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Quantitative Determination of Total L-Carnitine in Infant Formula, Follow-up Formula, and Raw Materials by Liquid Chromatography with Tandem Mass Spectrometry

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We developed a rapid and useful routine screening assay for total L-carnitine content in various infant formulas and materials by liquid chromatography coupled with a tandem mass spectrometric method (LC-MS/MS) and alkaline hydrolysis. For separation of L-carnitine, a multi-mode octadecylsilane (ODS) column was used that contained ODS ligands, anion ligands, and cation ligands to avoid using ion-pairing agents. The stable isotope L-carnitine-d_3 (m/z 165 → 103/85) was used in electrospray MS/MS in the multiple reaction monitoring mode with the ion transitions of m/z 162 → 103/85 for detection and quantitation of L-carnitine. Alkaline hydrolysis of short/medium chain (C2 – C15) acyl-L-carnitines in infant formula was analyzed by LC with quadrupole time-of-flight mass spectrometry (QTOF-MS). The majority of short/medium chain acyl-L-carnitines were hydrolyzed to free L-carnitine. The overall standard deviations for L-carnitine in infant formula, follow-up formula and raw materials ranged from 2.1 to 4.0. The overall mean recoveries ranged from 90.2 to 94.2%.

Keywords LC-MS/MS, L-carnitine, infant formula, raw materials, multi-mode ODS column

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Introduction

L-Carnitine (3-hydroxy-4-*N*-trimethylaminobutyric acid, Fig. 1) is an essential component of animal tissues, higher plants, and many microorganisms and is well recognized as having a fundamental role in lipid metabolism. This lipid metabolism is consistent with an increase in fatty acid β-oxidation and/or an enhancement in lipoprotein with the transport of long-chain fatty acids into the inner mitochondrial matrix in the form of acyl-carnitines as coenzyme A (CoA) esters. An additional role of L-carnitine in lipid metabolism is removal of potentially toxic short- and medium-chain acyl-CoA esters from mitochondria.[1,2]

L-Carnitine is a non-protein amino acid available to humans both through *in vivo* biogenesis involving lysine and methionine and from a variety of dietary sources, notably meat and dairy products.[3,4] In infants, this non-protein amino acid is an essential nutrient because they are unable to synthesize L-carnitine because of an immature biosynthesis pathway.[1,5] Human milk is reported to contain 0.5 – 1.5 mg/100 g L-carnitine, which is sufficient to maintain normal plasma concentration, but is lower than that of bovine milk (2.5 – 4.3 mg/100 g).[6,7] Therefore bovine milk-based formulas should generally meet the nutritional needs of infants unless L-carnitine concentrations are altered during manufacturing. However, certain bovine milk-based formulas assembled from depleted raw materials and all soy-based formulas are potentially L-carnitine deficient and may require supplementation.[1] The need to fortify infant formula with L-carnitine has been recognized since the late 1970s or the early 1980s.[8] Human and bovine milk are composed of free L-carnitine, short/medium chain acyl-L-carnitines (acid soluble), and long chain acyl-L-carnitines (acid insoluble). Long chain acyl-L-carnitines reportedly contribute 2 – 3% in milk; thus, quantitation of total L-carnitine content is commonly performed for free L-carnitine and short/medium chain acyl-L-carnitines.[3,9-11] To quantitate the total amount of L-carnitine content in milk, it is necessary to perform alkaline hydrolysis of short/medium chain acyl-L-carnitines.[12] We have previously reported the optimal alkaline hydrolysis conditions of short/medium chain acyl-L-carnitines.[13] In this study, we

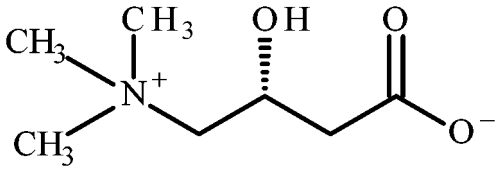

Fig. 1 Chemical structure of L-carnitine.

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used quadrupole TOF-MS with accurate mass analysis to evaluate the alkaline hydrolysis behavior of short/medium chain (C2 – C15) acyl-L-carnitines in infant formula and follow-up formula under our optimal conditions.

