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Application News

Liquid Chromatography Mass Spectrometry

# T Cell Media Analysis using Triple Quadrupole Mass Spectrometry

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## ■ Summary

A workflow that comprehensively evaluates commercially available T cell media using a Shimadzu LCMS-8050 triple quadrupole mass spectrometer is demonstrated in this application news. One hundred and twenty-five T cell culture medium components and secreted metabolites were analyzed simultaneously using a 17-minute method. A list of compounds that differentiate the two commercially available T cell culture media was easily obtained using this method.

## ■ Background

T cell-based immunotherapies have emerged to be a promising approach for diseases such as cancer. With the increasing demand for T cell manufacturing, more research has been devoted to optimizing the T cell expansion process.[1] 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. A Shimadzu LCMS-8050 triple quadrupole mass spectrometer, with its fast scanning and polarity switching speeds, has the capability to simultaneously analyze multiple compound groups with a short total run time. Using the established method from the Cell Culture Profiling Method Package version 2, one hundred and twenty-five compounds can be analyzed in just 17 minutes (Table 1). The scan and MRM data obtained on a triple quadrupole mass spectrometer can provide comprehensive profiling information of T cell media.

## ■ Materials and Methods

Two commercially available T cell media were purchased and investigated using a 17-minute chromatographic method on an LCMS-8050 triple quadrupole mass spectrometer. Mobile phases were water and acetonitrile with 0.1% formic acid. LC-MS grade formic acid, acetonitrile, and water were sourced from Honeywell. T cell media samples were subjected to a 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 (Fig. 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 in each T cell medium. Triplicate results were obtained for each sample. Method details for chromatography and mass spectrometry are included in Table 2. LabSolutions was used for data acquisition, and LabSolutions Insight was used for data analysis. In addition to the MRM method, a scan method was also developed (*m/z* 200-1000 in ESI positive and negative mode) for this analysis. Mass spectra were acquired for two serum-free and xeno-free T cell media from two different vendors, three embryo culture media from the same vendor (Early Cleavage Medium™ (ECM™), MultiBlast Medium , and Continuous Single Culture™ NXcomplete (CSCM NXC)), and one Dulbecco's Modified Eagle (DMEM) medium to comparatively evaluate T cell specific media by principal component analysis (PCA). PCA results were obtained by directly processing the data file in MS-DIAL, an open-source software.[2]

Table 1 125 registered compounds in the Cell Culture Media Profiling Method Package version 2.

| 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 | Internal Standard |
|---|---|
| Riboflavin | 2-Isopropylmalic acid |
| Niacinamide | |
| Nicotinic acid | |
| Pantothenic acid | |
| 4-Pyridoxic acid | |
| Pyridoxal | |
| Pyridoxalphosphate | |
| Pyridoxine | |
| Biotin | |
| 4-Aminobenzoic acid | |
| Folic acid | |
| Choline | |
| Ascorbic acid | |
| Cyanocobalamin | |
| Lipoic acid | |

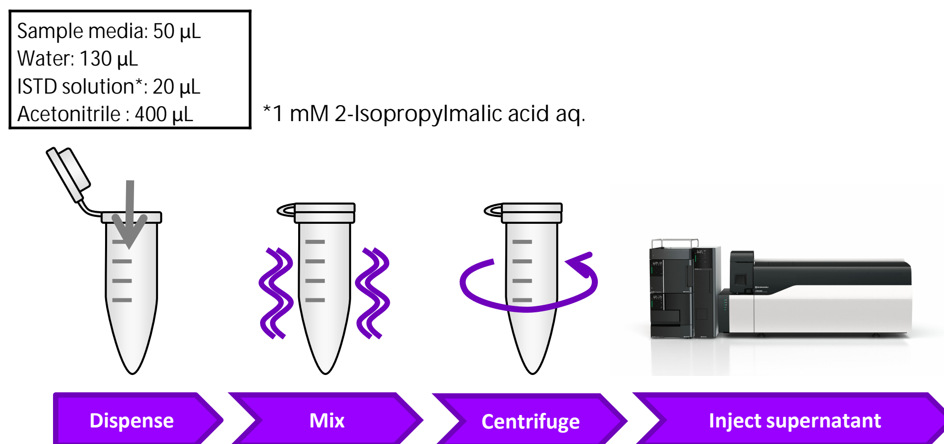


Fig. 1 Sample preparation procedure for cell culture profiling analysis.

