ELSEVIER

Journal of Chromatography A, 804 (1998) 279–287

JOURNAL OF CHROMATOGRAPHY A

Detection of counterfeit and relabeled infant formulas by high-pH anion-exchange chromatography–pulsed amperometric detection for the determination of sugar profiles

Lisa A. Kaine*, Karen A. Wolnik

US Food and Drug Administration, 1141 Central Parkway, Cincinnati, OH 45202, USA

Abstract

High pH anion-exchange separation with pulsed amperometric detection (HPAE–PAD) is used to characterize various milk-based, soy-based, and protein hydrolysate infant formulas based on carbohydrate profiles. Counterfeit and relabeled formulas are compared to authentics. Figures of merit are shown for glucose, fructose, lactose, sucrose, and maltose.

Keywords: Infant formulas; Food analysis; Carbohydrates

1. Introduction

Millions of units of infant formula are sold annually. Milk and soy-based formulas, available in powder, concentrate, or ready-to-feed forms, comprise the majority of the market. In addition, hypoallergenic protein hydrolysate formula is fed to infants with special needs such as milk allergy. While many infants are fed the same formula brand from birth until approximately their first birthday, others must switch brands due to lactose intolerance or milk protein allergy, or simply for economic reasons. There exists great potential for economic gain in counterfeiting and/or relabeling infant formulas due to the large volume of product sold and the wide variety in the price of some formulas, currently ranging from US$5 to US$20 per can of powdered product. While reaction to a change in formula brand/type may result only in fussiness or colic, a serious health risk may exist in the case where a milk-based formula is substituted for soy or protein hydrolysate.

Carbohydrate content as stated on milk-based formula labels is determined by difference: carbohydrate=total solids−(protein + fat + ash) [1]. Values for commercial U.S. infant formulas in powdered form vary only from approximately 0.43 to 0.56 g carbohydrate per g dry matter. However, differences in carbohydrate content are evident from examination of the ingredients list of various brands of infant formulas. Corn syrup solids, lactose, sucrose, maltodextrin, and starch are common ingredients used in different proportions (refer to Table 1). There may be differences between ingredients listed with the same name. For example, there are many grades of corn syrup solids available, and maltodextrins may be products of corn, rice, or other grain [2].

Scott and Hatina [3] used HPLC to determine carbohydrate content of some formulas. Samples

*Corresponding author.

0021-9673/98/$19.00
PII S0021-9673(97)01249-1

Table 1
Infant Formulas Labeled Ingredients[a]

Type	Brand	Ingredients
Milk-based	A	Nonfat milk, corn syrup solids, lactose, whey protein concentrate, oil
	B	Nonfat milk, lactose, oils, whey protein concentrate
	C	Hydrolyzed whey protein concentrate, oils, lactose, maltodextrin
	D	Whey, nonfat milk, oil, lactose
	E	Corn syrup solids, oils, milk protein isolate
	F	Nonfat milk, corn syrup solids, oils, maltodextrins
Soy-based	A	Corn syrup solids, oils, soy protein isolate, sugar
	B	Corn syrup solids, oils, soy protein isolate
	C	Maltodextrin, oils, soy protein isolate, sucrose
	D	Corn syrup solids, sucrose, soy protein isolate, oil
	E	Maltodextrin, oils, sucrose, soy protein isolate
Protein-Hyd	A	Corn s.solids, casein hydrolysate, oil, modified corn starch, dextrose
	B	Corn syrup solids, casein hydrolysate, oil, modified corn starch

[a] Label ingredients are listed in order of decreasing concentration.

were prepared in alcohol for monosaccharide and disaccharide analysis. Starch was determined by hydrolysis of a separate sampling and subsequent quantitation of glucose. Similarly, samples suspected of containing oligosaccharides were treated with amyloglucosidase to convert polymers to glucose. However, maltose was not separated from lactose or sucrose on the Bio-Rad Aminex resin-based column. High-pH anion-exchange separation with pulsed amperometric detection (HPAEC–PAD) has been shown to provide excellent resolution and sensitivity for carbohydrates [4,5]. Indeed, several researchers have used carbohydrate profiles to detect adulteration in high carbohydrate content foods. Low [6] used oligosaccharide profiles determined by HPAEC–PAD to detect adulteration of fruit juices, honey, and maple syrup with high fructose corn syrup, honey, and maple syrup. Cornell et al. [7] used trace oligosaccharides determined by capillary GC to detect sugar addition to citrus juices.

