

LC-MS OF KNOWN AND UNKNOWN PROSTAGLANDINS

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Human mesenchymal stem cells (hMSC) are multipotent cells found in human bone marrow. When hMSCs are co-cultivated with human T lymphocytes, they produce and secrete immunosuppressive factors that inhibit T-cell activation and effector functions. Thus, hMSC have been used therapeutically to prevent the rejection of allogeneic bone marrow transplants. We have found that hMSC in culture release prostaglandin E₂ (PGE₂) constitutively. PGE₂ is known to have immunosuppressive properties. However, these cells most likely also produce other prostanoids that may add to the effect of PGE₂.

Our aim was to develop a selective and sensitive method based upon large volume injection capillary LC-MS for determination of prostaglandins in cell culture supernatants and use this method to analyze cell culture supernatants.

Cells were grown in modified (without phenol red) RPMI 1640 (Roswell Park Memorial Institute medium) supplemented with 10% fetal calf serum. In the present method 250 µl cell culture supernatant were diluted four times, filtered, and 500 µl were analyzed using an on-line sample preparation using strong cation exchange (SCX) – reversed phase (RP) capillary liquid chromatographic (cLC) method with ion-trap mass spectrometric (IT-MS/MS) detection. Deuterated PGD₂ (PGD₂-d₄) and PGF_{2a} (PGF_{2a}-d₄) were used as internal standards. The method was used to analyze the prostaglandin production profile of hMSC-TERT cells during differentiation. In addition to the PGs included in the LC-MS method, an unknown was observed in some of the samples. Characterization of the unknown by LC-MS will be discussed.