurine	4.043	114.973539	3.6	0.106	0.99	10--2000
L-Threonine	4.043	110.676368	3.549038	0.519	0.9967	1-1000
Phosphoserine	4.031	109.867317	3.842958	0.242	0.9951	1-1000
Myo-inositol	3.962	113.237078	2.8	0.082	0.99	10--1000
Hydroxyproline (trans-4-hydroxy-L-proline)	4.106	108.448903	2.382373	0.019	0.9912	1-1000
Choline Chloride	4.182	114.565726	2.028092	0.01	0.9923	1-1000
Gamma-aminobutyric acid	4.106	109.188939	2.4323	0.01	0.99	10-1000
Phosphocholine	4.182	110.258974	0.861813	0.01	0.9934	1-1000
Cysteic acid	4.107	111.179558	3.987552	0.046	0.9938	1-1000
Glucose	4.119	118.646207	4.972933	0.159	0.99	10-1000
Glutamate	4.134	113.489666	2.419951	0.093	0.9912	1-1000
Alpha-ketoglutarate (AKG)	4.134	111.619	3.549592	0.232	0.991	1-1000
Citrulline	4.186	111.532171	1.679892	0.01	0.9933	1-1000
D-Ribose-5-phosphate (R5P)	4.163	104.123367	2.735784	0.01	0.9978	1-1000
D-+-Mannose	4.11	115.243221	4.204743	0.225	0.99	10-1000
Proline	4.549	106.830421	1.436056	0.032	0.9926	1-1000
L-2-Aminobutyric acid	4.318	110.00458	2.919053	0.058	0.9968	1-1000
L-Carnitine	4.463	104.613799	1.908832	0.032	0.9902	1-1000
Thiamine	4.457	99.197786	1.569257	0.459	0.99	1-1000
Creatinine	4.409	110.050239	3.287368	0.046	0.99	1-1000
Allantoin	4.41	108.391632	1.021607	0.136	0.9971	1-1000
Betaine	4.527	106.910284	1.239991	0.097	0.99	10-1000
L-Malic acid	5.262	106.424903	1.171313	0.043	0.9985	1-1000
UDP-Alpha-Glucose	4.768	93.688548	4.7	0.413	0.99	250-4000
Cytidine-5-monophosphate (CMP)	4.802	107.956783	0.750212	0.298	0.9957	1-1000
Pyruvic acid	5.188	106.400894	1.661415	0.023	0.996	1-1000
N-Acetyl - L-lysine	5.793	107.868091	0.849823	0.036	0.9963	1-1000
Orotic acid	5.84	104.924642	1.618095	0.078	0.9925	1-1000
Adenosine5-monophosphate (AMP)	6.336	100.329568	1.473234	0.331	0.9994	1-1000
2-Picolinic acid	6.37	83.254376	4.901125	0.323	0.9927	50-4000
N-alpha-Acetyl-L-glutamine	6.326	114.638825	2.811319	0.295	0.997	1-1000

Table 5. Linearity, Accuracy and Reproducibility Results for the Targeted Cell Media Components (n=6) (Continued).

Analyte	Retention Time	% Accuracy (Area counts)	Peak Area (%RSD)	Retention Time Deviation (%RSD)	R^2	Linear Range (µg/L)
Pyridoxal	6.533	86.84219	2.740547	0.437	0.99	1-500
Nicotinamide	7.263	99.999117	1.676528	0.191	0.9965	1-1000
Riboflavin B2	8.219	108.907008	5.092	0.029	0.996	1-1000
Citric acid	7.542	101.753165	3.772	0.085	0.99	1-1000
Adenosine	7.542	107.458785	0.934073	0.046	0.99	1-1000
Inosine	7.542	104.788415	2.66867	0.033	0.9903	1-1000
Valine	7.723	102.38453	4.528713	0.597	0.9911	1-1000
Tyrosine	7.542	104.737857	2.201719	0.06	0.9907	1-1000
Glutathione oxidized	7.529	106.880406	3.705432	0.079	0.9921	1-1000
N-acetyl-L-glutamic acid	7.545	107.36087	2.483384	0.064	0.9926	1-1000
Fumaric acid	7.545	108.647154	3.746862	0.005	0.99	1-1000
Hydroxybutyric acid	7.545	103.205781	3.727476	0.035	0.99	10-500
Xanthine	7.545	110.354032	4.733768	0.106	0.99	1-1000
Thymidine-5'-monophosphate (TMP)	7.545	108.225773	2.430082	0.099	0.999	1-1000
Nicotinamide adenine dinucleotide (NAD)	7.533	96.499176	2.9	0.053	0.99	1-1000
Hypoxanthine	7.533	109.416305	3.7086	0.033	0.9908	1-1000
Acetyl L-Carnitine (chloride)	7.533	109.606876	2.9	0.046	0.99	1-500
Phenylalanine	7.716	104.081883	0.96242	0.006	0.9997	1-1000
5'-Methylthioadenosine (MTA)	7.868	109.568911	1.318546	0	0.99	1-1000
Folic acid	7.933	112.727522	3.811369	0.013	0.99	1-1000
Tryptophan	8.097	103.926715	1.468588	0.035	0.9994	1-1000
Biotin	8.489	114.663008	4.5	0.016	0.9937	1-1000
4-Aminobenzoic acid (para-aminobenzoic acid)	8.578	104.97439	5.151269	0	0.99	10-1000
DL-indole-3-lactic acid (Indolelactic acid)	10.673	104.92533	3.901949	0.021	0.998	1-1000

Conclusion

A method, using the PerkinElmer QSight 420 LC/MS/MS was developed for the quantitative analysis of several components in cell culture media. This method covers a wide range of compounds including amino acids, vitamins, nucleoside pyrimidines and purines, nucleotides, organic acids, and carbohydrates. With its excellent reproducibility and sensitivity, this targeted work was designed to meet industry demands and to make cell culture media analyses fast, easy and effective.

Furthermore, an isotope dilution method was developed to limit possible errors from matrix effects or sample-preparation effects and allow for more accurate quantitation of components within the cell culture media.

References

1. Sellick CA, Croxford AS, Maqsood AR, Stephens G, Westerhoff HV, Goodacre R. Metabolite profiling of recombinant CHO cells: designing tailored feeding regimes that enhance recombinant antibody production. *Biotechnol. Bioeng*. 2011; 108:3025–31.

2. Villas-Bôas Silas G, Moxley Joel F, Åkesson M, Stephanopoulos G, Nielsen J. High-throughput metabolic state analysis: the missing link in integrated functional genomics of yeasts. *Biochem. J.* 2005; 388:669–77.

3. Arora M. Cell culture media: a review. *Mater. Methods.* 2013; 3:175.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

801313 PKI