Poster Reprint

ASMS 2019
ThP203

Analyses of water-soluble and fat-soluble vitamins in fruits juice by triple quadrupole LC/MS

Seiya Tanaka[1], Masako Yorishita [2]

[1] Agilent Technologies Japan, Ltd.

[2] ITO EN, Ltd.

Introduction

Vitamin analyses for nutrition.

Vitamins are present in various vegetables, fruits, supplements, and energy beverages for consumption. Water-soluble and fat-soluble vitamins in food are analyzed using HPLC or microbiological assay by AOAC methods[1] or official methods based on Japanese food hygiene guidelines[2]. These methods require considerable time and labor for measuring a variety of vitamins. We examined a new LC/MS/MS method using triple quadrupole mass spectrometer to analyze various vitamins simultaneously in fruits juice.

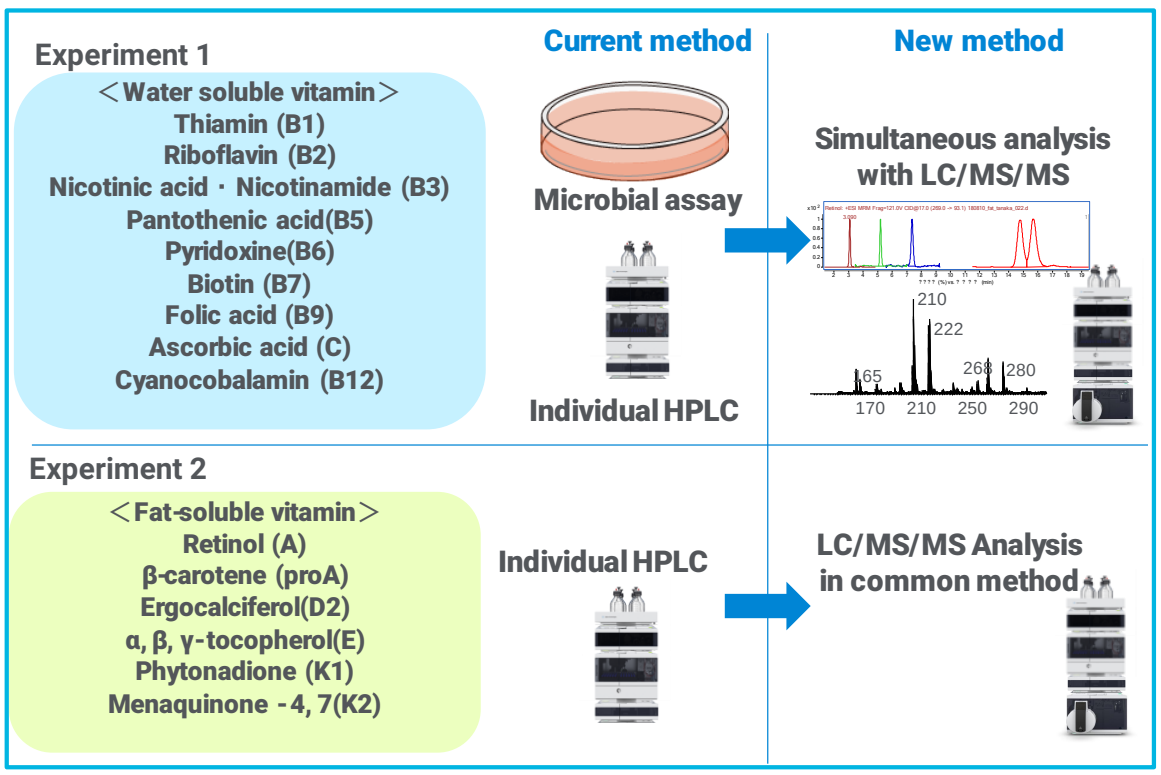


Figure 1 Outline of this study

Aim : To compare the results of a current official method and LC/MS/MS methods for the vitamin analysis in fruits juice.

Outline of experiment

Vitamin fortified beverage

Experimental 1

Water-soluble vitamin analysis by LC/MS/MS

Table 1 The method parameters for water-soluble vitamin in LC/MS/MS.