The object of this work was to develop a single HPAEC–PAD analysis to characterize the various commercially available infant formulas. This paper focuses on powdered formula since most of the cases of adulteration or relabeling investigated in this laboratory have involved powdered formula, rather than liquid concentrate or ready-to-feed forms. Carbohydrate profiles of counterfeit/ relabeled formulas are compared to known authentic products.

2. Experimental

2.1. Sample preparation

Samples (0.2 g) were dissolved in 100 ml deionized distilled water (DDW) and shaken mechanically for approximately 2 min. Approximately 4 ml of solution was passed through a 0.45-μm nylon 66 syringe filter (Alltech, Deerfield, IL, USA) and an activated C_{18} sample preparation cartridge (Maxi-clean, Alltech) using a disposable syringe. The first 3 ml through the filter/cartridge were discarded and the remaining sample collected for analysis. The C18 was activated by passing 3 ml of methanol followed by 6 ml of DDW through the cartridge. Recovery of 10 $\mu g\, g^{-1}$ standards of glucose, fructose, lactose, sucrose, and maltose through the method was quantitative (97–101%).

2.2. HPAEC–PAD analysis

Samples were analyzed on a model 4500 metal-free gradient ion chromatograph (Dionex, Sunnyvale, CA, USA). Carbohydrates were separated on a Carbopac PA-1 (Dionex) pellicular anion-exchange analytical column (250×4 mm) with Carbopac PA-1 guard column (50×4 mm). An automated sample module (ASM, Dionex), along with a Rheodyne Model 9126 injection valve (Rheodyne, Cotati, CA,

USA) with a 20 μl loop was used for sample introduction. The carbohydrates were detected by a pulsed electrochemical detection system (PED-1) in the integrated amperometry mode, using a gold working electrode and pH/Ag/AgCl reference electrode (all Dionex). The waveform E_1: 0.05 V for 0.40 s; E_2: 0.75 V for 0.19 s; E_3: −0.15 V for 0.39 s was used with integration from 0.20 to 0.40 s.

Monosaccharides and disaccharides were eluted with an isocratic step (150 m*M* NaOH for 22 min), followed by gradient elution of oligosaccharides (linear ramp from 150 m*M* NaOH to 150 m*M* NaOH–600 m*M* sodium acetate over 15 min). A re-equilibration step of 13 min at 150 m*M* NaOH preceded each injection. Eluent flow-rate was 1.0 ml min^{-1}. The 150 m*M* NaOH eluent was prepared by degassing DDW, then pipetting in 50% *w*/*v* NaOH (Fisher, FairLawn, NJ, USA) to minimize the amount of carbonate contamination to the mobile phase, since this contamination affects the retention times of carbohydrates. The 150 m*M* NaOH with 600 m*M* sodium acetate eluent was prepared in a similar manner, except that the sodium acetate (Aldrich, Milwaukee, WI, USA) was dissolved in DDW and diluted to volume prior to degassing and subsequent addition of the 50% NaOH.

3. Results and discussion

Visual observations of physical differences are often the first indication of infant formula counterfeiting or relabeling. Physical differences include changes in label printing such as font or number of pixels, can construction, product code, and formula color, texture, and odor. Microscopy methods are invaluable for detecting differences in product color and texture as well as the presence of lactose crystals. However, the microscopy methods may fail to detect lactose in milk-based formulations which were mixed as liquids and then spray-dried into powder. Due to the serious potential health risk associated with a milk-based formula substituted for a soy or protein hydrolysate formula, it is very important to determine whether or not a formula contains lactose.

