

VITAMIN B12 IN INFANT FORMULAS AND NUTRITIONAL PRODUCTS: COMPARISON BETWEEN ISO 20634 AND LC/UV DETECTION METHOD USING IMMUNOAFFINITY EXTRACTION.

I. Malaviolo, MF. Dubos, M. Delcamp, D. Guillonneau.



151 bis avenue Jean Jaurès - 33600 Pessac (FRANCE) - www.aquanal.fr

INTRODUCTION

Determination of vitamin B12 in food products by LC/UV detection using an immunoaffinity extraction step is a fast and simple method largely used. There are two approved methods within AOAC for infant formula and adult nutritionals according this procedure IAC [1st action - AOAC 2011.08 (1) and AOAC 2011.09 (2)].

Recently, ISO has released a new analytical method developed by the SPIFAN project and the Codex committee on methods of analysis and sampling (CCMAS) has endorsed the method as Type II.

The method described in the International Standard is equivalent to AOAC 2011.10 (3).

This new international standard ISO 20634:2015 (4) utilizes C8 or C18 SPE cartridges to concentrate and clean up sample extracts, size-exclusion and reversed-phase chromatography (column switching) to separate vitamin B12.

In both procedures (IAC or SPE) vitamin B12 is extracted from products with a sodium acetate buffer in the presence of sodium or potassium cyanide.

The single supplier for the IAC cartridges proposes 2 references which are codes P80 and P88.

