

## Cell Culture Media Monitoring in Cell and Gene Therapy Using the Bioaccord™ LC-MS System

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This is an Application Brief and does not contain a detailed Experimental section.

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### Abstract

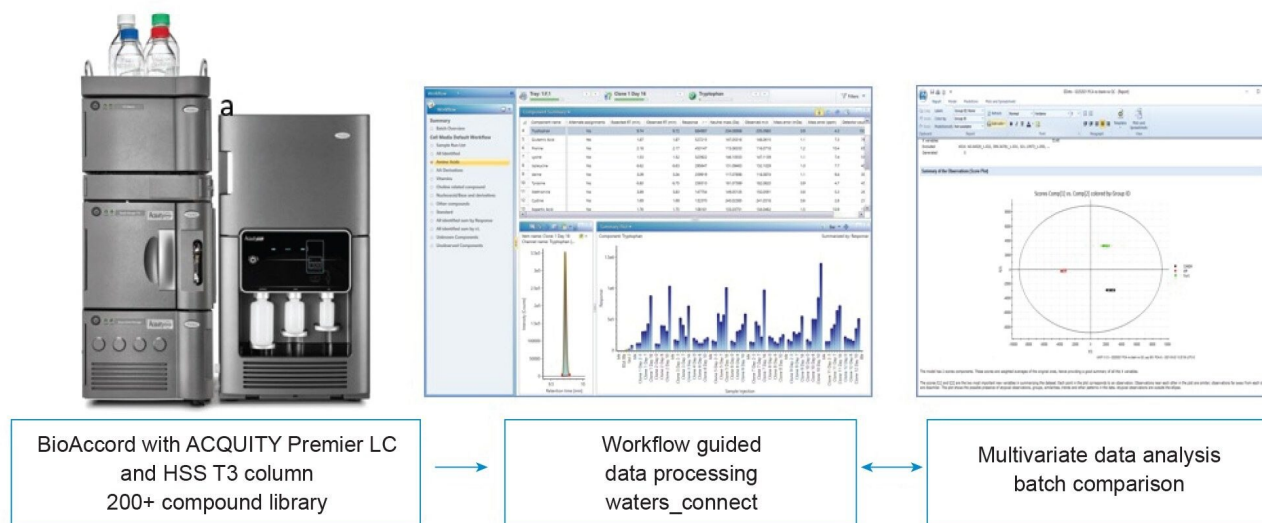
The HEK293 cell line is commonly used in the production of viral vectors and requires the use of cell culture media tailored for health and growth of these cell lines. Here, we demonstrate the use of the BioAccord Liquid Chromatography-Mass Spectrometry (LC-MS) System for the analysis of HEK293 viral vector media. Results show that the media contains typical classes of cell culture media compounds such as amino acids and vitamins. It also contains a lower amount of nucleic acids and nucleosides. In conclusion, the cell culture media method and workflow developed for protein production can be readily applied to viral vector based gene therapy.

### Benefits

- Easy-to-use LC-MS platform for rapid and comprehensive monitoring of cell culture media used in viral vector based gene therapy
  - One HRMS platform supporting media monitoring process analytics as well as product quality analysis in
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## Introduction

In viral vector based gene therapy, modified viruses are used as drug-delivery vehicles to introduce specific DNA sequences into cells. The HEK293 cell line, from immortalized human embryonic kidney cells, is commonly used in the production of viral vectors such as adenoviral (AV), adeno-associated viral (AAV), and retroviral vectors.<sup>1</sup> In this technology brief, methodology developed for cell culture media analysis based on the BioAccord high resolution mass spectrometry (HRMS) platform is applied for HEK 293 viral vector media analysis. Scheme 1 is a general description of the method package; further details are given in a previously published Waters application note.<sup>2</sup>



*Scheme 1. A schematic illustration of BioAccord/waters\_connect™ based workflow for media analysis (adopted from a Waters Appnote)<sup>2</sup>.*

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## Results and Discussion

HEK293 viral vector media was purchased from MilliporeSigma (p/n: 14385C). Since media composition was not disclosed in the product, the media was diluted at ratios of 1:100, 1:200, 1:500, 1:1000, and 1:2000 to facilitate positive compound identification, and help in identifying the best dilution ratio for routine monitoring. The diluent used was H<sub>2</sub>O containing 0.1% formic acid and 0.1 μM 3-chlorotyrosine as an internal standard. Basal media solutions, DMEM and IMDM at 1:100 dilution were also prepared. The solutions were subjected to LC-MS analysis using BioAccord as described previously.<sup>2</sup> A 17 amino acids calibration solution at concentrations from 0.01 to 10 μM were injected at the beginning and end of the sample analysis. A mass range of 50–800 *m/z* was used for data acquisition.