A gradient HPAEC–PAD method was developed

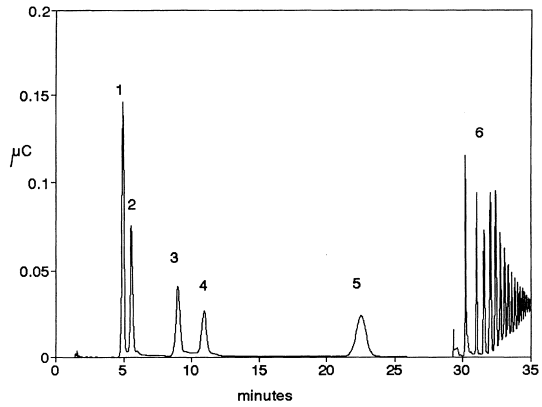

Fig. 1. HPAEC–PAD of carbohydrate standards. 1=glucose; 2=fructose; 3=lactose; 4=sucrose; 5=maltose; 6=maltodextrin.

to separate the major carbohydrates in infant formulas. Fig. 1 shows the separation of glucose, fructose, lactose, sucrose, maltose, and maltodextrin, and Table 2 demonstrates the figures of merit for all of these analytes except maltodextrin. Note that minimum detection limits (MDLs) are given for standards in DDW as well as for in formula matrix. The MDL for analytes in DDW were determined by calculating three times the standard deviation of the baseline noise divided by the peak height response for the analyte standard times the concentration of the standard. The MDL in formula, however, is dependent upon other carbohydrates in the matrix and is especially affected by disparate levels of analytes (eg. lactose in the presence of high levels of sucrose). MDLs in formula are given as the average estimated value based upon lowest levels of spikes which were observed in formula samples. This method was intended to profile each formula, not to determine total carbohydrate as declared on the label. The aforementioned monosaccharides and disaccharides account for approximately 100% of the labeled carbohydrate value for milk-based brands B and D, but only 3–70% for the remaining brands in this study. Maltodextrin was not quantitated by this method because the pattern of oligosaccharides may not be the same in a sample as in the maltodextrin standard. Retention time stability in this gradient method is especially important so that lactose is not

Table 2
Figures of Merit for Selected Carbohydrates[a]

Analyte	Slope	Intercept	Correlation	MDL[b]	MDL[c]
Glucose	$7.713 \cdot 10^{-6}$	0.2593	0.99996	0.011	0.05
Fructose	$8.202 \cdot 10^{-6}$	0.5741	0.99985	0.013	0.05
Lactose	$1.098 \cdot 10^{-5}$	0.5364	0.99977	0.020	0.17
Sucrose	$1.432 \cdot 10^{-5}$	0.1652	0.99999	0.019	0.15
Maltose	$1.372 \cdot 10^{-5}$	−0.0473	0.99912	0.053	0.20

[a]Working range: 1–100 μg/ml; Six level calibration.
[b]MDL=minimum detection limit (μg ml^{-1} standard solution) determined as 3×standard deviation baseline noise/(peak height response for analyte standard×standard concentration).
[c]MDL (mg/g) in formula matrix, estimated based on lowest level spikes observed in sample.

misidentified as the nearby peak, sucrose. The retention time of lactose (8.5 min) varied only by 0.2 min, and sucrose (10.2 min) by 0.3 min over the course of 27 continuous hours of analysis. No decrease in resolution between lactose and sucrose was observed after more than 100 injections of formula.

Lactose was spiked into soy-based (brand D) and milk-based lactose-free (brand E) formulas to ensure that lactose would be detected. Lactose was spiked into sucrose containing soy-based formula at 0.5 mg lactose g^{-1} powder and at 100 mg/g. The spikes were recovered at 154% for the low spike which was below the quantitation limit in this matrix, and at 125% for the higher spike level. Lactose spiked into lactose-free milk-based formula (which did not contain sucrose) at 20 mg g^{-1} was recovered 107%. Spikes of sucrose were recovered from milk-based formula B at 103% for 0.5 mg g^{-1} powder, and at 91% for 10 mg g^{-1}. Maltose spiked into milk-based formula B was recovered at 110%.

