Abstract of the presentation:

SEMIPREPARATIVE ISOELECTRIC FOCUSING OF AMPHOLYTES BY CARRIER AMPHOLYTE-FREE ISOELECTRIC FOCUSING (CAF-IEF) ON EA101 INSTRUMENT (Villa Labeco, SR)

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At the start of this presentation I included basic principles of capilary isoelectric focusation as main preparational capillary electromigrating methods of proteomic sample.

Then there are explained main principals CAF-IEF as an alternative to cIEF. In this presentation method CAF-IEF was carried out in commercially available instrument EA 101 (Villa Labeco, Slovakia), which was equipped with especially made column to allow dosing of larger samples (up to 0.5ml).

Continual mode [1] has been designed as an addition to CAF-IEF, which doesn't require change of electrolyte with the interruption of current. In this mode focusation of low-molecular pI marker (PIM 7.4) was carried out [2].

After that semipreparation and also pre-concentration of mixture proteins (cytochrome C, myoglobine and insuline) with dosing concentration of cca 0,03 mg/ml and volume 0,5 ml was tested.

In collected fractions there was an increase in concentration of cca 46 times for myoglobine, 45 times for cytochrome C and 4 times for insuline.

This work demonstrated that it is posible to achieve semipreparation with pre-concentration of proteins by CAF-IEF method with utilization of commercial fractionation valve. The advantage of CAF IEF with regards to a use for preconcentration and semipreparation is, that collected fractions of real sample should not contain complicated matrix, which usually interferes with other analysis'.