A Complex Mixture Where Quantities Really Count

Cell culture media is widely used in the production of biologics, biosimilars and microbial metabolites. As the development of biopharmaceuticals increases in the pharmaceutical industry, there is growing demand for the assessment of culture media for composition. Typical media contain a defined mixture of nutrients, the majority of which are small molecules. Precise control of the nutrient composition over time is critical for optimal cell culture performance and quality of the resulting product. Higher-order structure of biologics, critical to efficacy, may be jeopardized with changes in cell culture media.

NMR spectroscopy is well suited to the evaluation and monitoring of nutrient mixtures because of its unique characteristics of being quantitative, non-destructive and requiring only minimal sample preparation. The composition of culture media can be automatically analyzed for key components using AssureNMR™.

**Dulbecco’s Modified Eagle Media (DMEM)**

Fig. 1 1H NMR spectrum (800MHz) of Dulbecco’s Modified Eagle Media (DMEM) AT0683 containing 4.5 g/L glucose and L-glutamine. This media also contains a selection of amino acids, vitamins and inorganic salts.

Generating a Custom Method

Bruker’s AssureNMR is used to generate custom methods for automated component analysis. These methods can be designed for pure samples, such as APIs or reference standards, or for mixtures, such as culture media. A custom method for DMEM was generated using spectral database (SBASE) BBIOREFCODE.

Automated Quantification Results

Quantification of the signals may be performed using external or internal standards. External quantification standards are automatically acquired within the AssureNMR workflow and the information is recorded for use with AssureNMR in automation or with TopSpin. For an internal standard, the reference region and number of atoms are assigned in the AssureNMR method. Quantification and identification reporting can be customized to accommodate site specific needs.

Monitoring Nutrient Utilization and Product Formation

Preliminary tests indicate the AssureNMR method generated may be used to monitor nutrient utilization. For example, spectra acquired 3 hours after yeast was added to DMEM were analyzed by AssureNMR and identified the concentration of total D-glucose to decrease to 317 mg/L from an initial value of 4,450 mg/L in Figure 3. In this spectrum, ethanol and acetic acid, not originating in DMEM, were observed and quantified. Additionally, an unidentified intermediate was observed. The unidentified intermediate decreased over the course of the preliminary experiment (18 hours).

Summary

NMR provides a comprehensive evaluation of organic components in cell culture media. Results may be used for quality control assessment and studies of cell culture media optimization.

AssureNMR provides tools for generating customized analysis of complex mixtures, such as cell culture media.

Components are quantified in full automation using AssureNMR using external or internal references.

SBASEs populate AssureNMR methods for analysis of quantity and identity of components.

Preliminary results show that AssureNMR may be useful for monitoring nutrient utilization.