3.1. Within-lot and lot-to-lot repeatability

Prior to making comparisons between different types/brands of infant formulas, it is necessary to show within-lot and lot-to-lot repeatability. Five preparations from one lot each of one milk-based and one soy-based formula, as well as four preparations of another milk-based formula, were analyzed to demonstrate repeatability of carbohydrate values within the same lot (within-lot repeatability). Three to five different lots of each formula spanning production years 1994 to 1997 were analyzed to show repeatability of carbohydrate values between different lots of the same brand (lot-to-lot repeatability). The data is shown in Table 3, listed as average values obtained along with standard devia-

Table 3
Within-lot and lot-to-lot repeatability of monosaccharides and disaccharides determined by HPAE–PAD analysis of infant formulas

Formula	Lots	n[b]	mg carbohydrate g^{-1} powder[a]		
			Glucose	Lactose	Maltose
Milk-based D	1	5	14.3 (2.1)	441 (19)	
	5	2–5	8.3 (5.8)	435 (26)	
Milk-based B	1	4	0.8 (0.05)	522 (19)	
	3	2–4	trace[c]	528 (20)	
Soy-based D	1	5	14.1 (0.1)	123 (7)	19.5 (0.7)
	4	2–5	15.2 (2.0)	122 (18)	19.9 (2.4)
Soy-based D (European)	1	1	27.1	71.8	28.9

[a]Average value (standard deviation).
[c]n=number of samplings. For multiple lots, various numbers of samplings were performed.
[c]Trace indicates some samplings were below quantitation limit estimated at 0.15 mg glucose g^{-1} powder in this matrix.

tion in mg carbohydrate g^{-1} powder. Within-lot repeatability for the soy based formula was better than 6% for glucose, sucrose, and maltose. Lot-to-lot repeatability for this brand ranged from 12 to 15% for these analytes. It is very important to note that one lot of soy-based formula brand D manufactured and marketed in Europe was also analyzed, and found to be significantly different from the same brand US product. This study is currently limited to commercially available US formulations. Similar results to the variability observed in the soy-based US formula was also observed in milk-based formula B. More variability, however, was observed in the milk-based formula brand D. Within-lot repeatability was 14% for glucose, and 4% for lactose. While the lactose repeatability from one lot to another (6%) was similar to the within-lot repeatability, the amount of glucose from lot-to-lot varied substantially (70%). Two lots of milk-based brand D had glucose near 3 mg g^{-1}, while three lots had glucose near 14 mg g^{-1}. Therefore, glucose cannot be used solely to distinguish between milk-based formulas.

3.2. Milk-based formulas

Six authentic milk-based formulas were characterized by HPAEC–PAD (refer to Fig. 2). Based upon the authentic formula samples that were analyzed, all six formulas can be distinguished from one another, even considering lot-to-lot variability. Note formula brand E, which contains no lactose peak. The absence of a lactose peak is explained by the label

