T-Cell Media Analysis using Triple Quadrupole Mass Spectrometry

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1. Overview

A comprehensive evaluation of commercially available T-cell media was done using a triple quadrupole mass spectrometer

2. Introduction

T-cell based immunotherapies have emerged to be a promising approach for cancer treatment. With the increasing demand for T-cell manufacturing, more research has been devoted to optimizing the T-cell expansion process. The T-cell media composition is directly related to the quality of the final product. Therefore, a method that can comprehensively analyze and monitor the culture media is needed for media selection, research and development, T-cell harvest and expansion monitoring, and quality control. Triple quadrupole mass spectrometry with its fast scan and polarity switching speed has the capability to simultaneously analyze multiple compound groups with a short total run time. The scan and MRM data can provide comprehensive profiling information on T-cell media.

3. Method

Commercially available T-cell media were purchased and investigated using a 17 min chromatography method on an LCMS-8050 triple quadrupole mass spectrometer. Mobile phases were water and acetonitrile with 0.1% formic acid. T-Cell media samples were subjected to protein crash using acetonitrile and centrifugation at 15,000 rpm for 10 min at 4°C. Supernatants were further diluted with water prior to analysis. (Figure 1) One hundred and twenty-five cell culture related compounds including amino acids, nucleic acids, metabolites, sugars, and vitamins were simultaneously analyzed by MRM in both ESI positive and negative modes for each of the T-cell medium. (Table 1) Triplicate results were obtained for each sample.

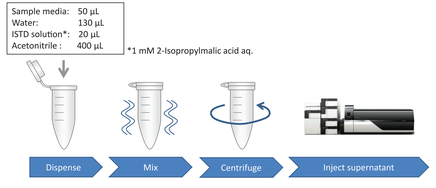

Figure 1. Sample preparation procedure for cell culture profiling analysis

Table 1. 125 registered compounds in cell culture media profiling method package.

Amino acids and their metabolites		Nucleic acids and their metabolites
1-Methylhistidine	Glycyl-glutamine	Adenine
2-Aminoadipic acid	Histidine	Adenosine
2-Aminobutyric acid	Homocysteine	Adenosine monophosphate
3-Hydroxyanthranilic acid	Hydroxykynurenine	Deoxyadenosine
3-Hydroxyisobutyric acid	Hydroxylysine	Deoxyadenosine monophosphate
3-Methyl-2-oxovaleric acid	Indole-3-acetic acid	Deoxyguanosine
3-Methylhistidine	Isoleucine	Deoxyguanosine monophosphate
4-Aminobutyric acid	Kynurenic acid	Guanine
4-Hydroxyphenyllactic acid	Kynurenine	Guanosine
4-Hydroxyproline	Leucine	Guanosine monophosphate
5-Glutamylcysteine	Lysine	Hypoxanthine
5-Hydroxytryptophan	Methionine	Inosine
5'-Methylthioadenosine	Methionine sulfoxide	Inosine monophosphate
5-Oxoproline	N-Acetylaspartic acid	Uric acid
Alanine	N-Acetylcysteine	Xanthine
Alanyl-glutamine	Ornithine	Xanthosine
Anthranilic acid	Oxidized glutathione	Xanthosine monophosphate
Arginine	Phenylalanine	3-Aminoisobutyric acid
Argininosuccinic acid	Pipecolic acid	3-Aminopropanoic acid
Asparagine	Proline	Cytidine
Aspartic acid	Putrescine	Cytidine monophosphate
Citrulline	Saccharopine	Cytosine
Cystathionine	S-Adenosylhomocysteine	Deoxycytidine
Cysteine	Serine	Deoxycytidine monophosphate
Cystine	Serotonin	Orotic acid
Formylkynurenine	Threonine	Thymidine
Glutamic acid	Tryptophan	Thymidine monophosphate
Glutamine	Tyrosine	Thymine
Glutathione	Urocanic acid	Uracil
Glycine	Valine	Uridine
		Uridine monophosphate

Sugars	Others
Gluconic acid	2-ketoglutaric acid
Hexose (Glucose)	Acotinic acid
Sucrose	Citric acid
Threonic acid	Fumaric acid
	Isocitric acid
	Lactic acid
	Malic acid
	Pyruvic acid
	Succinic acid
	Penicillin G
	2-Aminoethanol
	Glyceric acid
	NAD
	O-Phosphoethanolamine
	Taurine

Vitamins
Riboflavin
Niacinamide
Nicotinic acid
Pantothenic acid
4-Pyridoxic acid
Pyridoxal
Pyridoxalphosphate
Pyridoxine
Biotin
4-Aminobenzoic acid
Folic acid
Choline
Ascorbic acid
Cyanocobalamin
Lipoic acid

Internal Standard
2-Isopropylmalic acid

In addition to MRM, scan mass spectra (m/z 200-1000) were acquired for T-cell, In vitro fertilization (IVF), and Dulbecco's Modified Eagle (DMEM) media to comparatively evaluate T-cell specific media by principal component analysis (PCA).

