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High speed data acquisition and polarity switching MS/MS applied to water-soluble vitamin analysis using a novel multi-mode ODS separation

ASMS 2011 MP165

Tetsuo Tanigawa[1], Toshikazu Minohata[1], Kiyomi Arakawa[1], Jun Watanabe[1], Itaru Yazawa[2], Andrew Herman[3]

[1] SHIMADZU CORPORATION, Kyoto, Japan

[2] Imtakt Corporation, Kyoto, Japan

[3] Imtakt USA, Philadelphia, USA

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Introduction

Vitamins are essential for healthy growth and development and the need to analyze multivitamin products in the food and pharmaceutical industries is becoming increasingly important. However, the analysis of water-soluble vitamins (B and C) is compromised by the polar nature and poor retention characteristics.

A mixed mode bonded silica chemistry using ODS+cation+anion ligands has the advantage that it can be used to separate highly polar compounds and exhibits similar behavior to ODS columns for non-ionic compounds whilst the IEX ligands provide a normal phase interaction for separation. This column chemistry was applied to the simultaneous analysis of water-soluble vitamins using fast polarity switching and high speed data acquisition MS/MS analysis.

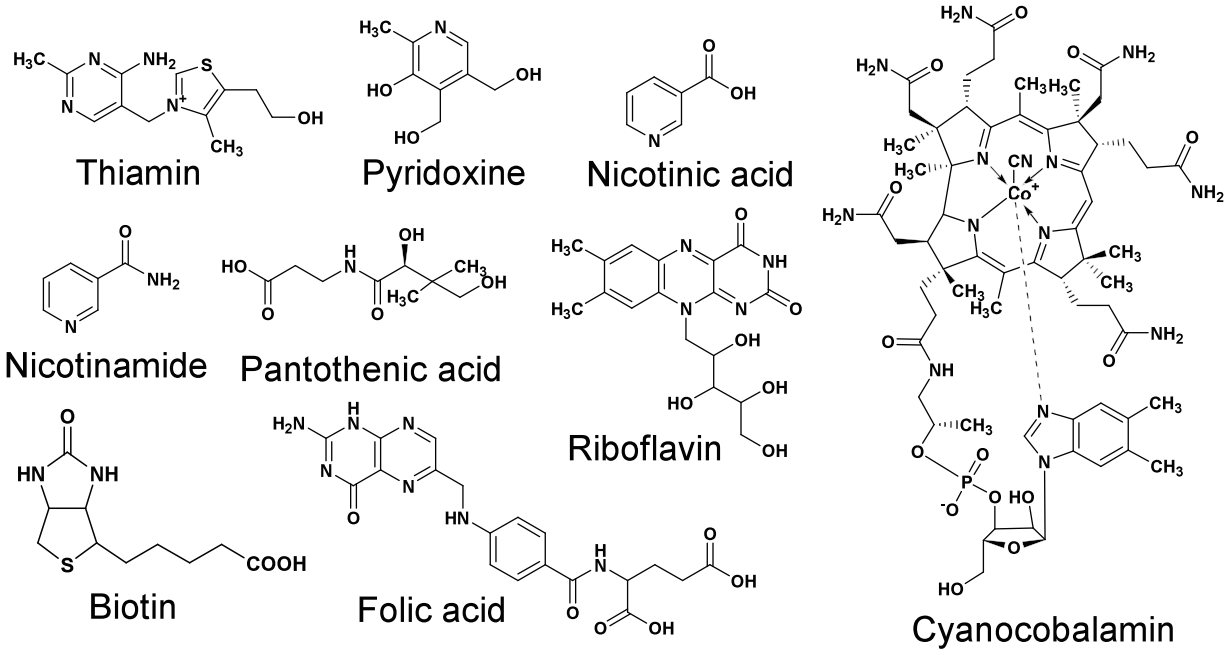

Fig. 1 Structre of 9 water-soluble vitamins

Materials and Methods

Water-soluble vitamins were determined by LC/MS/MS using a UFLC HPLC system coupled to a LCMS-8030 triple quadrupole mass spectrometer or LCMS-2020 single quadrupole mass spectrometer. Chromatographic separations were carried out using multi-mode column, Scherzo SM-C18 (2.0 mmI.D. x 150 mm, 3um) maintained at 40 °C. Vitamins were separated using a gradient elution with a flow rate of 0.2mL/min; solvent A, 5mmol/L ammonium formate / 0.1% (v/v) formic acid, and solvent B, acetonitrile.