Table 2 Analytical conditions

| | |
|---|---|
| LC Conditions | [LC-40 series] |
| Mobile Phase A | : Water containing 0.1% formic acid |
| Mobile Phase B | : Acetonitrile containing 0.1% formic acid |
| Chromatography Gradient | : 0% B -> 95% B |
| Total Run Time | : 17 minutes |
| Column | : Cell Culture Profiling Column (150mm x 2.1mm, 3 µm) (P/N 220-91581-10) |
| Flow Rate | : 0.35 mL/min |
| Column Oven Temperature | : 40 |
| Injection Volume | : 10 µL |
| MS Conditions | [LCMS-8050] |
| Ionization Mode | : ESI +/- |
| Acquisition Mode | : MRM and Scan |
| Scan Mass Range | : *m/z* 200-1000 |
| Nebulizing Gas Flow | : 3.0 L/min |
| Heating Gas Flow | : 10.0 L/min |
| Interface Temperature | : 300 ℃ |
| DL Temperature | : 250 ℃ |
| Heat Block Temperature | : 400 ℃ |
| Drying Gas Flow | : 10.0 L/min |

## ■ Results and Discussion

Six culture media from three subtypes (T cell media, IVF media, and DMEM) were analyzed using the MS scan method. Three embryo culture media from the same vendor, two serum-free and xeno-free T cell media from two different vendors, and one DMEM medium were investigated. Cell culture media PCA showed clear delineation between the different media subtypes (Fig. 2). Even though the two T cell culture media were sourced from two different vendors, they are still closely grouped on the PCA score plot and can be distinguished from the other media subtypes.

The two T cell culture media were also analyzed using the cell culture media profiling MRM method targeting one hundred and twentyfive cell culture related compounds. 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 (Fig. 3). In addition to the PCA plot, similarities of the two T cell culture media were further revealed by the abundance of the identical components observed in both media.

Significant differences between the two T cell culture media were also identified (Fig. 4). The discrepancies are especially apparent in glutamine (*m/z* 145.20 > 127.15; $t_R$: 1.2 min), alanyl-glutamine (*m/z* 216.00 > 154.05; $t_R$ : 1.5 min), pipecolic acid (m/z 130.10 > 84.05; $t_R$ : 2.3 min), and biotin (*m/z* 245.10 > 226.95; $t_R$ : 5.1 min).

