Pantothenic acid in infant formula and adult/pediatric nutritionals: comparison between AOAC Official Method 2012.16 and a liquid chromatography method with post-column reaction

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INTRODUCTION

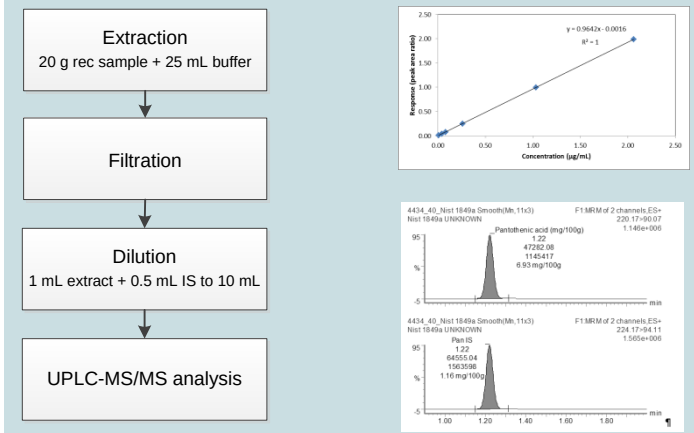
The determination of pantothenic acid in foodstuffs is usually accomplished by microbiological assay (MBA) based on the growth of *Lactobacillus plantarum*. Although very sensitive, the specificity of the MBA remains limited when applied to complex food matrices. In addition, this type methods are time consuming and generally exhibit poor precision. Indirect ELISA or radioimmunoassay methods have also been developed and applied to the quantification of pantothenic acid in milk and infant formulas with adequate sensitivity, but the lack of chromophore makes the detection quite unspecific, which could impair reliable quantification in more complex matrices (such as highly hydrolyzed hypoallergenic formulas).

Pakin et al.(I) reported LC separation with fluorescence detection after post-column derivatization, which is currently used by several European labs, mainly in France, for labeling and compliance demonstration. Highly selective MS/MS detection has also been proposed as reported by Rychlick(II). A method validated in fortified foods combining rapid sample preparation and specific mass spectrometry analysis was developed by Andrieux et al. (III). It has been proposed and accepted as First Action AOAC Official Method **2012.16**(IV) for the analysis of pantothenic acid in infant formula and adult/pediatric nutritionals.

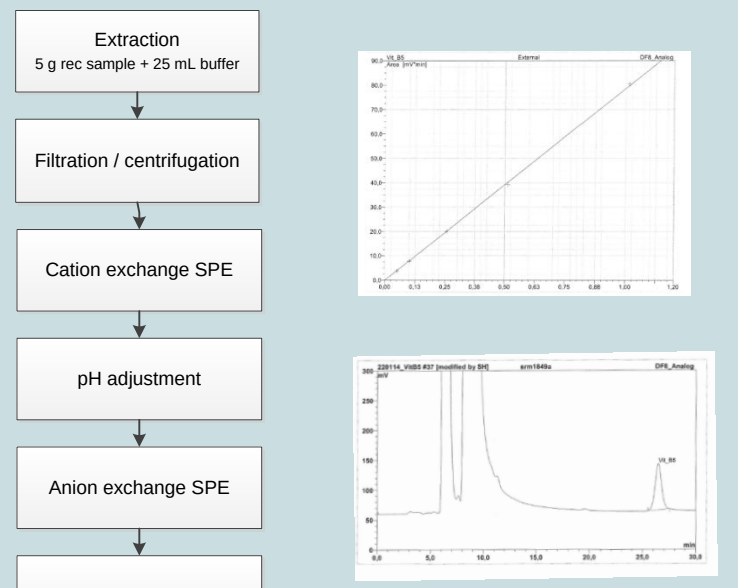
The use of Official Methods is required in many countries for compliance demonstration. In this respect it is important to know how results provided by methods currently used compare to the official ones, and the possible impact on compliance. The present work reports the comparison between AOAC Official Method **2012.16** and the liquid chromatography method used in several European laboratories(iv) for the analysis of pantothenic acid in infant formula and adult/pediatric nutritionals..