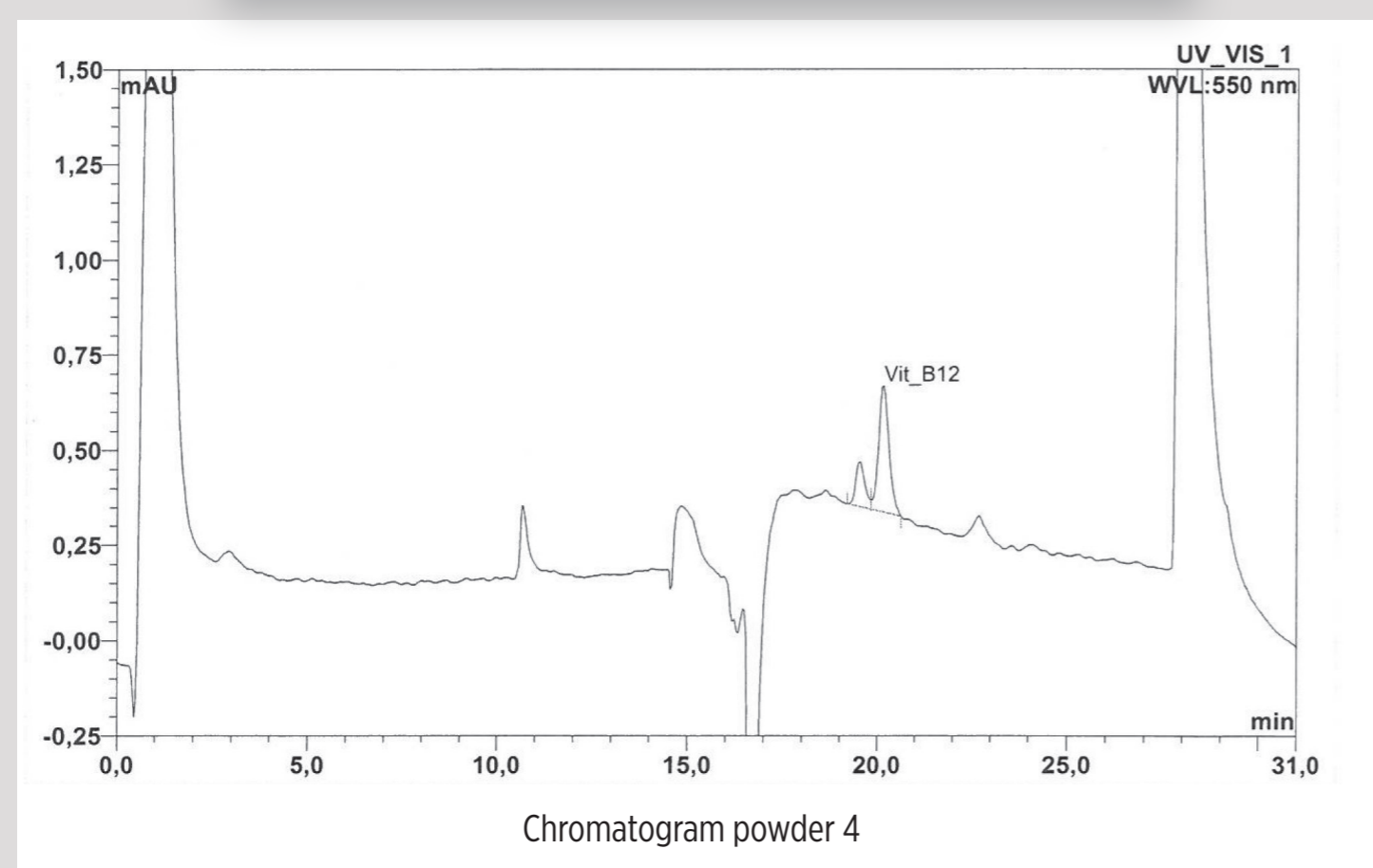
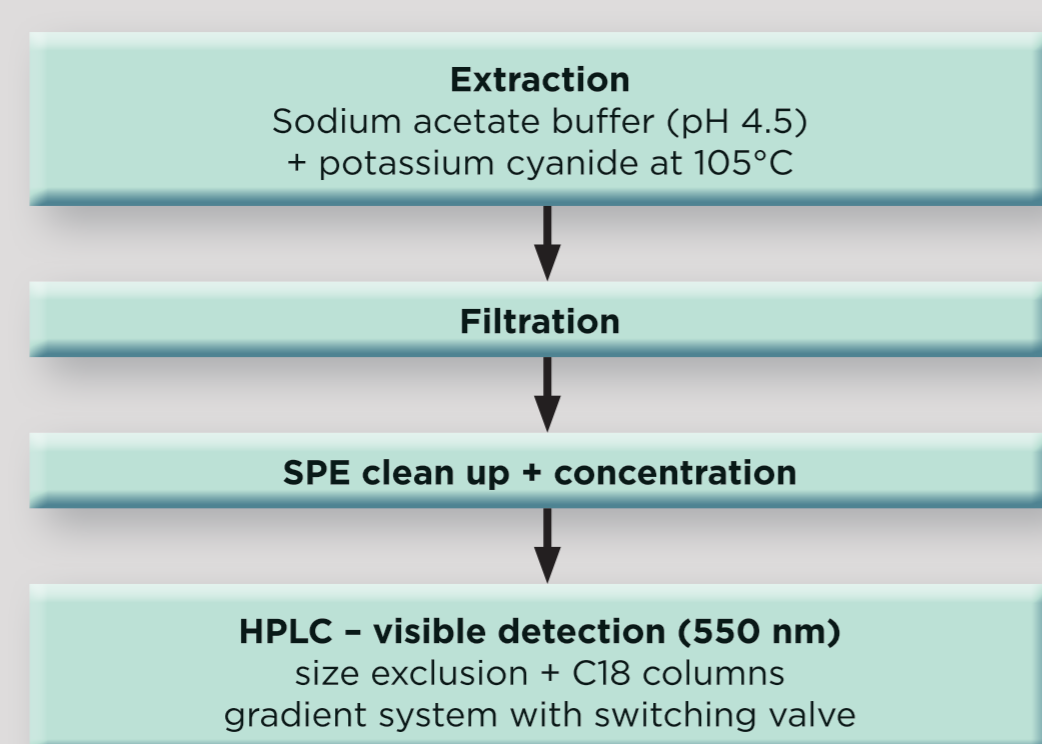
The bed volume of the antibody/gel mixture is the same for both formats but IAC P88 has a built-in reservoir that can accommodate 9 ml sample extract.

In cases of dispute and for calibration purposes the reference method should be recommended and it is important to know how results provided by methods currently used compare to the official one.

