FOOD COMPOSITION AND ADDITIVES

Rapid Determination of Thiamine, Riboflavin, Pyridoxine, and Niacinamide in Infant Formulas by Liquid Chromatography

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A simplified, simultaneous determination of vitamins B_1, B_2, B_3, and B_6 in supplemented infant formulas was developed from a single deproteinized sample extract, with analysis by reversed-phase, ion-pair chromatography with an acidified methanol–water mobile phase. The dioctylsulfosuccinate counter-ion facilitates unique retention of the pyridine-based vitamins (niacinamide and pyridoxine) and allows for concurrent measurement of both the pyridoxal and riboflavin 5′-phosphate endogenous components of milk. Other naturally occurring undetected vitamin congeners have minimal analytical significance. UV detection is used for niacinamide, and programmed fluorescence detection is used for riboflavin and the B_6 vitamins. Thiamine is routinely determined sequentially under modified elution conditions.

Vitamin fortification of infant formulas is allowed within defined prophylactic ranges to meet the nutritional requirements of the nonbreastfed infant and is subject to rigorous regulatory control of composition and labeling. The potential multiplicity of supplemental and endogenously occurring forms of individual vitamins present in formula represents significant analytical challenges, both from a compliance and clinical perspective.

Extraction is a critical factor influencing the analytical reliability for a comprehensive estimate of B-vitamin content, and in view of the wide range of protocols used, may significantly contribute to measurement uncertainty. Generally, a combination of acid hydrolysis and enzymatic digestion is used to release endogenous protein-bound and phosphorylated B-group vitamins. As free analytes and in a minimum of molecular forms, they can then be aggregated with supplemental vitamers during subsequent detection protocols. However, the content of infant formulas is declared on the basis of the recovery of vitamins added during manufacture, thereby simplifying the extraction protocols required and

minimizing the individual vitamin forms to be determined. Specifically, infant formula containing vitamins B_1, B_2, B_3, and B_6 is supplemented with thiamine hydrochloride, riboflavin [or occasionally riboflavin mononucleotide (FMN)], niacinamide, and pyridoxine dihydrochloride, respectively, at levels intended to both emulate human breast milk and compensate for processing losses.

Traditional measurement protocols based on microbiological assay are sensitive and provide a single, lactobacilli-specific estimate of biological activity through the combined response of all vitamers present. However, such methods are lengthy, manipulative, and generally of poor precision. Alternative compendial spectrophotometric or fluorimetric chemical methods are well established despite their potential for matrix interferences, while available gas chromatographic techniques (1) have not been generally adopted. The relative attributes of these compendial microbiological and chemical methods for nutritional labeling have been reviewed (2–4).

Rapid, reliable, and convenient procedures are required for routine compliance control and labeling of many fortified foods, and liquid chromatographic (LC) procedures, in general, have superseded alternative methodologies. Many such techniques for individual B-group vitamins in nonfortified and fortified foods are based on rigorous extraction protocols followed by either ion-suppression, ion-interaction, or ion-pair reversed-phase LC. These methods have been comprehensively reviewed in the literature (3–10).