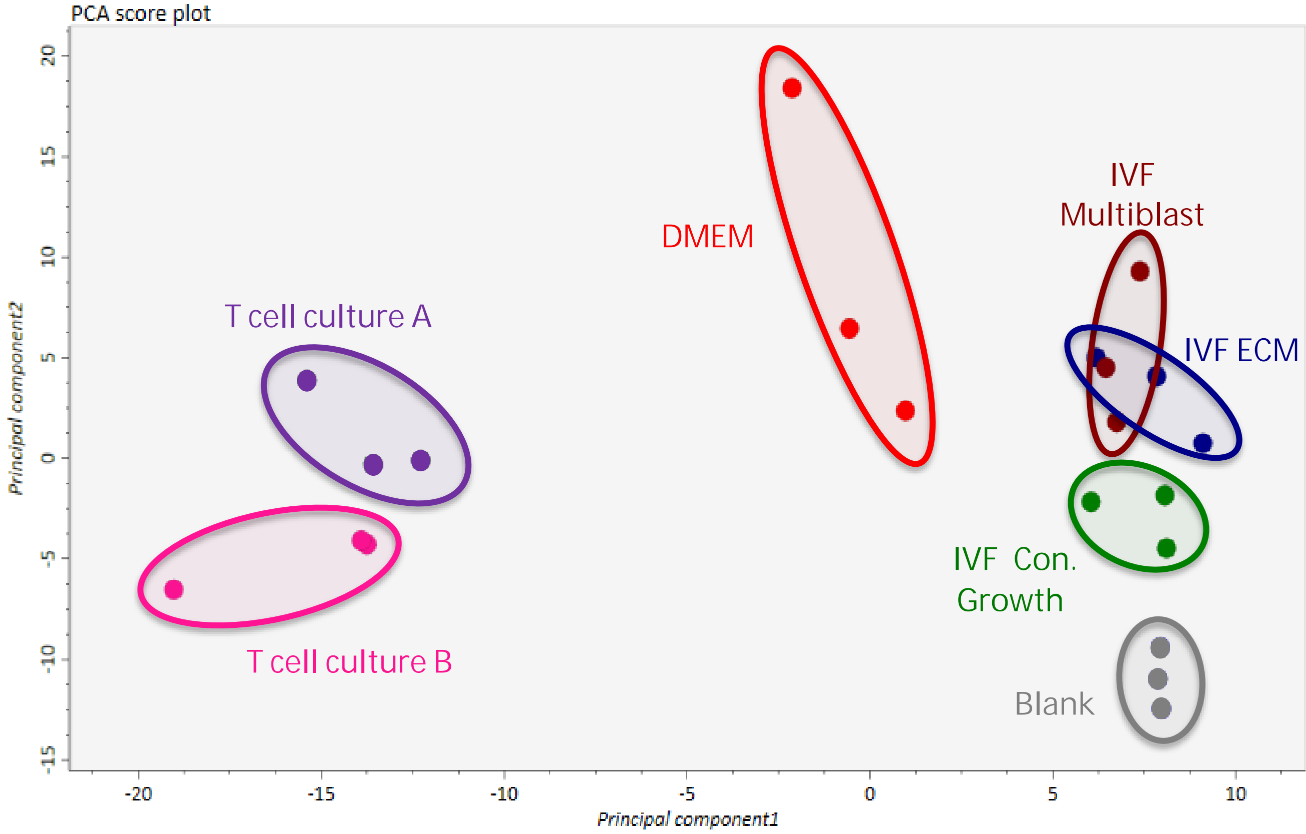


Fig. 2 Principal component analysis (PCA) score plot of the six culture media samples from three subtypes (T cell media, IVF media, and DMEM ) in triplicate.

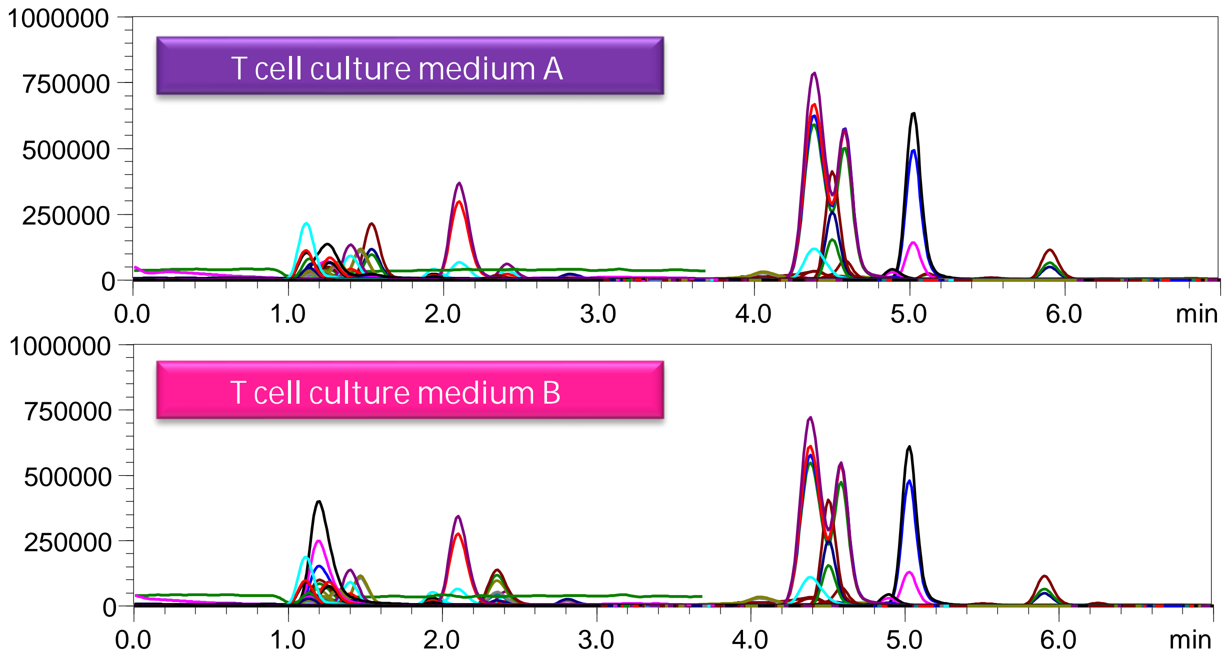


Fig.3 Chromatograms of two commercially available T cell culture media sourced from different vendors.