An overlaid extracted ion chromatogram (XIC) for the major compounds observed is shown in Figure 1 and representative trend bar plots for each compound class are shown in Figure 2. Results show that three major classes of compounds were detected in HEK293 media, amino acids, vitamins, and several nucleic acids and nucleosides. The most abundant compound class is amino acids, followed by vitamins. Nucleic acids and nucleosides are the least abundant compounds. The rapidly metabolizing amino acid, glutamine, was included in its stable dipeptide form as alanyl-glutamine. Compared to basal media DMEM and IMDM, HEK 293 viral vector media has in general a higher concentration of amino acids and vitamins. Nucleic acids and nucleosides are not typically present in basal media. Excellent LC-MS reproducibility for the top 35 compounds was observed at 1:200 and 1:500 dilution ratio is tabulated in Table 1. Based on the response observed at multiple dilutions, a dilution ratio of 1:500 is recommended for routine monitoring of the media components in a viral vector production process.

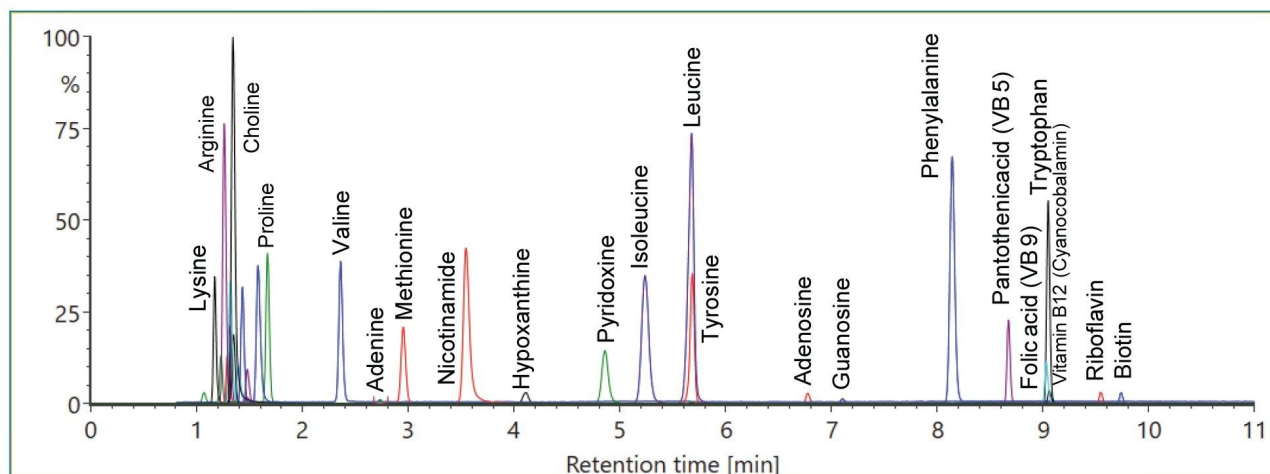


Figure 1. Overlaid XIC of the top 34 compounds observed in HEK 293 media under positive ion electrospray (ESI+) conditions. For display purposes, amino acids are extracted from the 1:2000 diluted sample, Vitamins and other compounds are extracted from the 1:100 diluted sample. The vitamin, myo-inositol is observed in negative ion mode (ES-) (data not shown).