Method	
LC	1260 Infinity II Prime pump (G7104A) 1260 Infinity II Vial sampler (G7129A) 1260 Infinity II Diode array detector (G7117A)
Injection	1 µL
Flow rate	0.3 mL/min
Mobile phase	A : 10 mM ammonium acetate + 0.2 % acetic acid B : 5 mM ammonium acetate + 0.1 % formic acid in methanol
Column	InfinityLab Poroshell 120 PFP (2.1 x 150 mm, 2.7 µm, P/N : 693775-408)
Sample solution	10 % methanol
MS	Ultivo triple quadrupole LC/MS
Ionization	ESI (Agilent Jet Stream, AJS)
Polarity	Positive
Scan type	Dynamic MRM
Dry gas temp.	200 ˚C
Dry gas flow	5 L/min
Sheath gas temp.	350 ˚C
Sheath gas flow	11 L/min
Capillary voltage	2000 V
Nebulizer	45 psi
Nozzle voltage	0 V

Table 2 MRM transition and calibration curve for water-soluble vitamins

Compound name	Polarity	Frag (V)	Transition	CE (eV)	RT (min)	Dynamic range (µg/L)	R^2
Biotin	+	82	245.1 -> 227.0	9	7.76	0.3 – 560	0.9998
Cyanocobalamin	+	160	678.3 -> 147.0	32	7.61	0.0986 – 49.3	0.9998
Folic acid	+	82	442.2 -> 295.0	9	7.74	0.093 - 185	0.9989
Nicotinamide	+	121	123.1 -> 80.1	21	4.81	0.5 - 200	0.9991
Nicotinic acid	+	121	124.1 -> 80.1	21	2.69	0.125 - 125	0.9984
Pantothenic acid	+	82	220.1 -> 90.1	9	5.94	0.132 - 1315	0.9996
Pyridoxine	+	82	170.1 -> 152.0	9	3.02	0.117 – 23.3	0.9992
Tryptophan	+	84	205.1 -> 146.0	18	7.65	Analyzed only	Analyzed only

※Frag (Fragmentor), CE (Collision energy)

Results and Discussion

Vitamin-fortified drink (Itoen, Ltd.) 10g were extracted with 10 % methanol and fill up to 100 mL. The sample were analyzed with LC/MS/MS and microbial assay.