In earlier reports, L-carnitine in milk and infant formulas has traditionally been determined by enzymatic assays, which were adapted from earlier methodologies for clinical determinations of L-carnitine in body fluids and tissues. Marquis *et al.* developed an enzymatic assay of L-carnitine in rat tissues based on the following reaction (1) catalyzed by carnitine acetyltransferase.[14]

$$\text{L-carnitine} + \text{acetyl-CoA} \rightleftharpoons \text{acetyl-L-carnitine} + \text{CoA} \quad (1)$$

After the reaction, the concentration of L-carnitine was determined indirectly by generating a color reaction between free CoA and 5,5′-dithiobis-(2-nitrobenzoic acid).[11] In recent years, efficient, stable, and useful systems using LC techniques[15,16] and LC-MS/MS of individual carnitine species[17,18] have been developed. For the LC analysis of L-carnitine, researchers have commonly used ion-pairing agents added to the mobile phase that reduce the polarity of L-carnitine and make it more suitably retained on reversed-phase stationary phases.[18,19] Although ion-pairing LC can improve the retention and selectivity of hydrophilic ionic and/or ionizable analytes, it often requires long equilibration times, a dedicated column, and usually an MS-incompatible mobile phase. Thus, a mixed-mode stationary phase utilizing simultaneous reversed-phase and ion exchange functionalities was used to increase the retention of L-carnitine without any mobile phase ion-pair additives. In recent work, a mixed-mode stationary phase and mass spectrometric detection with stable isotope dilution was applied for the determination of L-carnitine in infant formula.[20] A few methods using LC-MS/MS and/or LC-MS with stable isotope dilution have been used in attempts to quantitate L-carnitine content in infant formula.[18-20] In this study, we used 15 min alkaline hydrolysis, a multi-mode octadecylsilane (ODS) column,[20,21] LC-MS/MS with a stable isotope standard, and developed a more rapid routine method for quantitative determination of total L-carnitine content in infant formulas, follow-up formulas, and raw materials.

Experimental

Reagents and chemicals

Analytical standard L-carnitine hydrochloride (98%) and acetyl-L-carnitine hydrochloride (99%) were obtained from Sigma-Aldrich (St. Louis, MO). Internal standard (IS) L-carnitine-d_3 hydrochloride (99.9%) was obtained from C/D/N Isotopes (Quebec, Canada). Acetonitrile, formic acid, and ammonium formate were of LC-MS grade from Sigma-Aldrich. Perchloric acid ($HClO_4$), hydrochloric acid (HCl), and potassium hydroxide (KOH) were of analytical grade from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Water was processed through a Milli-Q purification system (Merck Millipore, Billerica, MA). L-carnitine standard stock solution of 320 μg/mL was prepared by dissolving 40 mg of L-carnitine hydrochloride in 100 mL of water and stored at –20°C for 1 year. An intermediate standard solution of 800 ng/mL was obtained by diluting the stock solution 400 times with water. L-carnitine working solutions were prepared in appropriate concentrations by diluting the intermediate standard solution with water and were stored at 4°C. IS stock solution of 820 μg/mL was prepared by dissolving 10 mg of L-carnitine-d_3 hydrochloride in 10 mL of water and stored at –20°C for 1 year. IS working solution of 820 ng/mL was obtained by diluting the stock solution 1000 times with water and stored at –20°C for 1 year.

Milk samples

Infant formula and follow-up formulas were manufactured by Wakodo Co., Ltd. (Tokyo, Japan) for the recovery and validation. In addition, other common infant and follow-up formulas and whole milk powder, skim milk powder, and whey protein concentrate powder were obtained from Japanese or foreign markets. Standard reference material (SRM) 1849a (the certified mass fraction value of total L-carnitine content was 13.6 ± 1.4 mg/100 g) was obtained from the National Institute of Standards and Technology (NIST).

Sample preparation for total L-carnitine content

Samples were prepared by dissolving 1.5 g infant or follow-up formula, 1.0 g whole milk powder or skim milk powder, and 0.25 g whey protein concentrate powder in 15 mL warm (50°C) water in a 50-mL glass beaker. A 5.0-mL aliquot of 1.2 M $HClO_4$ solution was added and mixed thoroughly, and the sample was filtered through a No. 5B filter paper (Advantec, Dublin, CA) into a 50-mL volumetric flask. The glass beaker was washed with 0.3 M $HClO_4$ solution, filtered twice, and filled to 50 mL with water. A 10-mL aliquot of the filtrate was added to 20 mL of 0.2 M KOH solution and allowed to stand for 15 min at 40°C. The pH was adjusted to 7.0 ± 0.5 with 1.0 M HCl solution. The solution was transferred to a 50-mL volumetric flask and brought to volume with water. A 100-μL aliquot was mixed with 50 μL of the IS working solution and 850 μL of water and then filtered through a 0.20-μm polytetrafluoroethylene syringe filter (Merck Millipore) before LC-MS/MS analysis.

