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Analysis of Carbohydrate-Deficient Transferrin (CDT) as a marker of chronic alcohol abuse. Comparative evaluation of a commercial fully automated multicapillary electropherograph with a single capillary electropherograph.

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INTRUDUCTION AND AIM: Carbohydrate Deficient Transferrin (CDT), is widely recognized as the most reliable marker of chronic alcohol abuse. The analytical methods now available for CDT determination include isoelectric focusing, immunoassays, HPLC and capillary electrophoresis (CE) [1]. Among these techniques, HPLC and CE have been proposed as reference/confirmation techniques, because of their ability to provide precise quantification by direct measurement of the separated peaks. In the recent years CE has become increasingly popular especially for its lower consumption of solvents and sample, its rapidity, ruggedness and minimum need of sample pre-treatment. Quite recently, a commercial instrument has become available in the market, which combines patented chemistry for high rapidity capillary zone electrophoresis (CZE) separation with a multicapillary instrumental configuration (Sebia, Evry, France), which, reportedly, provides high throughput and unmatched productivity in comparison with other separation methods.
The present work has been aimed at a validation of this new commercial system on the basis of the usual analytical chemistry parameters and by comparison with a validated CZE method.

MATERIALS AND METHODS: Multicapillary CZE was performed on a commercially available CE system for routine serum protein analysis (Capillarys™, Sebia, Evry, France), featuring eight uncoated fused silica capillaries operated in parallel and UV detection at 200 nm wavelength. All the reagents were provided in a commercial kit named CAPILLARYS™ CDT assay.
As reference, a validated CE method performed on a single capillary electropherograph (P/ACE MDQ, Beckman Coulter, Fullerton, CA, USA) fitted with an uncoated fused-silica capillary was used. The separation was carried out in 120 mM borate buffer adjusted to pH 8.0 containing 6 mM DAB under 20 kV at 30°C. Detection was by UV absorption at 200 nm wavelength. Serum pre-treatment included iron saturation by dilution 1:4 in 0.5 mM $FeCl_3$ aqueous solution [2]. The analyzed serum samples had been collected from 140 subjects randomly chosen in the general population undergoing clinical chemistry analyses for clinical purposes with %CDT values ranging from 0.5% to 8.4%.

RESULTS AND DISCUSSION: The analysis of Tf isoforms provided by the CAPILLARYS™ CDT assay showed all the relevant glycoforms separated from each other and clearly detectable, even in sera with low levels of total transferrin. The efficiency of the separation, calculated on the disialo-Tf peak, was about 15,000 theoretical plates/column. The resolution factors (R) calculated in the between adjacent peaks were: 1.23 for the peak couple disialo-Tf and trisialo-Tf, 1.67 for the couple trisialo-Tf and tetrasialo-Tf and 1.09 for the couple tetrasialo-Tf and pentasialo-Tf.

The analytical precision, evaluated by repeating 7 times the injection of the same serum sample in each of the seven capillaries, was acceptable, being the mean variation coefficients (CV) lower than 10% in both intra-run and between-run experiments.
Lacking a certified standard of CDT, the accuracy of the method was verified by comparison with a validated single capillary CZE method. The comparison of results from 140 serum samples analysed with the Sebia Capillarys method and with the reference CZE method showed a highly significant correlation, described by the following equation: $y = 0.981x + 0.2858$ ($R^2 = 0.9523$) (x: Sebia data; y: reference CE data).

On the basis of the above considerations and taking into account that the throughput of the instrument is 38 samples/hour, whereas that of the reference CZE method is about 4 samples/hour, it can be concluded that the multicapillary array configuration provides the highest productivity in CDT instrumental analysis with good analytical performances including reproducibility.

REFERENCES:

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