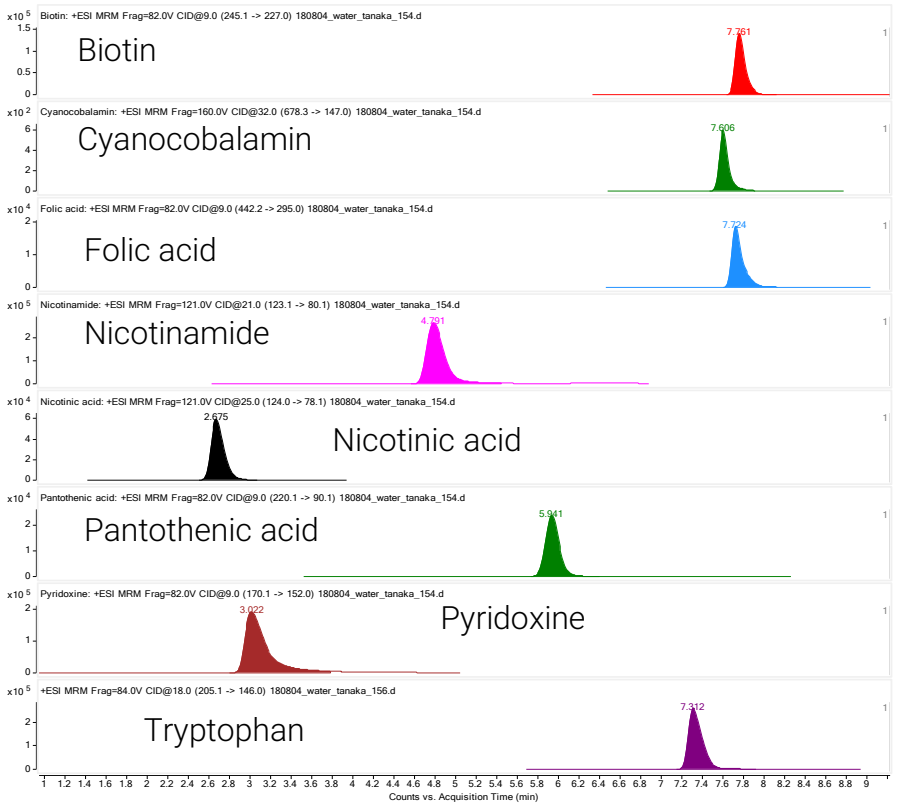


Figure 2 The MRM chromatograms of water-soluble vitamins in vitamin fortified beverage.

Table 3 Limit of quantitation of both methods microbial assay and LC/MS/MS.

	Limit of Quantitation (µg/L)	
Compound name	Microbial assay	LC/MS/MS
Biotin	0.024	0.3
Cyanocobalamin	0.002	0.099
Folic acid	0.016	0.093
Nicotinic acid	1	0.125
Pantothenic acid	1.6	0.132
Pyridoxine	0.1	0.117

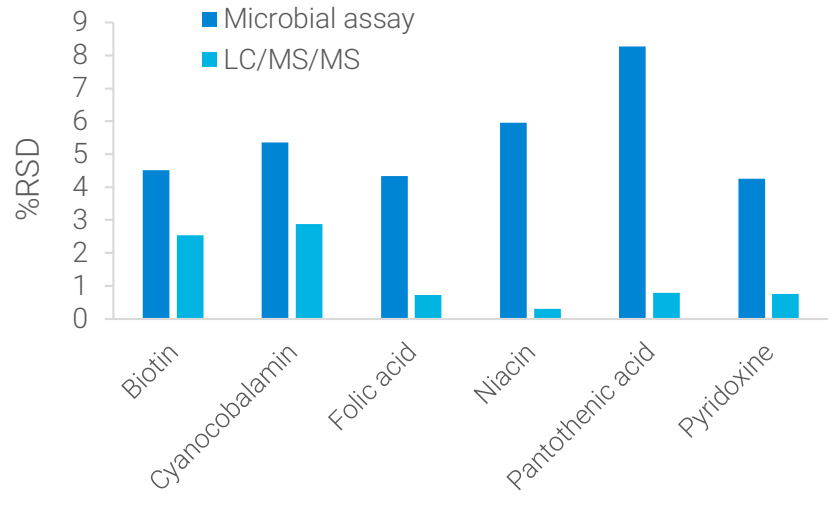


Figure 3 Repeatability of microbial assay and LC/MS/MS in water-soluble vitamins analysis

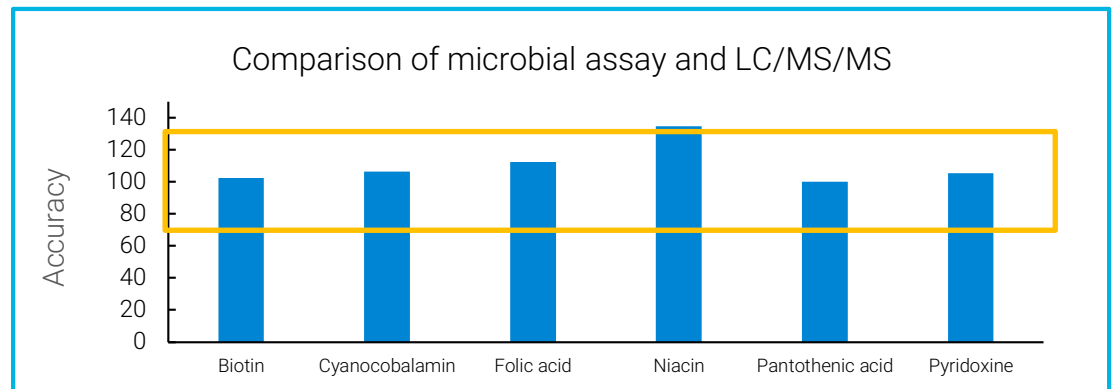


Figure 4 Quantitative results of water-soluble vitamin in vitamin fortified beverage.