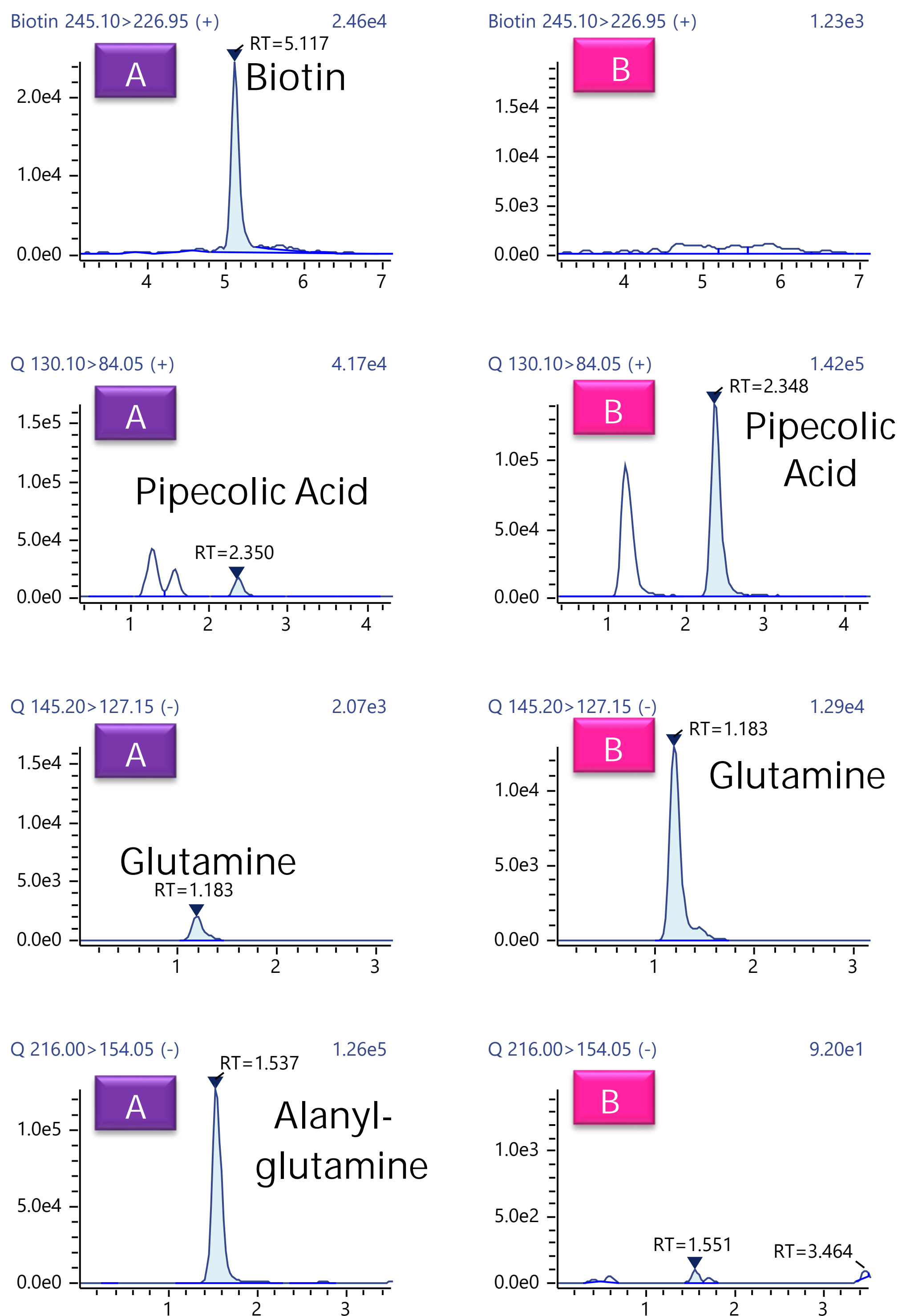


Fig. 4 Example chromatograms of differentiating compounds between T cell culture media A and B.

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 cell culture media to increase the cell survival rate during oxidative stress and high osmolarity conditions.[3] The intensity of pipecolic acid was significantly higher in T cell medium B in comparison to 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 as a substitute for glutamine in cell culture media.[4-5] 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 a measurably higher glutamine intensity. This data indicates that T cell medium A adopted the dipeptide method to release glutamine into the media.

## ■ Conclusion

This application news demonstrates a workflow for T cell culture media analysis using an LCMS-8050 triple quadrupole mass spectrometer. Data revealing the similarities and differences between the media samples were obtained and clear delineation was shown between the media subtypes by a PCA plot.

The fast scan speed and short polarity switching time of the LCMS-8050 allows the method to simultaneously analyze one hundred and twenty-five target compounds in 17 minutes.

This simultaneous multicomponent cell culture analysis method can be valuable in assisting media research and development as well as quality control. Time-lapse study can also 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.

## ■ References

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