4. Results

4-1. PCA on Multiple Media Subtypes

Six culture media from three subtypes (t-cell media, IVF media, and DMEM) were analyzed. Cell culture media PCA showed clear delineation between the different media subtypes. (Figure 2)

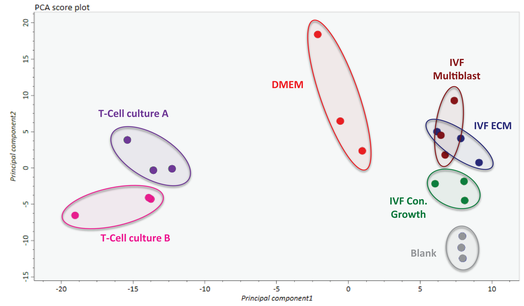

Figure 2. Principle component analysis score plot of the six culture media samples from three subtypes in triplicates.

4-2. Similarity and Differences between the T-cell Media

Two commercially available serum-free and xeno-free T-cell culture media were analyzed using the cell culture media profiling method. Sugar (glucose), nucleic acid metabolite (uridine monophosphate), amino acids and metabolites(cystine, asparagine, aspartic acid, serine, glycine, glutamine, threonine, glutamic acid, alanine, citrulline, ornithine, proline, lysine, 2-aminobutyric acid, histidine, argininosuccinic acid, arginine, 5-oxoproline, valine, methionine, pipecolic acid, tyrosine, isoleucine, leucine, and phenylalanine), and vitamins (niacinamide, pantothenic acid, folic acid, and riboflavin) were detected in both T-cell media. Significant differences between the two media were shown in glutamine (*m/z* 145.20 >127.15; Rt: 1.2 min), alanyl-glutamine (*m/z* 216.00 >154.05; Rt: 1.5 min), pipecolic acid (*m/z* 130.10 >84.05; Rt: 2.3 min), and biotin (*m/z* 245.10 >226.95; Rt: 5.1 min).

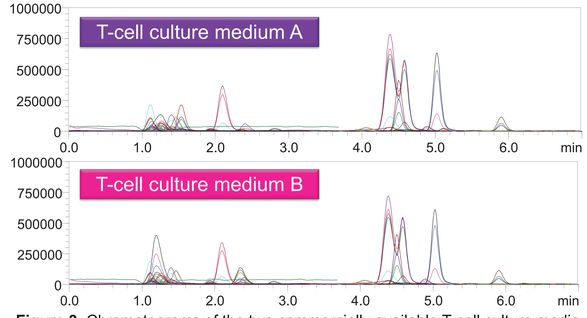

Figure 3. Chromatograms of the two commercially available T-cell culture media.

4-3. Differentiating compounds between the T-cell Media

As an essential vitamin, biotin, is indispensable for cell growth and fatty acid synthesis. It is found in many classical media except those based on Minimum Essential Medium Eagle (MEM). In this study, biotin was found in T-cell medium A, but not in T-cell medium B.

Pipecolic acid, a product of lysine metabolism, is likely to be included in the cell culture media to increase the cell survival rate during oxidative stress and high osmolarity conditions. The intensity of pipecolic acid was significantly higher in T-cell medium B in comparison to the T-cell medium A.

Glutamine is an essential amino acid for cell culture. However, it degrades into toxic ammonia. A more stable alanyl-glutamine dipeptide can be used to substitute glutamine in cell culture media. The gradual release of glutamine from the dipeptide is believed to allow for a more efficient energy metabolism and a higher production yield. Alanyl-glutamine was observed only in T-cell medium A while T-cell medium B had an extensively higher glutamine intensity. This data revealed that T-cell medium A adopted the dipeptide method to release glutamine into the media.

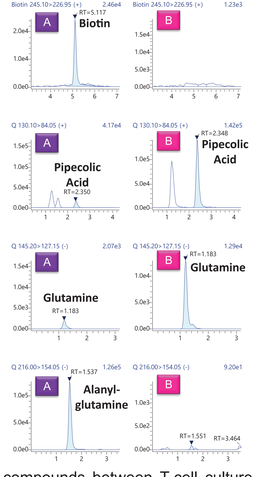

Figure 4. Example chromatograms of differentiating compounds between T-cell culture medium A and B.

5. Conclusion

- Six cell culture media from three different subtypes were analyzed using a LCMS-8050 triple quadrupole mass spectrometer and clear delineation was shown between the subtypes.
- The fast scan speed and short polarity switching time allows the method to simultaneously analyze 125 compounds in 17 minutes.
- Similarity and differences between the two T-cell culture media in sugars, nucleic acid and their metabolite, amino acids and their metabolites, vitamins, and other cell culture related compounds were obtained using the Cell Culture Profiling method package.
- Differentiating compounds such as glutamine, alanyl-glutamine, pipecolic acid, and biotin for the two commercially available serum-free and xeno-free T-cell culture media were found.
- This simultaneous multicomponent cell culture analysis method can be valuable in assisting media research and development as well as quality control.
- In the future, time-lapse study can be done during the T-cell and chimeric antigen receptor (CAR) T-cell expansion to monitor the cell uptake and secretion pattern for process optimization.
- Cell culture profiling method package along with LCMS-8050 triple quadrupole mass spectrometer provide a solution where numerous medium components and secreted metabolites can be analyze simultaneously in one run for T-cell culture media.