It has been relatively easy to establish simultaneous, multianalyte LC or capillary electrophoresis techniques to determine B-group vitamins in pharmaceuticals and vitamin premixes (11–17). However, complexity of the food matrix and low analyte level restrict the concurrent or simultaneous determination of added vitamins to fewer analytes. Thus, various combinations of thiamine, riboflavin, niacinamide, and pyridoxine have been successfully determined in supplemented foods and such procedures have been comprehensively reviewed (4, 10) or reported (18, 19). Applications to infant formulas have increasingly relied on a simple removal of protein by chemical precipitation, followed by reversed-phase LC with either an alkylsulfonic acid ion-pair reagent (18–20), or ion-suppression following solid-phase cleanup (21).
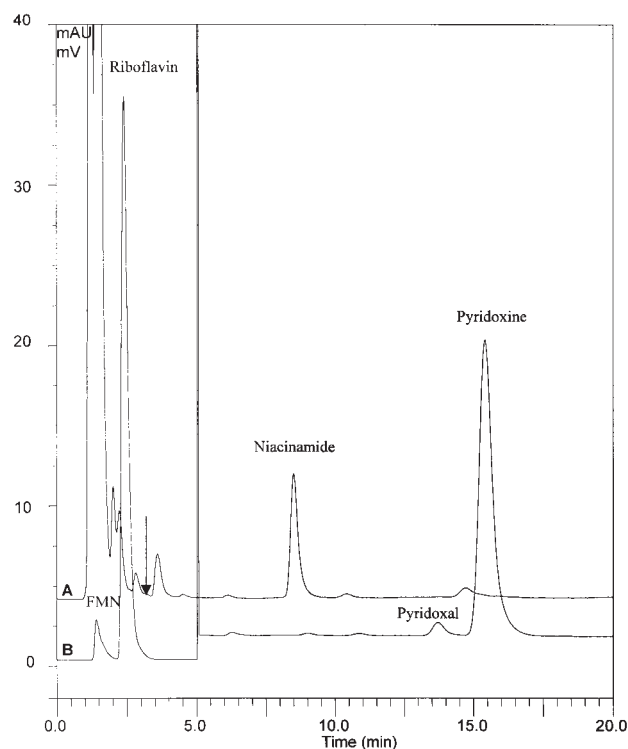
Figure 1. Chromatography of milk-based infant formula extract described for vitamins B$_2$ (riboflavin and FMN), B$_3$ (niacinamide), and B$_6$ (pyridoxal and pyridoxine). (A) UV at 258 nm; (B) programmed fluorescence (0–5 min, λ_{ex}: 450 nm, λ_{em}: 510 nm; 5–20 min, λ_{ex}: 290 nm, λ_{em}: 390 nm). Arrow = elution of niacin.

Although buffered eluents provide adequate selectivity when single vitamins are determined, the simultaneous measurement of the B-group vitamins more frequently demands incorporation of ion-pair modifiers to control selectivity. Although cationic alkylsulfonates have been used almost exclusively in applications to foods, significant benefits have been reported for dioctylsulfosuccinate applied to pharmaceuticals (11). The present study reports the rapid, simultaneous determination of riboflavin, FMN, niacinamide, pyridoxal, and pyridoxine, with consecutive measurement of thiamine in infant formulas with use of dioctylsulfosuccinate as counter-ion under reversed-phase LC conditions.

Experimental

Equipment

LC system.—Consisted of pump, autosampler, and programmable UV and fluorescence detectors in series, controlled by Class-VP software (Shimadzu, Tokyo, Japan). A 5 µm, end-capped, polymeric C$_{18}$ Luna Prodigy ODS (3) column (150 × 4.6 mm, 15.5% carbon content) and guard column (Phenomenex, Torrance, CA) was suitable, although equivalent columns may also be used.

Reagents

All reagents were AR grade unless specified.

(**a**) *Vitamin standards thiamine·HCl, riboflavin, FMN, niacinamide, pyridoxine·HCl, and pyridoxal·HCl.*—USP grade; Sigma (St. Louis, MO). Dried over phosphorus pentoxide (4 h at 85°C) and stored desiccated in the absence of light. Moisture content was also determined by Karl Fisher titration.

(**b**) *Methanol.*—LC grade.

(**c**) *Formic acid.*—99%.

(**d**) *Sodium dioctylsulfosuccinate.*—Sigma.

(**e**) *Potassium hydroxide*

(**f**) *Trichloroacetic acid (TCA)*

(**g**) *TCA extractant (0.6M).*—Prepared by dissolving 98.0 g in 1 L water and storing at 4°C for up to 6 months.

(**h**) *Water.*—≥18MΩ.

Standards

(**a**) *Individual stock vitamin standards.*—Prepared by dissolving 100 mg thiamine·HCl, 10 mg riboflavin, 10 mg FMN, 100 mg niacinamide, 10 mg pyridoxal·HCl, and 10 mg pyridoxine·HCl each in 200 mL water and storing at 4°C for up to 4 weeks, except for FMN and niacinamide, which were prepared daily.