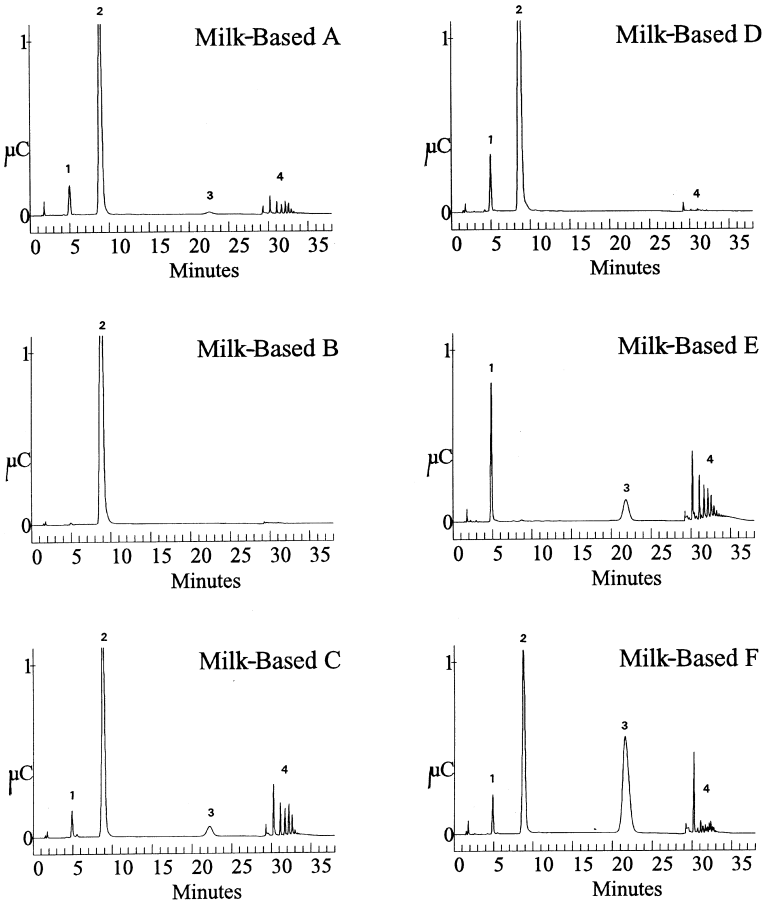

Fig. 2. HPAEC–PAD of various brands of milk-based powdered infant formulas. 1=glucose; 2=lactose; 3=maltose; 4=maltodextrin.

Table 4
Monosaccharides and disaccharides in milk-based infant formulas determined by HPAE–PAD

Brand	mg carbohydrate g^{-1} powder[a]			
	Glucose	Lactose	Sucrose	Maltose
A	10.7 (0.8)	492 (21)	0.7 (0.1)	4.1 (0.4)
B[b]	trace[c]	528 (20)	ND	ND
C	6.9 (0.2)	269 (27)	ND	19.0 (1.3)
D[d]	8.3 (5.8)	435 (26)	ND	ND
E[d]	34.8 (1.6)	ND	ND	39.5 (1.7)
F	9.6 (0.8)	175 (17)	ND	208 (52)

[a]Average value (standard deviation). Three samplings per lot.
[b]Average of three lots given, three samplings per lot.
[c]Trace indicates some samplings were below quantitation limit estimated at 0.15 mg glucose g^{-1} powder in this matrix.
[d]Average of two lots, three samplings per lot.

for this formula, which lists only corn syrup solids, oils, and milk protein isolate as major ingredients. In fact, this product is marketed specifically as lactose-free for infants with lactose intolerance. Table 4 details the monosaccharide and disaccharide composition of these six milk-based formulas, as de-