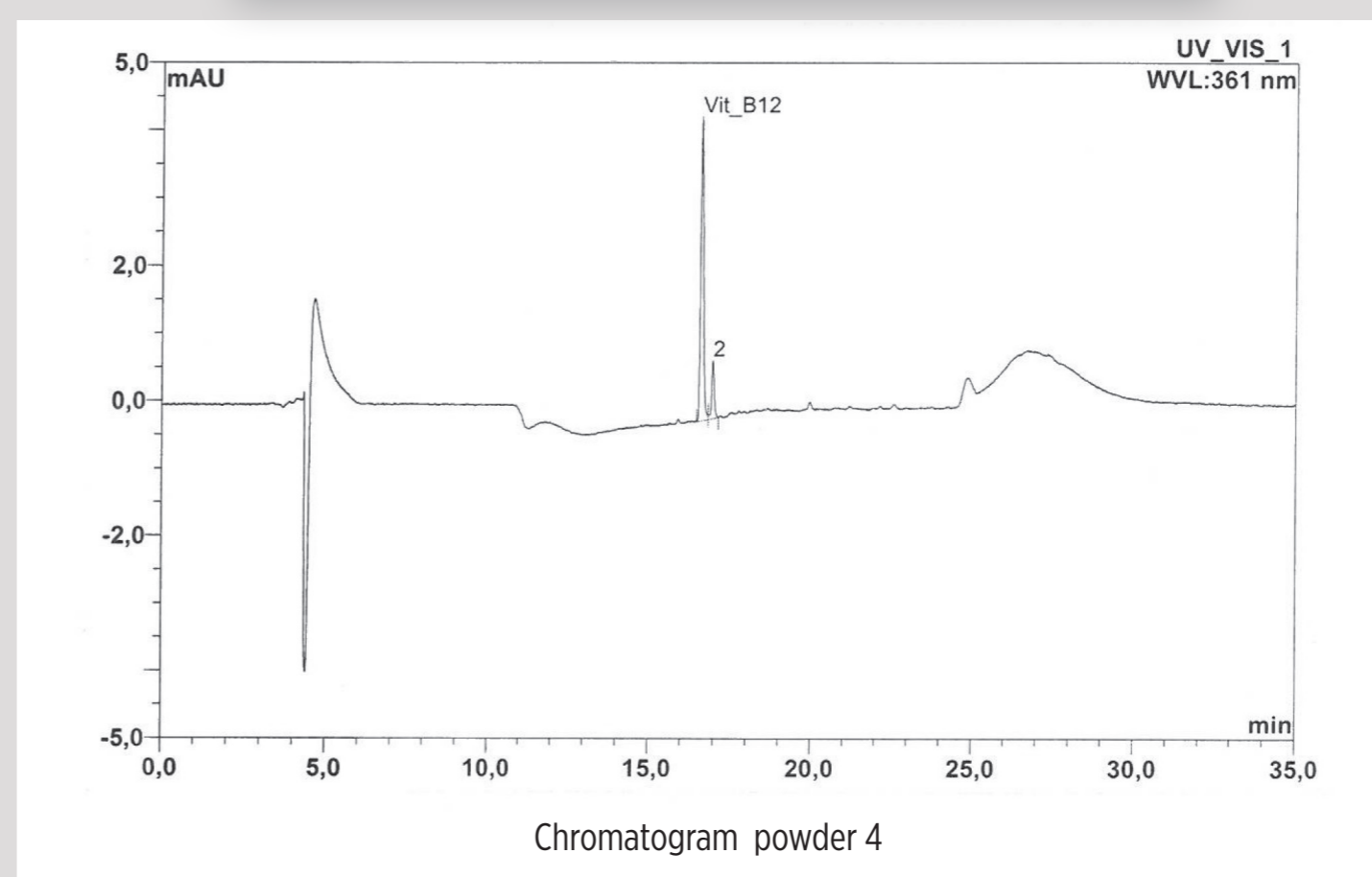
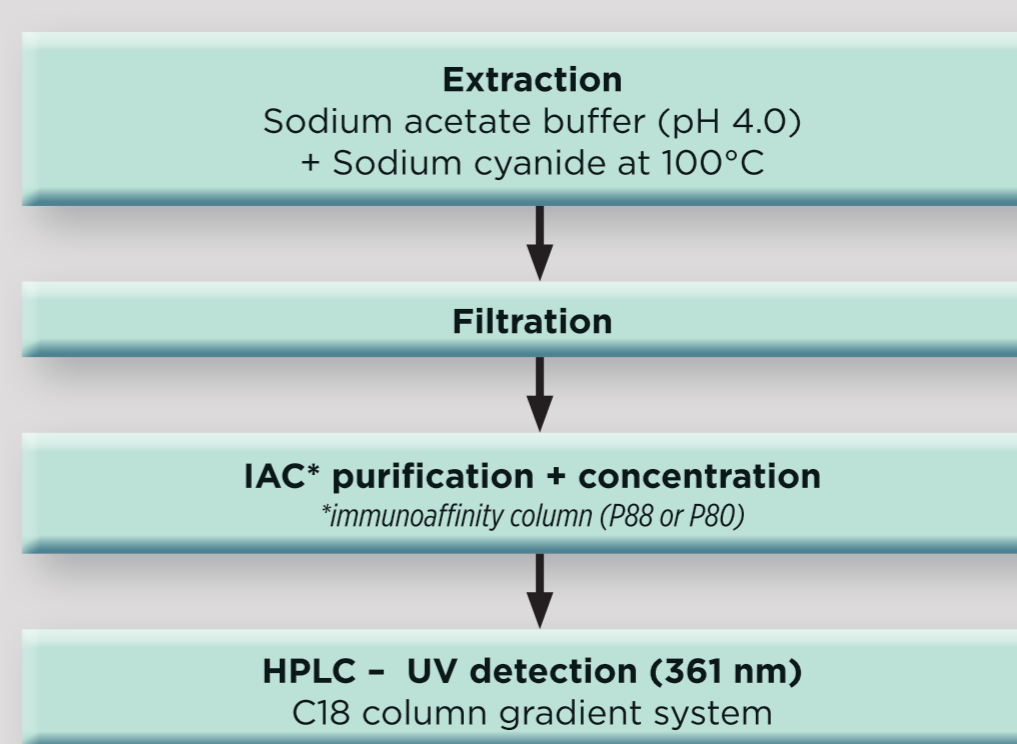
The present work reports the comparison between ISO 20634 and LC/UV detection with Immunoaffinity Extraction (according AOAC 2011.08) using both IACs references available. The comparison has been accomplished by single analysis of 11 samples (including a SRM) representing most of the products in the marketplace of infant formula and adult/pediatric nutritionals.