(**b**) *Combined working standard.*—Prepared daily by pipetting 5.0 mL riboflavin, 2.0 mL FMN, 5.0 mL niacinamide, 1.0 mL pyridoxal, and 5.0 mL pyridoxine stock standards into a 100 mL flask, adding ca 40 mL 0.6M TCA
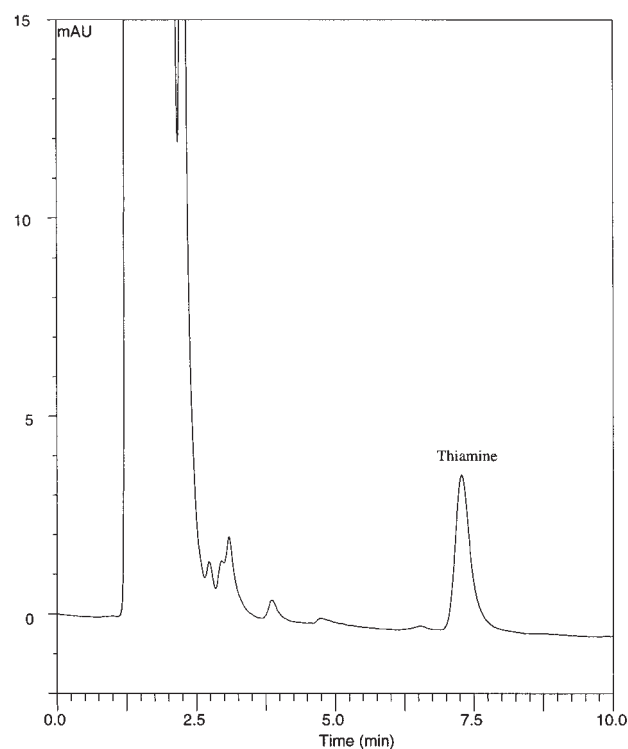
Figure 2. Chromatography of milk-based infant formula extract described for vitamin B$_1$ (thiamine). UV at 254 nm.

Table 1. Calibration and detection limits for vitamins

Vitamin	Range, µg/mL	Linear regression	r	LOD, µg/mL[a]	MDL, mg/100 g[b]
Thiamine	0.5–15.0	$y = 11.294x - 0.009$	0.9999	0.014 (0.72)	0.02
Riboflavin	0.1–3.0	$y = 334.030x - 0.370$	0.9997	0.004 (0.18)	0.04
FMN	0.02–1.0	$y = 492.304x - 0.124$	0.9990	0.002 (0.10)	0.06
Niacinamide	1.0–25.0	$y = 10.933x - 0.221$	0.9998	0.024 (1.20)	0.07
Pyridoxal	0.02–1.0	$y = 275.627x - 0.120$	0.9993	0.003 (0.14)	0.05
Pyridoxine	0.1–3.0	$y = 392.448x - 0.433$	0.9999	0.002 (0.09)	0.03

[a] $3 \times$ SD baseline noise (on-column, ng, 50 µL injection).

[b] SD from replicate sample analysis $\times$ $t_{(n-1,\ \alpha:0.01)}$.

and diluting to volume with water. A separate thiamine working standard was prepared daily by diluting 5.0 mL stock with ca 90 mL 0.6M TCA and diluting to 200 mL with water. Both working standards were serially diluted (1:2 and 1:20) to provide a 3-level calibration for each vitamin.

Mobile Phase

(**a**) *Simultaneous vitamin B_2, FMN, niacinamide, pyridoxal, and pyridoxine determination.*—Sodium dioctylsulfosuccinate (1 g) was dissolved in 250 mL methanol; 10 mL concentrated formic acid was added, the solution was diluted to 1 L with water, and pH was adjusted to 2.8 (range 2.7–2.9) with KOH (50%, w/w).

(**b**) *Vitamin B_1 determination.*—Sodium dioctylsulfosuccinate (1 g) was dissolved in 550 mL methanol; 10 mL concentrated formic acid was added, the solution was diluted to 1 L with water, and pH was adjusted to 4.4 (range 4.3–4.5) with KOH (50%, w/w).

Both mobile phases were filtered (0.45 µm) and degassed under moderate vacuum to minimize foaming and may be used for up to 3 days.