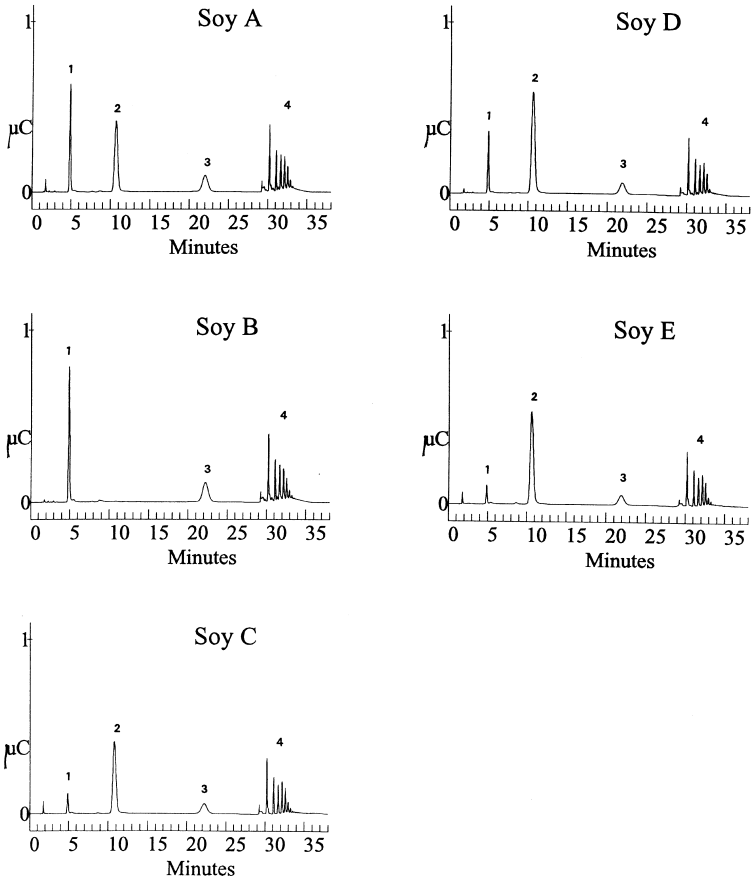

Fig. 3. HPAEC–PAD of various brands of soy-based powdered infant formulas. 1=glucose; 2=sucrose; 3=maltose; 4=maltodextrin.

termined by HPAEC–PAD. There are also differences in the region of the chromatogram from 28 to 33 min (maltodextrin region) which are shown in Fig. 2.

3.3. Soy-based formulas

A comparison of five different soy-based formula brands is shown in Fig. 3. No lactose was detected in any of the formulas (MDL in the matrix approximately 170 μg lactose g^{-1} powder). The profile for brand B is very distinct from the other soy-based formulas because no sucrose peak was detected. Brands C and E, however, are not easily distinguished from one another. Their labeled ingredients are very similar, except that sucrose is the fourth ingredient listed for brand C, and the third for brand E. Although the average sucrose value for brand E is approximately 50% greater than the average amount of sucrose in brand C (refer to Table 5), within-lot and lot-to-lot variability do not allow these two formulas to be distinguished from one another by this method. For these same reasons, brands A and D are difficult to distinguish from one another.

3.4. Protein hydrolysate formulas

Fig. 4 shows the chromatograms obtained for two brands of protein hydrolysate formulas. The two brands can be distinguished from one another based upon glucose quantitation (refer to Table 5) as well as differences in the patterns of peaks from 28–35 min.

Table 5
Monosaccharides and disaccharides in soy-based and protein hydrolysate infant formulas determined by HPAE–PAD

Type	Brand	mg carbohydrate g^{-1} powder[1]		
		Glucose	Sucrose	Maltose
Soy	A	25.6 (1.9)	105 (19)	30.4 (1.8)
	B[b]	32.8 (2.0)	1.8 (0.7)	35.0(2.3)
	C	5.1 (0.2)	91.3 (12)	18.7 (0.3)
	D[c]	15.1 (2.0)	122 (18)	19.9 (2.4)
	E[b]	4.8 (0.3)	152 (33)	18.9 (1.2)
Hydrol.	A	95.8 (9.6)	ND	25.4 (3.8)
	B[b]	25.2 (4.0)	ND	34.6 (1.6)

[a]Average value (standard deviation). Three samplings per lot.
[b]Average of two lots, three samplings per lot.
[c]Average of five lots, three samplings per lot.

