SHIMADZU

MP 313

Investigation of Human Embryo Culture Media Using a Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometer

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1. Overview

An identification method for organic compounds in human embryo culture media was developed using Shimadzu LCMS-9030, a high resolution Quadrupole Time-of-flight mass spectrometer.

2. Introduction

In vitro fertilization (IVF) is one of the most common and effective type of assisted reproductive technology (ART) to treat infertility. The success rate of ART relies heavily on the quality of the embryo' development. Therefore, analysis of embryo culture media for both research and development as well as quality control is crucial. Q-TOF was popular in identifying unknown compounds because of its high resolution mass and MS/MS spectrum.
In this poster, three kind of human embryo culture media were investigated. The energy substrates, amino acids, antioxidant and antibiotic were identified using accurate mass and MS/MS library.

3. Methods

Shimadzu LCMS-9030, a high resolution Quadrupole Time-of-flight mass spectrometer, was used for developing an identification method for organic compounds in human embryo culture media. The Ultra high-performance liquid chromatography, LC-30A, was used for separating those compounds. The LC separation conditions was used according Shimadzu LC/MS/MS Method package for Cell Culture Profiling. A cell culture MS/MS library was used for confirm the compounds in culture media. Instrument conditions are listed in Table 1.

A cell culture MS/MS library containing 96 compounds was built using product ion scan data under a spreading collision energy of 35+/- 17 eV. Three types of human embryo culture media, Early Cleavage Medium (ECM) , Multi Blast Media (MBM), and Continuous Single Culture-NX Complete (CSCM), were analyzed using Data Dependent Acquisition (DDA) technique with a scan range of 10-600 *m/z*. Any ion counts that reached the threshold of 100 triggered a MS/MS scan with a range of 50-600 *m/z*.

Table 1 Instrument Acquisition Parameters

Acquisition Parameters	
HPLC Instrument	LC-30A
Injection Volume	10 µL
MS Instrument	LCMS-9030
Interface	ESI +/-
Interface Temp.	300 °C
DL Temp.	250 °C
Heat Block Temp.	400 °C
Nebulizing Gas Flow	2 L/min
Heating Gas Flow	10 L/min
Drying Gas Flow	10 L/min
Scan Range	*m/z* 50-600
DDA Range	*m/z* 10-600

Shimadzu LCMS-9030

Samples were prepared with 12 times dilution using acetonitrile and water. Sample preparation procedure is shown in Figure 1. A protein precipitation step was incorporated prior to injection using centrifugation at 15,000 rpm for 10 minutes at 4° C.

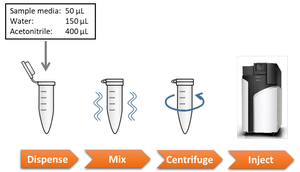

Figure 1. Sample preparation procedure

4. Results

96 analyte standards were used to develop LC separation method and MS analysis method. All analytes had good respond on ESI positive or negative mode. The chromatogram of them show in Figure 2.
Three kinds of embryo culture media, ECM, MBM, and CSCM-NXC, were analyzed using a LCMS-9030. Only one amino acid was detected in the ECM. However, multiple amino acids, both of essential and non-essential amino acids, were found in MBM. This result indicated that more amino acids are necessary for blastocyst stage embryo. Since CSCM-NXC media is used for the entire duration of embryo development, numerous amino acids were detected in as expected. Other than amino acids and sugars, an antioxidant (Citrate or citric acid, m/z 191.0197) were detected in all of three medias. A pH indicator (Phenol Red, m/z 353.0489) were identified in ECM and MBM, but not in CSCM-NXC. All of identified compounds are shown in Table 2.

