Title: **Separation and characterization of microRNAs by capillary electrophoresis with ultraviolet detection and mass spectrometry.**

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MicroRNAs (miRNA) are a class of small endogenous RNA molecules of about 19-23 nucleotides that act as regulators of genes. Aberrant expression of these oligonucleotides has been associated with a plethora of diseases ranging from nervous system diseases, heart diseases, inherited diseases and different types of cancer. The increasing association between miRNA malfunction and diseases coupled with the advances in detection and characterization of these kinds of analytes have turned miRNAs into an interesting topic of study, especially within the molecular biology field. Due to their stability towards pH, temperature and other factors, applications of microRNAs in forensic studies have also been studied.

In this work we have optimized a capillary electrophoresis with ultraviolet detection method (CE-UV) that allows separating a mixture of synthetic miRNAs with similar nucleotide sequence and length. Compatibility with on-line mass spectrometry detection was also considered during the optimization. The method was validated in terms of repeatability of migration times and peak heights, linearity and limits of detection (LOD).

For characterization of miRNAs matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was used, where mass accuracy and other quality parameters were evaluated and compared between different modes. Better LOD than the CE-UV method and good mass accuracy were achieved using one of the miRNAs as an internal standard for the calibration, though loss of repeatability of peak area and height was observed, making this method unsuitable for quantitative analysis. Sequencing of microRNAs with the tandem mass spectrometry (MS/MS) mode was discarded due to instrument limitations.

Keywords: Capillary electrophoresis, MALDI-TOF-MS, Mass spectrometry, MicroRNA, Optimization, Separation.