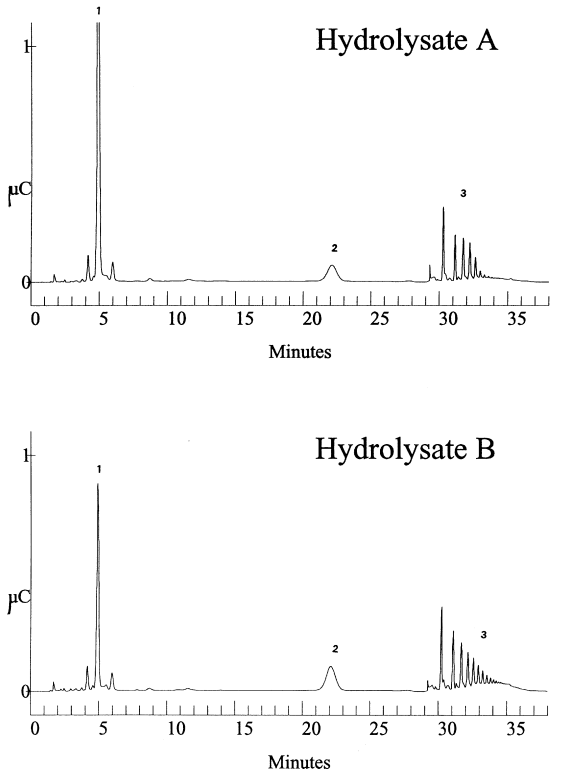

Fig. 4. HPAEC–PAD of various brands of protein hydrolysate powdered infant formulas. 1=glucose; 2=maltose; 3=maltodextrin.

3.5. Overall formula comparisons

Chromatograms for milk-based formula E, soy-based formula B, and protein hydrolysate formulas A and B are very similar to one another in that none contains lactose or sucrose at appreciable levels, but all contain glucose and maltose. Hydrolysate formula A can be distinguished from the other formulas by the glucose level which is significantly higher in hydrolysate formula A than the other aforementioned formulas. Small differences were observed in the maltodextrin region for the milk-based formula, soy-based formula and protein hydrolysate formula listed above. Preliminary results in our laboratory show that capillary electrophoresis analysis for proteins could be used to distinguish these formulas from one another and identify the lactose-free formula (milk-based formula E) as indeed milk-based.

A blind study was conducted using unknown analytical preparations of nine various infant formula

samples. The solutions were analyzed and the best estimate of the identity of each blind sample was made. Milk-based brands A, B, D (two samples) and soy-based brands B and D, as well as hydrolysate formulas A and B were all correctly identified. No estimate of match was made for the remaining blind sample (milk-based formula C). The dilution of the sample was such that the lactose peak in the chromatogram was smaller than expected, thus no match was suggested.

3.6. Suspected counterfeit samples

A number of samples suspected of being counterfeit and/or relabeled infant formula based upon visual observations and investigative information were received in our laboratory. Samples were analyzed by HPAEC–PAD and compared to authentic samples. The purpose of the comparison was multifold: (1) Determine if the powder was the brand declared on the label; (2) if the powder was not as labeled, determine if it was milk-based, due to possible health risk; (3) compare the powder to other US commercially available infant formulas to identify products simply relabeled; and (4) attempt to relate various suspected samples.

Fig. 5 shows the chromatograms obtained for six suspected counterfeit samples. All of these samples were collected in cases related to counterfeiting milk-based formula B. Although all of the suspect samples were milk-based as evidenced by the large amounts of lactose, none of the profiles matched that