METHODS

Method 1: AOAC Official Method 2012.16



Method 2: LC-FLD with post-column reaction(I)



METHOD COMPARISON

The comparison was accomplished by replicate analysis of 13 samples (including a Standard Reference Material with certified value for pantothenic acid) representing most of the products within the category present in the marketplace. Duplicate analysis on different days were performed in each of the two laboratories participating to the comparison.

RESULTS

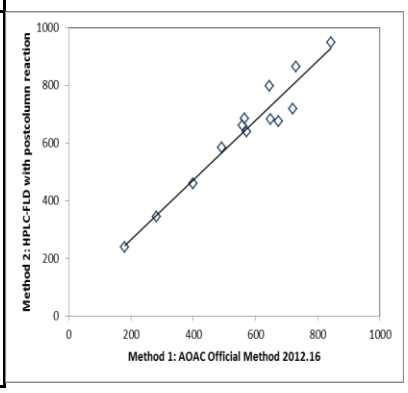
All results were averaged and the final result reported as µg/100 g of pantothenic acid in reconstituted final product. Reconstitution rate was 25 g of powder into 200 g of water.

Results on Standard Reference Material (SRM 1849a Infant/Adult Nutritional Formula) were equivalent for both methods and not statistically different from certified value (682 ± 19 µg/100 g).

The comparison between average results in all different matrices is shown below. Although both methods compare well when performing linear regression analysis (slope not different from 1 and intercept not different from 0, r^2 = 0.94) a systematic bias was detected (p-value 0.004) corresponding, in average, to about 10-15 % of the pantothenic acid content in a regular infant formula (70 µg/100 g).

	Pantothenic acid (µg/100g)	
Sample description	Method 1 AOAC 2012.16	Method 2 HPLC-FLD
SRM 1849a (Infant/Adult Nutritional Formula)	719	719
Adult Nutritional Powder Milk Protein Based	281	345
Child Formula Powder	644	798
Follow-up Formula Powder Milk Based	648	682
Infant Elemental Powder	730	864
Infant Formula Powder Milk Based 1	492	584
Infant Formula Powder Milk Based with probiotics	571	641
Infant Formula Powder Partially Hydrolyzed Milk Based 1	399	461
Infant Formula Powder Partially Hydrolyzed Milk Based 2	842	950
Infant Formula Powder Soy Based	564	686
Infant Formula Powder Whey Predominant	674	675
Infant Formula RTF Milk Based	558	661
Infant Formula RTF Milk Based - SPIFAN Blank Milk	180	238

*All results reported in µg/100 g of reconstituted or "ready-to-feed" product



CONCLUSION

Two different methods for the analysis of pantothenic acid in infant formula and adult/pediatric nutritionals have been compared. Method 1 was the AOAC Official Method 2012.16, which is based on buffer extraction and UPLC-MS/MS analysis. Method 2 was the method published by Pakin et al.(I), based on buffer extraction followed by successive anion and cation exchange clean-up steps.

The two methods provide comparable results in terms of accuracy. A slight systematic bias was observed, with slightly higher results obtained using method 2 (around 10-15 %); this difference will, in principle, not compromise compliance when used for the analysis of infant formula and adult/pediatric nutritionals.

The two methods can both be used for the analysis of pantothenic acid infant formulas and adult/pediatric nutritionals for compliance demonstration.

References
I. Pakin, C., Bergaentzlé, M., Hubscher, V., Aoudé-Werner, D., & Hasselmann, C. (2004) *J. Chromatogr. A* 1035, 87–95
II. Rychlik, M. (2003) *Analyst* 128, 3–8
III. Andrieux, P., Fontannaz, P., Kilinc, T., Campos Giménez, E. (2012), *J. AOAC Int*., 95, 143-148
IV. Official Methods of Analysis (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method 2012.16

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