An inter-laboratory study to extend the scope of the CEN biotin method by HPLC with post-column derivatization and fluorimetric detection

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Introduction

The existing European biotin method, EN 15607 (2009) was normalized on the basis of a French inter-laboratory study organized in 2000 in accordance with ISO 5725-2. The precision data were established thanks to different samples : breakfast cereals – infant milk powder – nutritive orange juice – green peas with ham (baby food) – lyophilized chicken soup. The biotin contents varied from 15 µg/100g to 200 µg/100g. This method is widely used in Europe for the control of food labeling and health claims.

However, a lot of products available on the market with a nutritional labeling may show a lower content of biotin. It is also the case of many infant formula and adult nutritional products.
Our objective was to study the possibility to extend the scope of the existing method to lower contents with the aim to reach the LOQ fixed within the SPIFAN Biotin SMPR.

Materials and methods

A collaborative study was recently performed with three laboratories to re-evaluate the performance of the CEN method.
The samples used for this study included a certified reference material SRM1849a and 4 samples with a low biotin content ranged from 1.2 to 13 µg/100g (infant formula powder and liquid – adult nutritional formula).
One laboratory has also fulfilled an in-house validation study with a six points calibration curve from 0,0015 µg/ml to 0,150 µg/ml.

D-biotin is extracted from food after an enzymatic treatment and quantified by HPLC with post-column binding reaction.
The complexion of d-biotin with avidin appears to be very specific. On that account, this protein, covalently bound to a fluorescent marker, fluorescein 5-isothiocyanate, can be used as a reagent for a post-column binding of d-biotin.

Results

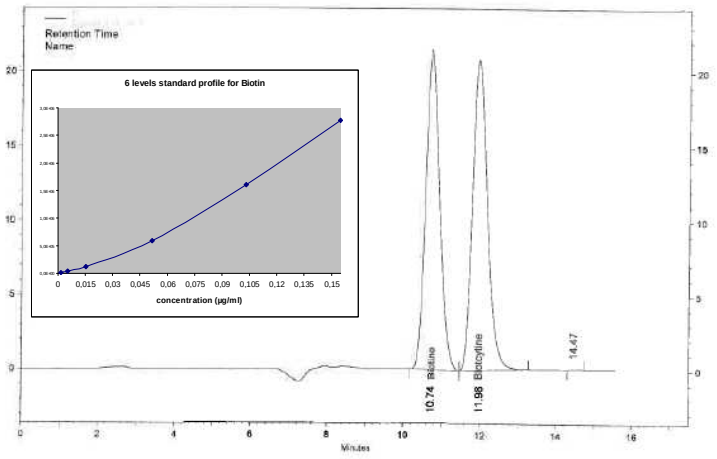

Figure 1 : Fluorimetric chromatogram of a 0,15 µg/ml standard mixture of biotin and biocytin, a minoritary form of B8 vitamin. These two compounds are correctly separated. Standard profile of biotin shows that the calibration curve follows a quadratic model : $y = ax^2 + bx$ between 0,0015 and 0,15 µg/ml.

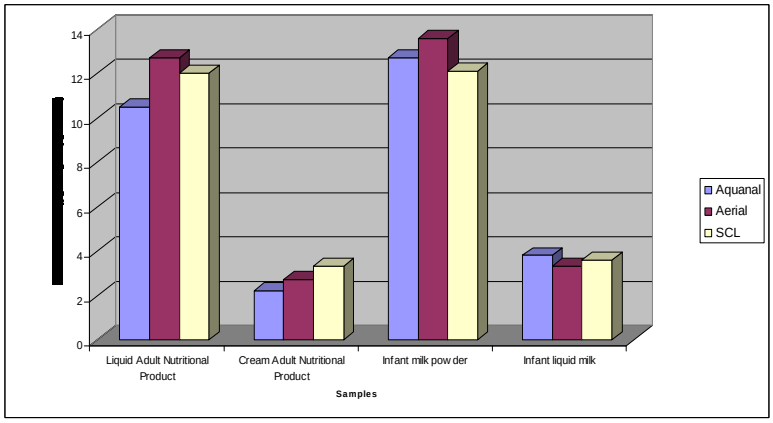

Figure 3 : Collaborative results on low biotin content samples obtained with three independent triplicate analysis (27 results for each matrix). Comparable results are obtained between the three laboratories.

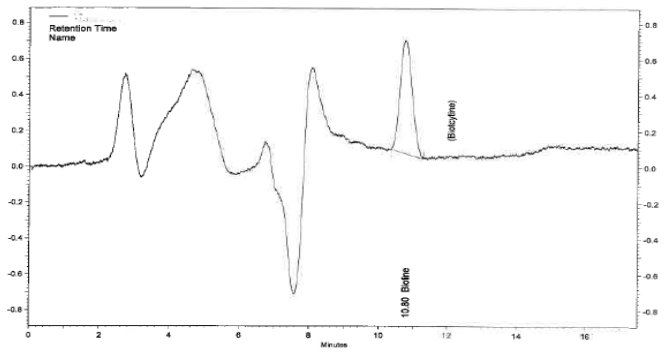

Figure 2 : Fluorimetric chromatogram of a cream adult nutritional product obtained using a Kinetex C18 column 100 x 4,6mm (2,6µm). A very low biotin content can be reached with this method : 0,008 µg/ml in the extract equivalent to 3 µg/100ml in the sample (see peak 1 of biotin)

Method validation :

The laboratory of Strasbourg[3] fulfilled the validation of the method according to NF V03-110 (2010), adapted by the French government laboratories, from 0,0015 µg/ml to 0,150 µg/ml. The main figures are as below :

Recovery :	95 to 105 % on all the studied matrices
Limit of Quantitation (LOQ) :	0,0015 µg/ml for an injected extract (0,1µg/100g for a reconstituted infant milk powder)
Repeatability (RSDr) :	5 % (for the high and medium biotin contents) 9 % (for the lowest biotin contents)
Uncertainty :	± 25 %

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Conclusion

The method reaches the main requirements of the SPIFAN Biotin SMPR for Infant formula and Adult / Pediatric formula.
With the extension of the scope, this method can be used on a very large range of matrices with different d-biotin contents.
The method is also able to quantify the d-biocytin content which is useful because d-biocytin has a vitamin activity and can be found in some samples.