Sample Preparation

All operations were performed in subdued light. Infant formulas or milk powder (6.00 g), or ready-to-feed formulas (30 mL) were weighed accurately into a 100 mL Schott flask or disposable container. Powdered samples were initially dissolved with 30 mL warm water (<40°C), 30 mL 0.6M TCA solution was added, and flasks were shaken for 15 min. An aliquot was filtered (0.45 µm) into an autosampler vial and was ready for either sequential vitamin B_1, or simultaneous vitamin B_2, FMN, niacinamide, pyridoxal, and pyridoxine determination by LC.

For samples of established homogeneity, the method may be scaled down for high sample throughput requirements by direct dispersion in 0.3M TCA (1 g in 10 mL). For products of poor homogeneity, a representative test sample was reconstituted in water (30 g with 150 mL), and a 30 mL aliquot was treated as described.

LC Analysis

The LC system and column were equilibrated routinely with the appropriate mobile phase at 2 mL/min for 10–15 min,

Table 2. Method precision for in-house control infant formula sample

Vitamin	Mean, mg/100 g[a]	RSD_r, %[b]	IRSD_R, %[c]	RSD_R, %[d]	RSD_r:RSD_R	HORRAT[e]
Thiamine	0.67 (30)	3.02 (10)	3.33 (31)	5.60 (60)	0.54	0.47
FMN	0.14 (22)	7.73 (6)	12.24 (22)	16.28 (76)	0.47	1.07
Riboflavin	1.36 (26)	3.97 (6)	8.25 (26)	8.60 (76)	0.46	0.80
Niacinamide	9.23 (34)	4.26 (8)	4.04 (34)	6.80 (76)	0.63	0.84
Pyridoxal	0.13 (25)	5.21 (6)	9.79 (25)	13.02 (76)	0.40	0.85
Pyridoxine	0.58 (25)	3.20 (6)	5.77 (24)	5.40 (76)	0.59	0.44

[a] Mean (n).

[b] Within-run precision (n).

[c] Intermediate precision from in-house control charts (n).

[d] Between-laboratory precision (n).

[e] Measured RSD_R:predicted RSD_R.

Table 3. Recoveries (%) of multivitamin standards added to infant formulas

Vitamin	A[a]	B[b]	C[c]	D[d]
B$_1$ (thiamine)	91–103 (14)	83.2 (20)	70.8 (5)	93.1 (4)
B$_2$ (riboflavin + FMN)	88–103 (7)	92.9 (20)	79.9 (10)	95.1 (6)
B$_3$ (niacinamide)	93–100 (9)	86.7 (20)	84.8 (9)	83.2 (4)
B$_6$ (pyridoxal + pyridoxine)	90–107 (12)	92.9 (20)	61.3 (10)	92.3 (5)

[a] Estimated by standard addition to a range of infant formulas (*n*).

[b] Estimated against NIST SRM 1846 infant formula reference values (*n*).

[c] Estimated against NIST RM 8435 wholemilk reference values, B$_6$: pyridoxal only (*n*).

[d] Estimated against reference digestion methods for total vitamins (*n*).

although a new column required up to 2 h to establish baseline and retention stability. Programmable detection settings were for riboflavin and FMN (fluorescence λ_{ex}: 450 nm, λ_{em}: 510 nm), niacinamide (UV 258 nm), pyridoxal and pyridoxine (fluorescence λ_{ex}: 290 nm, λ_{em}: 390 nm), and thiamine (UV 254 nm). System suitability was confirmed based on a resolution factor of >2 for both the FMN–riboflavin and pyridoxal–pyridoxine separations.

Working standards and sample extracts (50 μL) were injected sequentially with periodic reinjection of calibrants throughout the schedule and peak areas acquired under PC control. Typical retention times (2 mL/min) were FMN, 1.4 min; B$_2$, 2.2 min; niacinamide, 8 min; B$_6$-al, 13 min; B$_6$-ine, 15 min; and B$_1$, 15 min. The mobile phase was recycled continuously at low flow rate between analysis batches for up to 3 days. During long-term shutdown, the system was flushed with methanol–water (50 + 50, v/v) and the column was stored in this eluent.