Table 4 Quantitative results of water-soluble vitamin in vitamin fortified beverage.

Compound name	Unit	Microbial assay Concentration (※1 n=6-12)	LC/MS/MS Concentration (n=6)	Accuracy % (LCMSMS / Microbial assay)
Biotin	µg/100g	31.2	31.9	102.2
Cyanocobalamin	µg/100g	1.6	1.7	106.2
Folic acid	µg/100g	269	302	112.3
Niacin	mg/100g	5.42	7.29	134.5 ※2
Pantothenic acid	mg/100g	3.1	3.1	100
Pyridoxine	mg/100g	0.94	0.99	105.4

※1 Microbial assay was performed at n=12 and outliers were excluded.
※2 Different standards were used for both methods in quantitation of niacin.

We developed the simultaneous analysis of water-soluble vitamin with LC/MS/MS, the reproducibility of this method is less than 5 % . With the exception of niacin, the accuracy of quantitative concentrations were as good as 70-130 %.

Experimental 2

Fat-soluble vitamins in LC/MS/MS

Table 5 Method parameters for fat-soluble vitamin in LC/MS/MS.

Method	
LC	1260 Infinity II Prime pump (G7104A) 1260 Infinity II Vial sampler (G7129A) 1260 Infinity II Diode array detector (G7117A)
Injection	5 µL
Flow rate	0.9 mL/min
Mobile phase	A : 0.1 % Formic acid, B : Methanol
Column	YMC J2sphere ODS-H80 (3.0 x 75 mm, 4 µm, YMC CO., LTD.)
Sample solution	Acetonitrile
UV length	455 nm (Carotene), 325 nm (Retinol), 294 nm (Tocopherol)
MS	Ultivo triple quadrupole LC/MS
Ionization	ESI (Agilent Jet Stream, AJS)
Polarity	Positive
Scan type	Dynamic MRM
Dry gas setting	300 ˚C, 10 L/min
Sheath gas setting	350 ˚C, 10 L/min
Capillary voltage	3000 V
Nebulizer	45 psi
Nozzle voltage	0 V

Table 6 MRM transitions and calibration curve for fat-soluble vitamins

Compound name	Polarity	Frag (V)	Transition	CE (eV)	RT (min)	Dynamic range (µg/L)	R^2
α-carotene	+	160	537.5 -> 445.3	15	14.75	0.987 – 49.36	0.9989
β-carotene	+	160	537.5 -> 445.3	15	15.67	0.901 – 45.06	0.9991
Vitamin K1 (phytonadione)	+	160	451.4 -> 187.0	25	7.36	0.104 – 5.21	0.9995
Retinol (free)	+	121	269.0 -> 91.1	40	3.08	0.049 – 49.25	0.9993
α-tocopherol	+	160	431.4 -> 165.0	17	5.19	0.121 – 24.16	0.9991

※Frag (Fragmentor), CE (Collision energy)