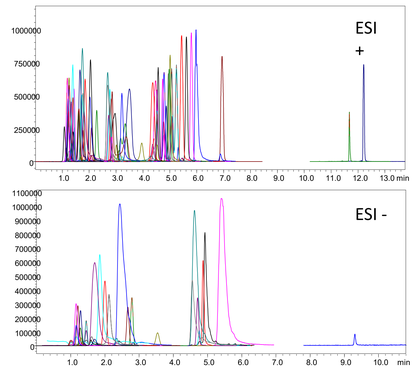

Figure 2 Chromatograms of 96 compounds in neat standard at 5 µM or 10 µM (Positive mode, 68; Negative, 28)

Table 2 Compounds identified in the three human embryo culture media

Type	ECM	MBM	CSCM-NXC
Energy Substrates	Glucose; Pyruvic acid; Lactic Acid;	Glucose; Pyruvic acid; Lactic Acid;	Glucose; Sodium Lactate; Sodium Pyruvate;
Amino Acids	Taurine	Alanine; Arginine; Asparagine; Aspartic Acid; Glutamic Acid; Glycine; Histidine; Isoleucine; Leucine; Lysine; Methionine; Phenylalanine; Proline; Serine; Taurine; Threonine; Tryptophan; Tyrosine; Valine	Alanine; Arginine; Asparagine; Aspartic Acid; Cystine; Glutamic Acid; Glycine; Histidine; Isoleucine; Leucine; Lysine; Methionine; Phenylalanine; Proline; Serine; Threonine; Tryptophan; Tyrosine; Valine
Other	Sodium Citrate; Phenol Red; Alanyl-glutamine	Sodium Citrate; Phenol Red; Alanyl-glutamine	Sodium Citrate; Alanyl-glutamine

In figure 3, the existence of isomers is evidently shown the chromatograms of CSCM-NXC. Some amino acids, such as leucine and isoleucine, are not distinguishable based on accurate mass because they have the same molecular formula. However, the constitutional isomers with different chemical structures can be differentiated according to their MS/MS spectra. Figure 4 shows the library searching results of isoleucine and leucine. The two isomers can be easily identified using a MS/MS library.

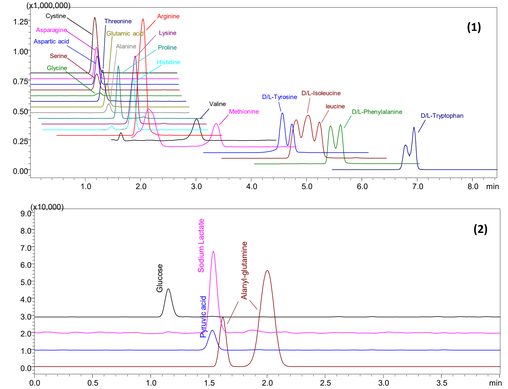

Figure 3 Chromatograms of CSCM-NXC. (1): Positive; (2): Negative

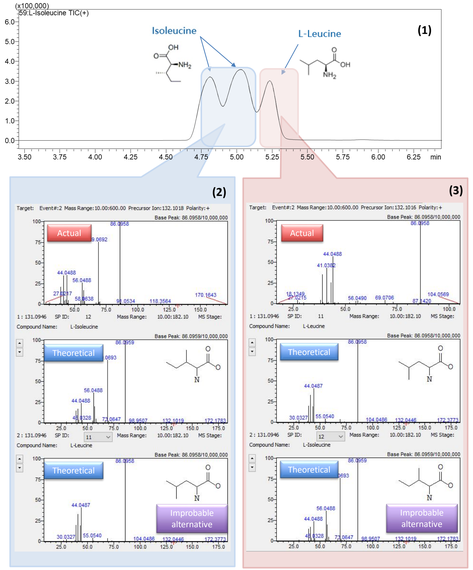

Figure 4 Chromatograms of isoleucine and leucine (1) and library searching results of isoleucine (2) and leucine (3)

5. Conclusions

An identification method for organic compounds in human embryo culture media was developed using a Shimadzu LCMS-9030. The energy substrates, amino acids, antioxidant and pH indicator in 3 types of medias were identified using accurate mass and MS/MS library. Significant differences in composition were observed in different media. The MS/MS library was beneficial in identifying isomers.

6. Reference

Biggers JD. Fundamentals of the design of culture media that support human preimplantation development. In: Van Blerkom J, ed. Essential IVF. Norell, MA: Kluwer Academic Press 2003; 291-332.