LC-MS/MS analysis

An acquity ultra performance LC system coupled to a Xevo TQ MS triple quadrupole MS with an ESI source (Waters, Milford, MA) was used to perform the analyses. The column oven temperature was set at 40°C, and the autosampler temperature was set at 10°C. The injection volume was 5 μL. Separation was achieved on a Scherzo SS-C18 (100 mm × 2.0 mm i.d., 3.0 μm) column (Imtakt, Kyoto, Japan) with mobile phase A consisting of 0.3% formic acid aqueous solution and mobile phase B consisting of 100 mM ammonium formate aqueous solution/acetonitrile (50:50, v/v). The flow rate was set at 0.3 mL/min. A linear gradient was used with the following parameters: 10 – 30% B from 0 to 5 min, 30 – 90% B from 5 to 6 min, 90% B from 6 to 9 min, and 90 – 10% B from 9 to 10 min. To achieve maximum sensitivity for the analytes, the analysis was performed in the positive ion mode. Nitrogen was used as the nebulizer gas. The parameters for ESI-MS were as follows: capillary voltage, 3.5 kV; source temperature, 150°C; desolvation temperature, 350°C; desolvation gas flow, 800 L/h; and cone gas flow, 50 L/h. Cone voltages and collision energies were optimized for the analytes. The multiple reaction monitoring (MRM) mode was used to perform quantitative analysis. L-carnitine transitions were m/z 162 > 103 and 162 > 85. IS transitions were m/z 165 > 103 and 165 > 85. The LC-MS/MS instrument was controlled by the MassLynx and Quanlynx software (Version 4.1, Waters).

LC-QTOF-MS analysis for investigation of alkaline hydrolysis of short/medium chain acyl-L-carnitines

An Agilent Technologies 1200 Series LC system was used

(Santa Clara, CA). The conditions were as described above. The detector was an Agilent 6510 QTOF-MS equipped with an ESI source. The instrument was operated with the ESI source in the positive ion mode. The following conditions were used: Vcap voltage, 3500 V; fragmentor voltage, 120 V; skimmer voltage, 65 V; OCT 1 RF voltage, 750 V; drying gas flow, 10 L/h; nebulizer, 45 psig; and gas temperature, 300°C. The MS monitoring range was from *m/z* 50 to 1500 using a reference mass solution (API-ROF Reference Mass Solution Kit, hexakis, purine, and ammonium trifluoroacetate from Agilent Technologies) for the identification of short/medium chain acyl-L-carnitines in infant formula or follow-up formula.

Calibration curves and quantitation of L-carnitine

L-Carnitine calibration standards were prepared at six concentration levels, 8, 16, 40, 80, 160, and 320 ng/mL, each containing 40 ng/mL IS. The MRM chromatogram for L-carnitine was a sum of signals for the transitions *m/z* 162 > 103 and 162 > 85. Similarly, the MRM chromatogram for the IS was a sum of signals for the transitions *m/z* 165 > 103 and 165 > 85. The QuanLynx software automatically calculated a response ratio, defined as the L-carnitine peak area divided by the IS peak area. Additionally, the software automatically plotted L-carnitine standard curves and calculated the concentration (ng/mL) of L-carnitine in the samples. We used the following equation to calculate the concentration (mg/100 g) of L-carnitine in milk powder: L-carnitine concentration (mg/100 g) = L-carnitine concentration. (ng/mL)/sample weight (g) × 0.25.

Method validation

Validation experiments were performed in a single laboratory on a single LC-MS/MS instrument. Linearity was evaluated against a calibration curve (n = 10). Precision was evaluated by repeatability. Repeatability was calculated from the analyses of infant formula, follow-up formula, raw materials, and SRM 1849a in duplicate on each of six days. Accuracy was evaluated by analysis of SRM 1849a and by performing spike recovery studies with infant formula, follow-up formula, and raw materials in duplicate on each of three days. In the recovery studies, each sample was fortified with L-carnitine reference standard at approximately 100% of the L-carnitine amount in each unspiked sample. Each sample was fortified with acetyl-L-carnitine at approximately 100% of the L-carnitine amount in each unspiked sample. The following equation was used to calculate the recoveries for each spiked sample:

$$\text{Recovery (\%)} = [(\text{empirical spiked concentration} - \text{average empirical unspiked concentration})/\text{theoretical spiked concentration}] \times 100$$

Each average empirical unspiked concentration was the average L-carnitine concentration of each unspiked sample in the precision studies. Detection and quantitation limits were not validated because all milk powders contained concentrations of L-carnitine well above these limits.