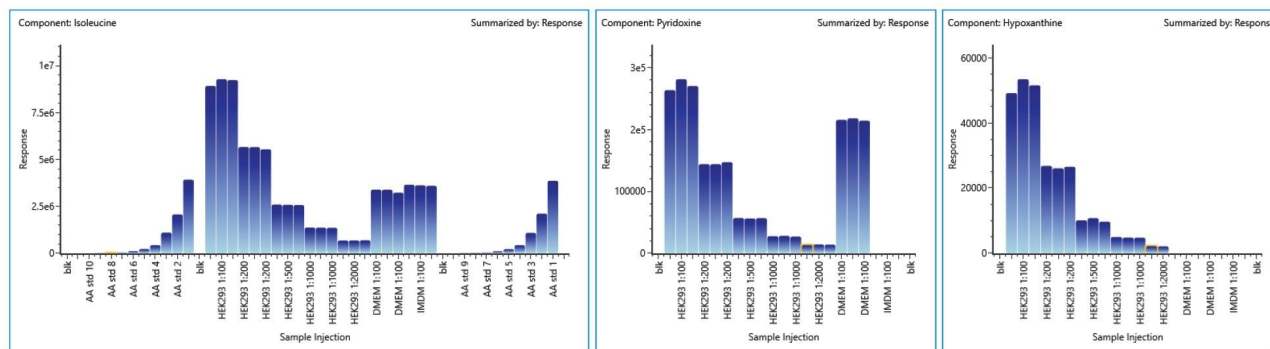


Figure 2. Representative bar trend plots of compound response (triplicate injection/sample). (A) amino acid example, isoleucine, showing standard calibration curve at the beginning and end of injection. Isoleucine is present in standard and all media samples. (B) vitamin example, pyridoxine, showing the compound is present in HEK293 media and DMEM media, and not present in IMDM media as expected. (C) nucleobase example, hypoxanthine, showing the compound is present in HEK293, and not in DMEM and IMDM media.

Compound	observed r.t (min)	Average response at 1:500 dilution ratio	%RSD at 1:500 dilution ratio	%RSD at 1:200 dilution ratio
Alanine	1.36	1.3E+04	2.2	1.7
Alanyl-Glutamine	1.48	4.1E+05	1.1	0.2
Arginine	1.26	2.2E+06	0.8	1.0
Asparagine	1.32	8.3E+05	0.7	1.0
Aspartic Acid	1.35	4.8E+05	1.2	0.9
Cystine	1.29	3.8E+05	0.7	1.1
Glutamic Acid	1.43	9.9E+05	0.5	0.5
Glycine	1.29	2.4E+03	6.1	4.4
Histidine	1.23	2.8E+05	0.2	0.4
Isoleucine	5.23	2.6E+06	0.5	1.2
Leucine	5.67	4.0E+06	0.6	2.7
Lysine	1.17	7.7E+05	1.5	0.6
Methionine	2.95	1.1E+06	0.6	0.9
Phenylalanine	8.14	3.5E+06	0.9	0.5
Proline	1.67	1.2E+06	1.3	2.5
Serine	1.31	5.3E+05	0.2	0.8
Threonine	1.39	2.8E+05	1.1	1.2
Tryptophan	9.05	2.0E+06	1.1	0.2
Tyrosine	5.68	1.2E+06	1.2	4.5
Valine	2.36	1.3E+06	1.4	2.4
Biotin	9.74	5.5E+03	4.3	0.8
Choline	1.34	3.1E+05	0.8	0.5
Folic acid (VB 9)	9.03	2.2E+04	1.9	0.7
Glucose/Myo-inositol	1.51	5.1E+04	1.3	0.9
Nicotinamide	3.55	1.6E+05	1.8	0.5
Pantothenic acid (VB 5)	8.67	4.0E+04	1.9	1.0
Pyridoxine	4.86	5.7E+04	0.5	1.3
Riboflavin	9.55	5.3E+03	0.7	1.3
Thiamine	1.58	9.8E+04	1.9	2.0
Vitamin B12 (Cyanocobalamin)	9.06	6.9E+03	3.9	1.0
Adenine	2.73	2.5E+03	12.4	2.9
Adenosine	6.78	6.9E+03	5.8	3.6
Cytidine	2.92	3.0E+03	5.4	4.1
Guanosine	7.11	3.2E+03	6.1	3.3
Hypoxanthine	4.11	1.0E+04	5.4	1.4

Table 1. Summary of response and reproducibility for the top 35 compounds based on three replicate injections.

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*The table is ordered according to compound class and compound name.*

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## Conclusion

A cell culture media method developed using the BioAccord LC-MS System has been employed for the analysis of HEK293 viral vector media. The analysis revealed HEK293 contains more than 35 compounds with compound classes including amino acids, vitamins, and nucleic acid and nucleosides that can be easily detected and monitored. The data suggests that in addition to cell culture and microbial media monitoring in protein production, the cell culture media analysis methodology is applicable for general media monitoring in gene therapy.

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## References

1. Tan E. *et al.* "HEK293 cell line as a platform to produce recombinant proteins and viral vectors; Front Bioeng. Biotechnol. 2021, 9 796991.
  2. Alelyunas YW, Wrona MD, Chen W, Monitoring Nutrients and Metabolites in Spent Cell Culture Media for Bioprocess Development Using the BioAccord LC-MS System With ACQUITY Premier Waters Application Note [720007359](#), 2021 September.
  3. Zhang X, Koza SM, Yu YQ, Chen W. Optimizing Adeno-Associated Virus (AAV) Capsid Protein Analysis Using UPLC and UPLC-MS, Waters Application note [720006869](#), May 2020.
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