Extraction for fat-soluble vitamins in LC/MS/MS

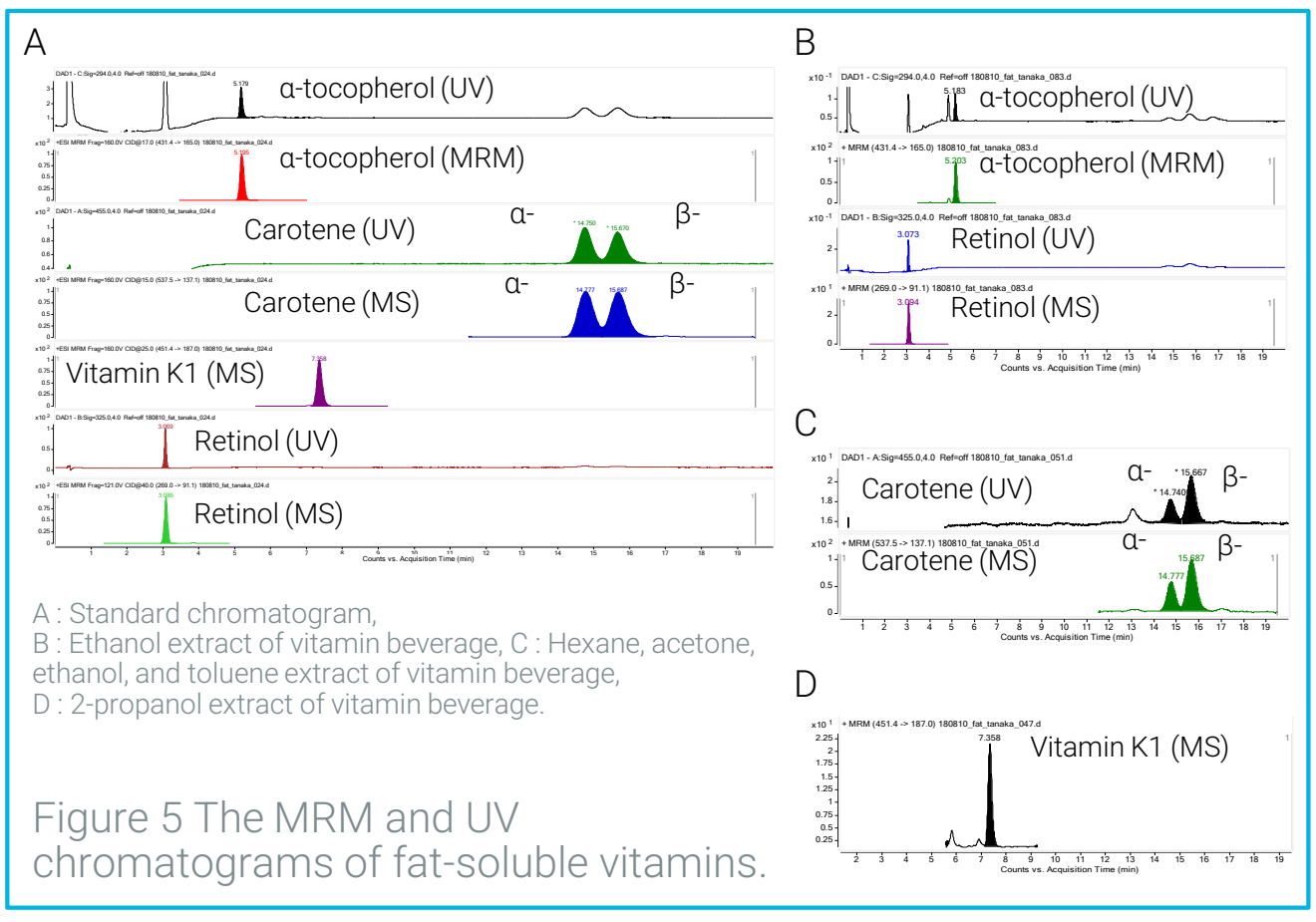
Vitamin fortified drink (Itoen, Ltd.) were extracted by each method , as shown in Table 7.

Table 7 Sample pretreatment

Compound	Extraction	Final sample solution
Retinol	After hydrolysis with KOH, extracted with hexane and ethyl acetate	Ethanol
α-, β-carotene	Hexane : Acetone : Ethanol : Toluene	Hexane : Acetone : Ethanol :Toluene
Vitamin K1 (Phytonadione)	Extracted with hexane and ethyl acetate	2 - Propanol

Results and Discussion

Fat-soluble vitamins were analyzed with HPLC or LC/MS/MS. By connecting a diode array detector in front of the mass spectrometer, UV and MS data can be acquired simultaneously.



A : Standard chromatogram,
B : Ethanol extract of vitamin beverage, C : Hexane, acetone, ethanol, and toluene extract of vitamin beverage,
D : 2-propanol extract of vitamin beverage.

Figure 5 The MRM and UV chromatograms of fat-soluble vitamins.