Determination of total L-carnitine content in commercial infant formulas and follow-up formulas

Twelve commercial products were collected from retail outlets, and their total L-carnitine content was determined by the described procedures.

Results and Discussion

Sample preparation

For quantitation of total L-carnitine content in infant formula, follow-up formula, and raw materials, it is necessary to remove protein and perform alkaline hydrolysis of short/medium chain acyl-L-carnitines. The alkaline hydrolysis conditions must assure that only short/medium chain acyl-L-carnitines are hydrolyzed and that L-carnitine is not decomposed. We have previously reported the optimal alkaline hydrolysis conditions for infant formula, follow-up formula, and raw materials. The optimal alkaline hydrolysis conditions are 0.2 M KOH solution (sample solution pH 13) at 40°C for 15 min.[13] In this study, alkali-hydrolyzed short/medium chain acyl-L-carnitines were investigated in infant and follow-up formulas; LC-QTOF-MS was used for the analysis, and accurate *m/z* values were calculated against standards. The *m/z* values, accurate mass results (ppm), and detection levels of short/medium chain acyl-L-carnitines are shown in Table 1. In the infant and follow-up formula samples, short/medium chain acyl-L-carnitines were of C1, C2, C3, C4, C5, and C8 types. The results showed that short/medium chain acyl-L-carnitines from C1 to C4 were completely hydrolyzed under the conditions used in the LC-QTOF-MS chromatogram. Most peaks were not detected (peak response <5000). In the case of C5 in the infant and follow-up formula samples, the peak responses were markedly decreased by 60 and 62%, respectively. On the other hand, the peak responses of C8 in the infant and follow-up formula samples without hydrolysis were 9460 and 7237, respectively, which represented lower concentration levels of medium chain acyl-L-carnitines of the C8 type. Because the unhydrolyzed sample peak levels were so low, the peaks of hydrolyzed C8 would not be detected in this system. Thus, the total short/medium chain acyl-L-carnitines were mostly changed to free L-carnitine. With respect to the hydrolysis conditions, several conditions have been reported; 1 M KOH solution at 40°C for 60 min,[11] 2 M KOH solution at 50°C for 30 min,[18] and 1 M HCl solution at 110°C for 15 min.[20] In comparison with these conditions, our optimal alkaline hydrolysis conditions are more safe, rapid and useful for the routine quality control assay of various formula samples.

LC-MS/MS analysis of L-carnitine

We used a multi-mode ODS column that contained ODS ligands, anion ligands, and cation ligands. The biggest benefit of the multi-mode columns was that the selectivity could be optimized by adjusting the mobile phase ionic strength, pH, and/or organic solvent. Consequently, these columns are not only complementary to reversed-phase columns but also provide different selectivities under different conditions.[22–25] The presence of both reversed-phase and ion-exchange functionalities requires no ion-pairing agent in the mobile phase to separate highly hydrophilic charged analytes, thus simplifying the mobile phase and making the method compatible with MS.[20,21] Previous reports suggest that mixed-mode columns capable of both reversed-phase and ion-exchange separation modes can be of some value for improved separation of polar substances.[26,27] Actually, these columns with gradient mode showed significant improvements in the peak shape of oligonucleotides.[26] Moreover, Stevenson *et al.* indicated that the retention mechanisms of these columns were changed under these pH environments for the mobile phase.[27] In this study, the separation of L-carnitine was investigated based on these reports. Mobile phase A was 0.3% formic acid aqueous solution and mobile

Table 1 Analysis of the alkaline hydrolysis of short/medium chain (C2 – C15) acyl-L-carnitines in infant formula and follow-up formula by LC-QTOF-MS