MRM transitions and responses were optimized for individual compounds in both positive and negative ionization. The LCMS-8030 acquired positive and negative data using a polarity switching speed of 15msec and a data acquisition scan speed of 15000u/sec together with a pause time of I msec.

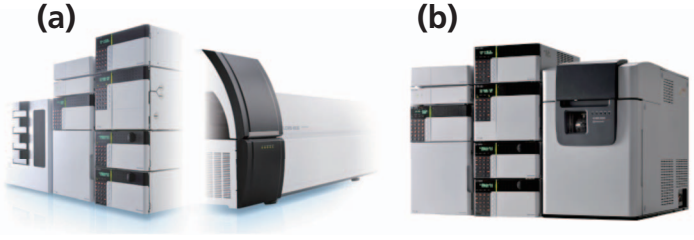

Fig. 2 (a) LCMS-8030 triple quadrupole mass spectrometer
(b) LCMS-2020 single quadrupole mass spectrometer

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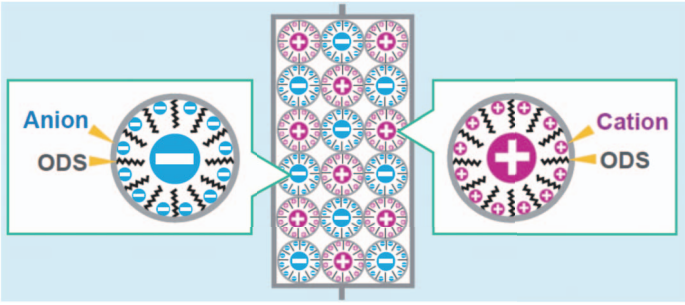

Fig. 3 Scherzo SM-C18 column; ODS column consists of C18 + anion + cation ligand

SM-C18 contains ODS+cation+anion ligands and can be used to separate highly polar compounds. Unlike other mixed-mode RP columns, Scherzo SM-C18 contains ODS ligands, and shows similar behavior to conventional ODS columns for non-ionic compounds. In addition, the IEX ligands on SM-C18 are highly polar and provide normal phase mode. This allows for both RP + NP separation mode.

Results

Method Development for Water-soluble Vitamins

A mixed mode ODS+cation+anion ligands bonded phase has been used for the separation of water-soluble vitamins using ammonium formate buffer and acetonitrile. The results show that this phase exhibits high selectivity for water-soluble vitamins with differing retention mechanisms. Anion exchange interaction influenced the retention of nicotinic acid (B3) and L-ascorbic acid (C), nicotinamide (B3) was retained using a weak cation exchange interaction and the chromatographic behaviour of riboflavin is almost completely derived from hydrophobicity (similar to conventional ODS).

The method resolved 9 water-soluble vitamins simultaneously, each compound was detected by SIM or an optimized MRM transition using fast polarity switching (15msecs), high scan speed (15,000u/sec) and short pause times (1msec). Folic acid (B9) was detected using negative ion, all other water-soluble vitamins were detected using positive ion. Calibration curves and chromatograms for the vitamins between 0.5 and 500 pg/uL were generated (r2 typically >0.99). Linearity of the MS/MS response was observed over three orders of magnitude with limits of quantitation in the 0.5 pg/uL range for all of the analytes.