METHODS

Method 1 : SPE procedure - ISO 20634



Methods 2 and 3: IAC procedure (P80 or P88)

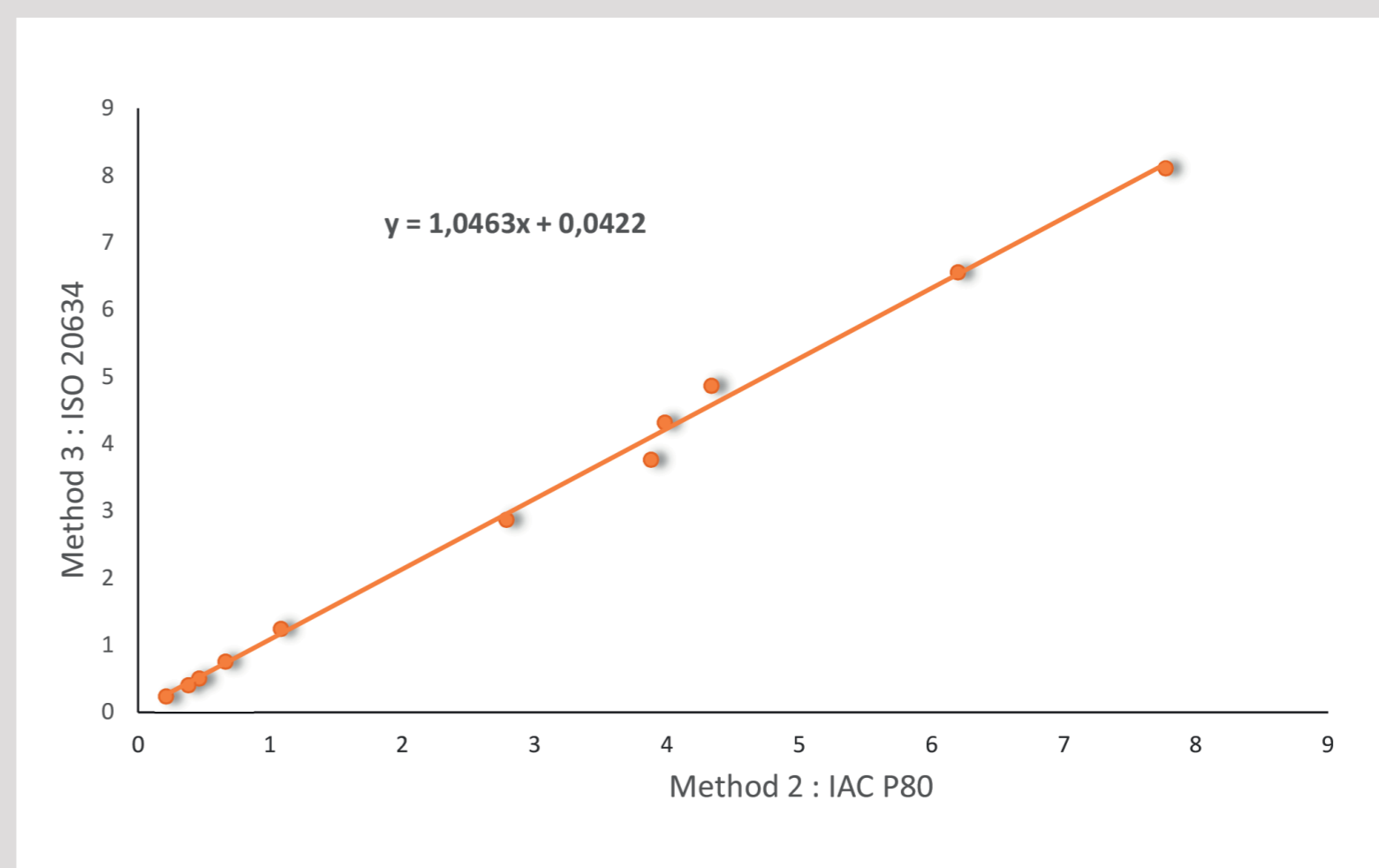
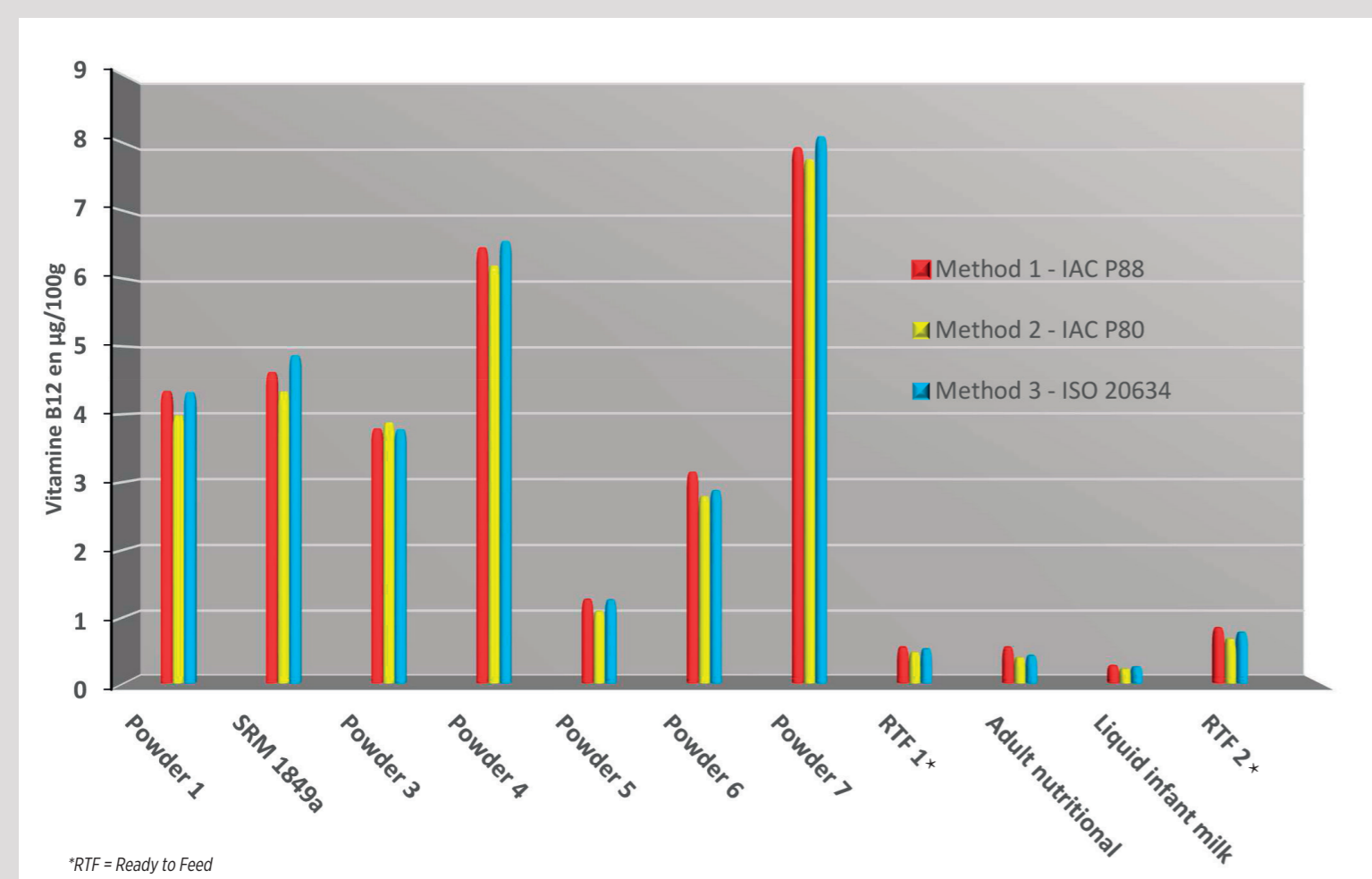