Calculation

Quantitation was achieved under software-controlled linear regression analysis of 3-level calibration standards and interpolation. Alternatively, simplified quantitation could be performed manually against a single calibrant level according to the following equation:

$$\text{B-group vitamin, mg/100 g} = \frac{A_{sam}}{A_{std}} \times C_{std} \times V \times \frac{100}{W}$$

where A_{sam} = peak area of vitamin in sample extract; A_{std} = peak area of vitamin in working standard; C_{std} = concentration of vitamin in working standard (mg/mL); 100 = conversion to 100 g basis; V = final volume of sample (usually 60 mL); W = weight of sample taken (usually 6 g).

Results

Chromatography

Figures 1 and 2 show chromatography obtained for a typical milk-based infant formula.

FMN and pyridoxal, both of which contribute to the endogenous vitamins B$_2$ and B$_6$ pool present in bovine milk, are re-

solved from the supplementary forms added during infant formula production, thus allowing their concurrent analysis. Niacinamide, although not naturally present in milk or added to infant formula, is retained as an incompletely resolved peak under these specific conditions.

Method Performance

Detection linearity was verified for each vitamin by least-squares regression analysis of multilevel calibration standards, with calculated correlation coefficients, r > 0.999 (Table 1). Also tabulated are the instrumental detection limits (3σ) estimated for each vitamin by serial dilution of calibration standards and method detection limits (MDL) based on replicate analysis of an infant formula containing low supplementation levels.

Method precision was determined under repeatability, intermediate reproducibility, and reproducibility conditions for an in-house control infant formula sample (Table 2).

The relationship between measured reproducibility and that predicted by the Horwitz function $2^{(1-0.5\log C)}$ is considered to be a significant indicator of interlaboratory precision (22). In this study, a mean HORRAT value of 0.75 (range, 0.44–1.07) complied with the acceptable limiting values of 0.5–2.0. The mean RSD$_r$:RSD$_R$ value measured 0.52 (range, 0.40–0.63), which conforms with the accepted guideline of 0.5–0.7 for the relationship between repeatability precision and reproducibility precision (22).

Recovery was evaluated by the standard addition technique, whereby authentic multivitamin standards were added to a range of infant formula samples (Table 3). Recoveries estimated from replicate analysis of NIST SRM 1846 milk-based infant formula are also tabulated. For this material, the overall means (mg/100 g) and confidence intervals (α = 0.05) compared with assigned values were 0.91 ± 0.02 (1.09 ± 0.15) for B$_1$, 1.62 ± 0.02 (1.74 ± 0.10) for B$_2$, 5.49 ± 0.12 (6.33 ± 0.76) for B$_3$, and 0.78 ± 0.04 (0.84 ± 0.10) for B$_6$. *Note*: SRM assigned values report total vitamin content based on single-analyte reference methods.

Predictably, somewhat lower recovery estimates of the exclusively endogenous vitamins contained in NIST RM 8435 unsupplemented whole milk powder (mg/100 g) compared

Table 4. Survey of milk-based infant formulas (mg/100 g)