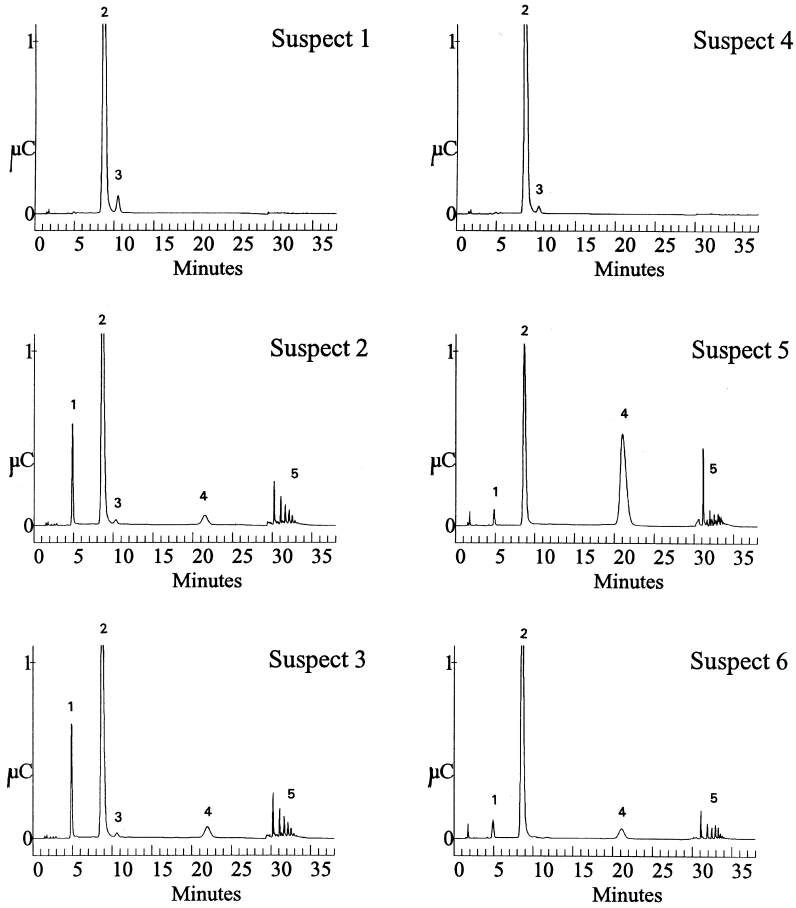

Fig. 5. HPAEC–PAD of several suspected counterfeit infant formula samples. 1=glucose; 2=lactose; 3=sucrose; 4=maltose; 5=maltodextrin.

of brand B although they were all labeled as such. Sucrose was detected in suspect samples 1–4 ranging from 1.7 to 17.5 mg g^{-1}, but none of the authentic brands in this study contained that much sucrose. Sucrose was indeed only detected in brand A at 0.7 mg g^{-1}. The profiles of the suspected counterfeits 1–4 also did not match any of the authentics, however suspected counterfeit sample 5 was similar to milk-based brand F and suspected counterfeit sample 6 was similar to milk-based brand A. The profiles of counterfeits 1 and 4 matched one another, and the profiles of samples 2 and 3 matched each other as well. Grouping counterfeit samples may indicate that these samples are related to one another, perhaps produced by the same source. Other techniques used on these samples detected differences from the authentic product such as the identity of the fill gas and the presence of starch crystals.

4. Conclusions

An HPAEC–PAD method has been developed to profile the water soluble carbohydrate content of powdered infant formula. Glucose, lactose, sucrose, maltose, and maltodextrin were determined in six milk-based, five soy-based, and two protein hydrolysate brands of infant formula. The carbohydrate profiles are used along with the results of other analytical techniques to make best estimates of the identity of an unknown powder.

Acknowledgements

Thanks to Brenda Zimmer for sample preparation. Thanks to Cheryl Flurer for capillary electrophoresis work.

References

[1] P. Cunniff (Ed.), Official Methods of AOAC International, AOAC International, Gaithersburg, MD, 16th ed., 3rd rev., 1997, method 986.25.
[2] M.B. Jacobs, The Chemical Analysis of Foods and Food Products, Van Nostrand, Princeton, NJ, 3rd ed., 1958.
[3] F. Scott, G. Hatina, J. Food Sci. 53 (1988) 264–269.
[4] M.R. Hardy, R.R. Townsend, Proc. Natl. Acad. Sci. USA 85 (1988) 3289–3293.
[5] Dionex Application Note 67, Polysaccharide Analysis: Maltodextrins, Dextrans, Inulin and Other Oligosaccharides', Dionex, Sunnyvale, CA, 1990.
[6] N. Low, Am. Lab., April (1996) 35M–35W.
[7] V. Cornell, W. Ooghe, P. Sandra, J. High Resolut. Chromatogr. 18 (1995) 286–288.