METHODS COMPARISON & RESULTS

The comparison between results in all different matrices obtained with the three methods is shown below.

Comparable results were obtained with the three methods. Results on Standard Reference Material (SRM 1849a Infant/Adult Nutritional Formula) were equivalent and not statistically different from referenced value ($4.82 \pm 0.85 \mu\text{g}/100 \text{g}$).

Although both procedures (IAC and SPE) compare well when performing linear regression analysis.



CONCLUSION

Two methods with different extract sample processing (IAC and SPE) for vitamin B12 analysis by LC in infant formula and adult/pediatric nutritionals have been compared. Moreover, the both references of IACs cartridges available have been tested.

Both procedures, ISO 20634 (SPE) and method using immunoaffinity extraction (whatever IAC reference used) provide comparable results.

REFERENCES:

- (1) AOAC 1st action 2011.08 - Vitamin B12 in Infant Formula and Adult Nutritionals - Liquid Chromatography/UV Detection with Immunoaffinity Extraction. Campos-Giménez *et al.* : J. AOAC International Vol. 95, N° 2, 2012
- (2) AOAC 1st action 2011.09 - Vitamin B12 in Infant Formula and Adult Nutritionals - HPLC After Purification on an Immunoaffinity Column. Kirchner *et al.* : J. AOAC International Vol. 95, N° 4, 2012
- (3) AOAC 1st action 2011.10 - Vitamin B12 in Infant Formula and Adult Nutritionals - HPLC. Schimpf *et al.* : J. AOAC International Vol. 95, N° 2, 2012
- (4) ISO 20634:2015 - Infant formula and adult nutritionals - Determination of vitamin B12 by reversed phase high performance liquid chromatography (RP-HPLC).