Short/medium chain acyl-L-carnitines	M_W[a]	M_W	Calculated *m/z* value	Actual *m/z* value[b]/ppm[c]	Detected *m/z* value/ppm			
					Infant formula		Follow-up formula	
	Average	Mono	$[M+H]^+$	$[M+H]^+$	Hydrolysis	Non-hydrolysis	Hydrolysis	Non-hydrolysis
C0	161.1995	161.1052	162.1131	162.1142 (–6.8)	162.1138 (–4.3)	162.1140 (–5.6)	162.1135 (–2.5)	162.1143 (–7.4)
C2	203.2363	203.1158	204.1236	204.1251 (–7.3)	ND[d]	204.1249 (–6.4)	ND	204.1255 (–9.3)
C3	217.2628	217.1314	218.1392		ND	218.1409 (–7.8)	ND	218.1409 (–7.8)
C4	231.2892	231.1471	232.1549		ND	232.1568 (–8.2)	ND	232.1571 (–9.5)
C5	245.3157	245.1627	246.1705		246.1721 (–6.5)	246.1704 (–0.4)	246.1696 (–3.7)	246.1720 (–6.1)
C6	259.3421	259.1784	260.1862		ND	ND	ND	ND
C7	273.3686	273.1940	274.2018		ND	ND	ND	ND
C8	287.3951	287.2097	288.2175	288.2198 (–7.9)	ND	288.2185 (–3.5)	ND	288.2211 (–12.5)
C9	301.4215	301.3353	302.2331		ND	ND	ND	ND
C10	315.4480	315.2410	316.2488		ND	ND	ND	ND
C11	329.4744	329.2566	330.2644		ND	ND	ND	ND
C12	343.5009	343.2722	344.2801		ND	ND	ND	ND
C13	357.5274	357.2879	358.2958		ND	ND	ND	ND
C14	371.5538	371.3036	372.3114		ND	ND	ND	ND
C15	385.5803	385.3192	386.3271		ND	ND	ND	ND

a. Molecular weight. b. Analysis of standard solutions (C1, C2, and C8). c. The relative mass accuracy, (*m/z* (experimental) – *m/z* (calculated))/*m/z* (calculated), is given in parts per million (ppm). d. Not detected is "peak abundance is <5000".

phase B was 100 mM ammonium formate aqueous solution/acetonitrile (50:50, v/v). The appearance of L-carnitine peak is singular and symmetric at 3.53 min. The IS (L-carnitine-d_3) peak demonstrates chromatographic behavior that matches that of native L-carnitine (Fig. S1, Supporting Information).

The MS conditions were initially developed by analyzing L-carnitine and IS solutions in the ESI-positive mode, which confirmed the predominant $[M+H]^+$ ions at *m/z* 162 and 165 for L-carnitine and the IS, respectively. The $[M+H]^+$ ions were chosen as precursor ions for ESI-MS/MS analysis and resulted in fragmentation of the parent $[M+H]^+$ ions into product ions. The transitions of *m/z* 162 > 85 and 162 > 103 were chosen for MRM of L-carnitine because the *m/z* 85 and 103 ions were the most intense in the product ions. Similarly, the ion transitions of *m/z* 165 > 85 and 165 > 103 were chosen for MRM of the IS. A single peak appeared at 3.47 – 3.58 min in all chromatograms, which confirmed the presence of L-carnitine in the milk samples. There was no interfering peak in any chromatogram despite minimal sample cleanup (Fig. S2, Supporting Information). The MRM chromatograms for the IS were similar and showed no interfering peak. Signal suppression of approximately 2 – 3% typically occurred when the hydrolyzed samples were analyzed, but the change in signal intensity was corrected against the IS.

Method validation

Linearity was evaluated for L-carnitine at 8 – 320 ng/mL by using 6-point standard curves. Table 2 shows the average results for least-squares linear regression analysis of the standard curves (n = 10). Linearity was obtained for L-carnitine with a corresponding correlation coefficient >0.999. Precision of the developed analytical method was determined by calculating repeatability. Repeatability was calculated from the analyses of infant formula, follow-up formula, raw materials, and SRM 1849a in duplicate on each of six days. The overall method RSDs were <5% (Table 3). Accuracy was evaluated by analysis of SRM 1849a. The value of SRM 1849a by our method was 13.9 ± 0.6 mg/100 g, with an RSD value of 4.5% (Table 3). Comparison with the SRM value (13.6 ± 1.4 mg/100 g) showed equivalence. The accuracy was also based on the recovery of

Table 2 Results for least-squares linear regression analysis of L-carnitine standard curves (n = 10)