Product[a]	B_1[b]		B_2[b]		B_3[b]		B_6[b]	
	Test method[c]	Range (label)[d]	Test method	Range (label)	Test method	Range (label)	Test method	Range (label)
FO	1.10 (8)	0.83–1.25 (0.70)	2.00 (9)	1.10–2.60 (1.0)	11.2 (13)	7.8–14.2 (7.2)	0.99 (9)	0.71–1.29 (0.65)
FO	1.03 (1)	0.80–1.26 (0.70)	2.20 (2)	1.60–2.52 (1.4)	9.93 (4)	7.8–12.0 (7.2)	0.82 (2)	0.60–1.08 (0.50)
3+	1.96 (15)	0.80–1.26 (0.70)	2.51 (12)	0.80–1.26 (0.65)	23.3 (15)	10.3–16.0 (8.5)	2.80 (11)	0.70–1.26 (0.65)
6+	2.72 (5)	1.84–2.78 (1.60)	2.59 (5)	2.09–3.60 (1.5)	25.2 (8)	22.0–37.0 (18.5)	4.14 (5)	2.58–4.68 (2.40)
IF	0.84 (7)	0.63–0.94 (0.55)	1.59 (4)	0.89–2.10 (0.65)	8.94 (15)	7.0–11.0 (6.0)	0.72 (4)	0.53–0.96 (0.45)
FO	0.92 (4)	0.83–1.24 (0.70)	1.69 (5)	1.34–2.30 (1.1)	9.30 (12)	6.7–11.1 (5.8)	1.07 (5)	0.71–1.29 (0.65)
IF	0.83 (16)	0.63–0.94 (0.50)	1.85 (10)	0.89–2.10 (0.7)	9.09 (15)	7.0–11.0 (6.0)	0.83 (11)	0.53–0.96 (0.45)
IF	0.39 (3)	0.33–0.48 (0.30)	0.84 (7)	0.50–0.90 (0.40)	6.57 (8)	5.0–8.5 (4.5)	0.47 (6)	0.26–0.60 (0.23)
IF	0.50 (4)	0.36–0.70 (0.33)	1.45 (1)	0.70–1.40 (0.63)	3.29 (12)	2.1–4.0 (1.9)	0.31 (1)	0.21–0.60 (0.19)
IF	0.39 (5)	0.35–0.48 (0.33)	1.23 (5)	0.51–1.50 (0.44)	7.50 (8)	6.0–9.0 (5.4)	0.49 (5)	0.32–0.60 (0.28)
FO	0.54 (5)	0.39–0.70 (0.36)	1.72 (7)	0.70–1.90 (0.63)	3.38 (14)	2.2–4.0 (2.0)	0.45 (7)	0.33–0.60 (0.30)
IF	0.68 (3)	0.47–0.78 (0.52)	1.33 (1)	0.86–1.43 (0.95)	7.61 (1)	5.8–9.6 (6.4)	0.60 (1)	0.47–0.78 (0.52)
Goat FO	0.70 (9)	0.60–0.89 (0.45)	1.25 (6)	0.80–1.80 (0.80)	3.33 (4)	2.2–3.9 (2.2)	0.57 (6)	0.35–0.63 (0.35)
Goat IF	0.69 (2)	0.60–0.90 (0.45)	1.13 (8)	0.72–1.60 (0.80)	5.83 (8)	5.3–7.0 (5.3)	0.41 (8)	0.28–0.50 (0.28)

[a] Abbreviations: FO, follow-on formula; IF, infant formula. All formulas were milk-based except 2 goat milk-based products.

[b] B_1: thiamine, B_2: riboflavin + FMN, B_3: niacinamide, and B_6: pyridoxal + pyridoxine.

[c] Mean (replicates).

[d] Specification range (label declaration).

with assigned values were 0.13 ± 0.02 (0.18 ± 0.05) for B_1, 0.85 ± 0.03 (1.06 ± 0.32) for B_2, 0.62 ± 0.10 (0.74 ± 0.09) for B_3, and 0.11 ± 0.02 (0.19 ± 0.06) for B_6. Nevertheless, the use of reference materials is invaluable for assessing method accuracy, because comparability of data based on independent analytical techniques is indicative of an unbiased estimate of analyte level. Table 3 also includes comparisons with single-vitamin methods used for foods (23), whereby conventional digestion schemes ensure quantitative recovery of all forms of these vitamins present in a food matrix.

A wide range of surveyed infant formulas yielded data that complied with targeted specification ranges and label claims (Table 4). Vitamin content was typically higher than declared levels, consistent with formulation overages generally recommended during infant formula production.

Discussion

The comprehensive analysis of the B-group vitamins at endogenous levels in natural foods requires a rigorous extraction scheme with which to release free, phosphorylated, and protein-bound forms. Such schemes, generally incorporating both acid and enzymatic procedures, have been well reviewed (3–5, 24). However, for supplemented foods, such comprehensive techniques may be less justified, and abbreviated sample preparation strategies incorporating protein removal have been reported to provide a reliable nutritional estimate of these vitamins in infant formulas (19, 20, 25–27) and in milk (28, 29). In such methods, protein is removed with trichloroacetic, perchloric, tungstic, sulfosalicylic, or mineral acid protein precipitants, although lead acetate has also been advocated. The present study selected trichloroacetic acid, based both on its effectiveness in removing milk proteins and the absence of artifacts under the chromatographic conditions described.