Table 8 Limit of quantitation of both methods HPLC and LC/MS/MS.

	Limit of Quantitation (µg/L)	
Compound name	HPLC	LC/MS/MS
α-carotene	24.68	0.987
β-carotene	22.53	0.901
Vitamin K1 (phytonadione)	Fluorescence	0.104
Retinol (free)	2.46	0.099
α-tocopherol	12.08	0.121

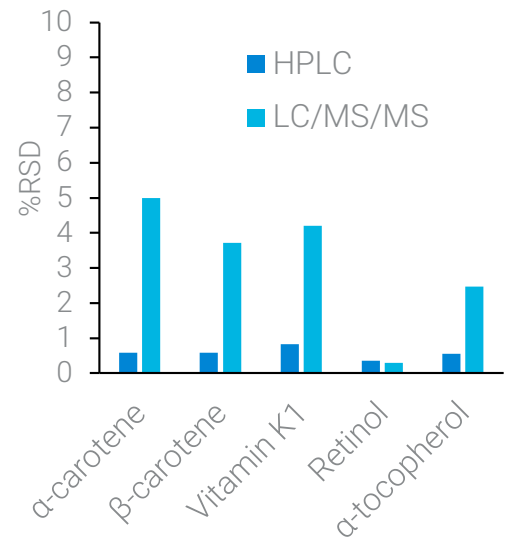


Figure 6 Repeatability of HPLC and LC/MS/MS in fat-soluble vitamin analyses.

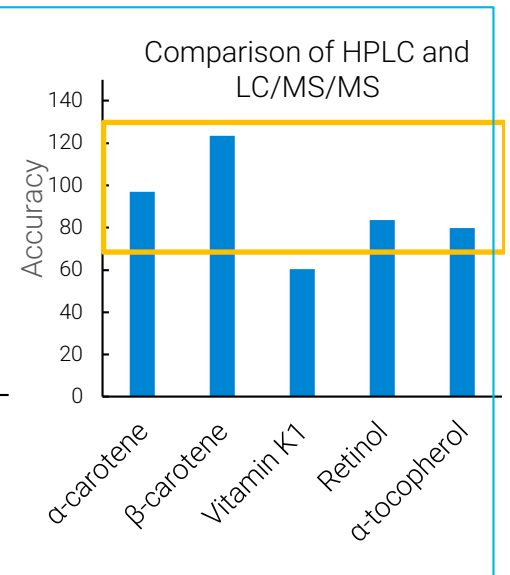


Figure 7 Quantitative results of fat-soluble vitamin in vitamin fortified beverage.

Table 9 Quantitative results of fat-soluble vitamin in vitamin fortified beverage.

Compound name	Unit	HPLC Concentration (n=6)	LC/MS/MS Concentration (n=6)	Accuracy % (LCMSMS / HPLC)
α-carotene	µg/L	455	441	96.9
β-carotene	µg/L	1047	1292	123.4
Vitamin K1 (phytonadione)	µg/L	6.8	4.1	60.3
Retinol (free)	µg/L	774	648	83.7
α-tocopherol	mg/L	11.14	8.90	79.9

Five fat-soluble vitamin were well separated, with good linearity (R^2>0.99) and a repeatability of less than 5%.With the exception of vitamin K1, the accuracy was within 70-130%. Compared to HPLC, LC/MS/MS was more than 10 times high sensitivity.

Conclusions

The novel LC/MS/MS method allowed easily, faster, and more accurate analysis compared with existing HPLC and microbial assays.

The quantitative values were almost identical in the microbial assay and the LC / MS / MS method. The fat-soluble vitamin, vitamin K1, was slightly different. It is necessary to evaluate the difference from the true value using certified reference materials (CRM).

References

[1] Official Methods of Analysis (OMA) of AOAC INTERNATIONAL Online.

[2] Standard Methods of Analysis in Food Safety Regulation in Japan. 2016

This information is subject to change without notice.

© Agilent Technologies, Inc. 2019
Published in USA, June 2, 2019

Agilent
Trusted Answers