	Slope	Intercept	r^2
Mean	0.9146	1.0586	0.9999
SD	0.0219	0.4933	0.0001

Table 3 Repeatability values from unspiked milk samples

Milk sample	L-carnitine concentration/mg 100 g^{-1} [a]	RSD, %
SRM 1849a	13.9 ± 0.6	4.5
Infant formula	10.4 ± 0.4	4.0
Follow-up formula	14.5 ± 0.5	3.3
Whey protein concentrate powder	82.4 ± 1.7	2.1
Whole milk powder	15.7 ± 0.4	2.5
Skim milk powder	20.7 ± 0.7	3.3

a. Number of replicates: 2 × 6. Values represent means ± SD.

Table 4 Recovery values from spiked milk samples[a]

Milk sample	Spiked L-carnitine[b]		Spiked acetyl-L-carnitine[c]	
	Recovery, %[d]	RSD, %	Recovery, %[d]	RSD, %
Infant formula	93.4 ± 3.7	4.0	104.1 ± 6.4	6.1
Follow-up formula	93.4 ± 3.3	5.3	104.1 ± 9.3	8.3
Whey protein concentrate powder	94.2 ± 3.4	3.6	109.3 ± 5.8	5.3
Whole milk powder	91.8 ± 4.9	5.3	98.9 ± 8.2	8.3
Skim milk powder	90.2 ± 3.9	4.4	103.2 ± 6.7	6.5

a. Number of replicates: 2 × 3. b. Spike level: 100% of the L-carnitine amount in each unspiked milk powder. c. Spike level: 100% of the L-carnitine amount in each unspiked milk powder. d. Values represent means ± SD.

Table 5 Total L-carnitine content in commercial infant formulas and follow-up formulas

Product[a]	Total L-carnitine concentration/mg 100 g^{-1} [b]	Label claim concentration/mg 100 g^{-1}
1	10.2	10.0
2	11.2	nd[c]
3	3.8	nd
4	9.5	nd
5	9.6	10.0
6	14.4	14.4
7	9.3	nd
8	8.0	nd
9	6.2	nd
10	8.6	nd
11	14.1	10.0
12	10.7	10.0

a. 1 – 6 are infant formulas and 7 – 12 are follow-up formulas. b. Mean, $n = 3$. c. nd: not declared.

L-carnitine content from infant formula, follow-up formula, and raw materials overspiked with L-carnitine at approximately 100% of the unspiked L-carnitine concentrations. The overall mean recoveries of L-carnitine were between 90.2 and 94.2%, with RSD values between 3.6 and 5.3%. The accuracy of the total L-carnitine determination was ascertained by overspiking with acetyl-L-carnitine. In addition, we used acetyl-L-carnitine to assess the completeness of the hydrolysis procedure and the accuracy of the total L-carnitine determination. Acetyl-L-carnitine was overspiked into samples at a concentration that would provide a free L-carnitine concentration of approximately 100% of that in each unspiked sample after hydrolysis. The overall mean recoveries of acetyl-L-carnitine content were between 98.9 and 109.3%, with RSD values between 5.3 and 8.3% (Table 4). Recovery values of spiked acetyl-L-carnitine were approx. 10% higher than those of spiked L-carnitine. We have reported the stability of L-carnitine under the hydrolysis conditions; 0.2 M KOH solution at 40°C for 60 min,[13] but several percent of L-carnitine was degraded. In case of spiked acetyl-L-carnitine, the hydrolysis was terminated when the free L-carnitine was produced from acetyl-L-carnitine. On the other hand, spiked L-carnitine was degraded a few percent during the hydrolysis process. We thought that a few percent degradation of the L-carnitine emerged as the difference between the recovery values.

Determination of total L-carnitine content in commercial infant formulas and follow-up formulas

The twelve various commercial formulas were assayed, and the total L-carnitine content is presented in Table 5 together with declared label claim when available. The formulas had diverse total L-carnitine content ranging from 3.8 to 14.4 mg/100 g because of their complex and varied compositions.

Conclusions

In conclusion, our method is sensitive, reliable, can be easily performed, does not require the use of ion-pairing reagents, and can be successfully applied to the determination of total L-carnitine content in infant formula, follow-up formula, and raw materials. The assay is rapid and useful for routine measurement of total L-carnitine content in various infant formulas and quality control samples.

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Supporting Information

Supporting information shows MRM chromatograms of L-carnitine and IS. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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