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QUALITY CONTROL OF RESIDENT STEM CELL BY HIGH PRESSURE LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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In 2017 the Cell Factory, the first pharmaceutical industrial unit in Piedmont, received the AIFA (Agenzia Italiana del Farmaco) authorization to prepare adult stem cells for cell and gene therapies purposes.
Cell and tissue production must be done following the GMP (Good Manufacturing Practices) and GLP (Good Laboratory Practices) rules, exactly as for drug development. This is because the cell culture products have been recognized from European Union as drugs.
In particular, the cell factory produces resident stem cell for cell therapy in adult patients affected by acute liver failure (ALF).
The cells were cultured in presence of fetal bovine serum (FBS) and some cytokines, and a quality control is required to exclude the presence of anti-inflammatory drugs, such as ketoprofen, and pro-inflammatory agents, such as cytokines, a family of small proteins involved in immune system response.
The aim of the research was to develop analytical methods based on high pressure liquid chromatography (HPLC) and mass spectrometry (MS) to identify and quantify impurities traces in stem cells produced by the Cell Factory.
It was well documented that MS is one of the best tool to investigate and quantify presence (amount) of molecules in biological samples [1]. In particular, tandem mass spectrometry (tandem MS) is recommended to quantify small molecules [2]; instead, high resolution (HR) MS, such as the Orbitrap technology, is endorsed for intact proteins and peptide quali and quantification [3].
We started the quality control process with the quantification of ketoprofen in stem cells with ultra (U)HPLC (Nexera, Shimadzu) coupled through an ESI source to a tandem MS triple quadrupole (QTRAP5500, Sciex). Samples were spiked with ketoprofen d3 (internal standard) and combined with acetonitrile to allow protein precipitation. Sample were vortex mixed and centrifuged. Clear supernatants were placed into a vial and analyzed by UHPLC tandem MS.
To quantify ketoprofen in samples, three calibration curves were prepared. A solution of water/acetonitrile 1/1, FBS (fetal bovine serum) and growth medium were used respectively as matrix/solvent and compared in the study of matrix effect. The matrices were spiked with known concentration of ketoprofen and ketoprofen d3 (fixed). The column was a Kinetex sub 2 µm particle size (Phenomenex) and the elution was obtained used 0.1% formic acid in water and acetonitrile with a flow of 400 µL min^{-1}. The quantification of ketoprofen with tandem MS was done in MRM (multi reactions monitoring) following the transitions

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253>209 *m/z* and 256>212 *m/z* for ketoprofen and ketoprofen d3 respectively. The obtained LLOQs (lower limits of quantitation) were 0.1 µg/L for calibration curve in solvent, 7.5 µg/L both for calibration curve in FBS and in growth medium. The analyzed samples did not show the presence of ketoprofen over LLOQ. No signal for the analyte molecule was detectable.
To control the presence of pro-inflammatory agents, cytokines in this case, we moved to a HRMS instrument (Orbitrap Fusion, Thermo Scientific) with a nanoHPLC (Ultimate 3000, Thermo Scientific) for the chromatographic separation. We evaluated two cytokines: the epidermal growth factor (EGF, 54 amino acids) and the fibroblast growth factor 2 (FGF2, 146 amino acids) with molecular weights of 6348.8 Da and 16397.4 Da respectively. Biological samples (growth medium with and without fetal bovine serum and stem cells) were analyzed both with top down and bottom up approaches. Samples preparation was done following various purification steps, and for bottom up analysis we used trypsin as enzymatic substrate for protein digestion. A PepMap RSLC C18, 2 µm, 100 Å, 75 µm × 50 cm and a PepMap C18, 5 µm × 5 mm, 100 Å. (both from Thermo Scientific) were used as separation and preconcentration columns. Eluents were 0.1% formic acid in water and in acetonitrile (flow 300 nL min^{-1}) for RSLC column, and 0.05% trifluoroacetic acid in water:acetonitrile 8:2 (flow 5 µL min^{-1}) for the preconcentration one. Full mass spectra were acquired with 500 k of resolution in a *m/z* range from 300 to 2000; data dependent analysis were picked up with 60 k of resolution in HCD (high collision dissociation) as activation type.
The calibration curves were obtained in 5mM bicarbonate buffer and in growth medium, with and without fetal bovine serum. The LLOQ was 10 µg/L of EGF and FGF2 in all matrices.
Also in this case, the analyzed samples did not show the presence of cytokines over LLOQ. No signal for the compounds was detectable.
In conclusion, the developed methods based on high pressure liquid chromatography and mass spectrometry were suitable to detect traces of compounds defined as impurities for stem cells.

References

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