Although ion-suppression has been successful in simple pharmaceutical applications, the structural dissimilarity of B-group vitamins has generally necessitated the use of ion-pair techniques for analysis of the more complex food matrixes. The cationic alkylsulfonates have been advocated almost exclusively, despite the selectivity of such separations yielding poor retention of niacinamide and pyridoxine, and high retention of riboflavin (18–20, 25, 26, 30–32). Unfortunately, the selective and sensitive detection of niacinamide in the presence of early-eluting food matrix excipients is difficult under these conditions, because only UV is available to monitor this vitamin.

The sodium salt of dioctylsulfosuccinate has not been commonly advocated as a counter-ion under reversed-phase ion-pair procedures, although its unique functionality was previously described for pharmaceutical applications (33–35) and for B-group vitamins (11, 36). Compared with conventional alkylsulfonates, the enhanced retention conferred to nitrogenous structures may be attributed to the highly lipophilic double-branched alkyl functionality of the dioctylsulfosuccinate counter-ion. Thus, under the described acidic eluent conditions, protonated niacinamide and pyridoxine are selectively retained, thereby facilitating the more secure use of UV detection for niacinamide. However, retention of B-group vitamins is not solely a function of analyte pKa, and other properties, including polarity, are involved. Thus, retention of the relatively hydrophobic riboflavin is largely a function of eluent polarity, and despite eluting early, the specific fluorescence properties of both riboflavin and FMN allow their unequivocal detection.

Thiamine is highly retained under the conditions described for the other vitamins, and an eluent of higher pH and methanol content is required to facilitate its convenient analysis. Although thiamine may be successfully determined simultaneously under gradient elution conditions (11), lengthy re-equilibration mediates against the expediency of this technique. It is therefore preferable to analyze for thiamine sequentially rather than concurrently, although the same test sample extract is used. The sensitive and specific measurement of thiamine has been routinely achieved at both endogenous and supplemented levels under the described chromatographic conditions, using post-column thiochrome formation with fluorescence detection. However, for supplemented infant formulas and other similar foods, UV detection is sufficiently sensitive, thereby simplifying the chromatographic configuration for routine compliance analysis.

The B-group vitamins are synthesized by rumen microorganisms in the cow and are expressed in bovine milk mainly in their free, nonphosphorylated forms (37, 38). Thus, most vitamin B_1 occurs as free thiamine (27), B_2 as free riboflavin with a minor contribution from flavin monoucleotide (FMN) and flavin adenine dinucleotide (FAD; 25, 39), B_3 principally as free niacinamide (38), and B_6 as free pyridoxal with appreciable amounts of pyridoxamine (25). The literature consensus is generally supported by data obtained for the NIST RM 8435 whole milk, where recovery of the endogenous free B-vitamins ranges at 61–85% of the assigned values. Recoveries for the supplemented NIST SRM 1846 infant formula are, however, almost quantitative, based on concordance with assigned value intervals established from reference methods measuring total vitamin content. Such data confirm the efficacy of the described extraction protocol in recovering both free supplemental and most endogenous vitamins.

Each vitamin is quantitated by the external standard technique following linear regression analysis and interpolation. FMN may, however, be measured either against authentic FMN as described, or against a riboflavin standard, based on their equivalent fluorescence yield (39). Although spectrophotometry (444 nm) may be used to measure vitamin B_2, fluorescence is preferred in view of both the relatively low levels and poor retention of endogenous milk FMN.

A wide range of C_{18}-based analytical columns yield comparable selectivity for the B_2, B_3, and B_6 group, despite the relatively low pH and high aqueous content of the eluent. Although innovative embedded-hydrophilic octadecylsilane columns have been developed to operate under such conditions, this application offers no advantage over conventional type B columns.

Conclusions

A rapid method is described involving a simple extraction procedure and ion-pair reversed-phase LC determination of vitamins B_1, B_2, B_3, and B_6 in infant formulas. The described multianalyte protocol facilitates measurement of both supplementary and the majority of endogenous vitamins, without the need for multistage acid and/or enzyme extraction schemes. The almost quantitative estimate of aggregate vitamin content provides a convenient and fit-for-purpose strategy for routine analysis of infant formulas for either compliance or nutritional labeling requirements, with superior precision compared with single-vitamin reference methods.

Acknowledgments

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