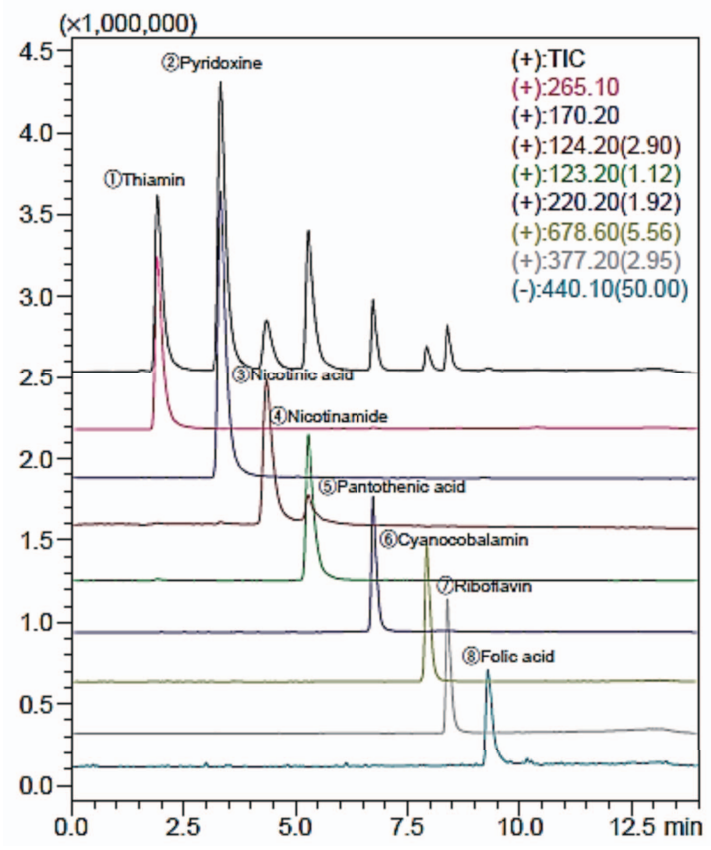

Fig. 4 LC-MS simultaneous analysis of 8 vitamins

HPLC condition

Column : Scherzo SM-C18, 150 x 2 mm
Solvent A : 5 mmol/L ammonium formate + 0.1% formic acid
B : acetonitrile
Flow rate : 0.2mL/min
Gradient table : 0 - 55 %B (0-10 min)
0 %B(10.01-20 min)
Sample : 1,000 pg/uL each, 1uL

Mass condition

LCMS-2020, SIM mode

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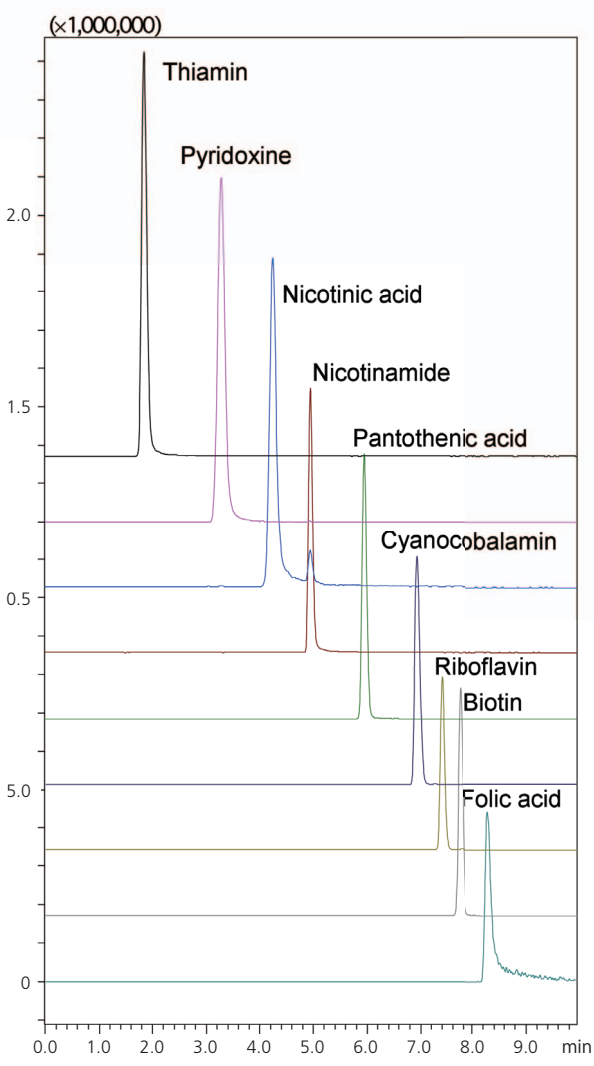

HPLC condition
Same as above condition
Mass condition
LCMS-8030, MRM mode

Compound	Transition
Thiamin	266>122
Pyridoxin	170>152
Nicotinic acid	124>80
Nicotinamide	123>80
Panthothenic acid	220>90
Cyanocobalamin	678.6>147
Riboflavin	377>243
Biotin	245>227
Folic acid	442>295

Fig. 5 LC-MS/MS simultaneous analysis of 9 vitamins

(a)

	m/z	range (ng/mL)	coefficient (r2)
Thiamin	226.1>122.1	0.5-500	0.9999
Pyridoxin	170.1>152	1-500	0.9993
Nicotinic acid	124>80	5-1000	0.9993
Nicotinamide	123>80	5-1000	0.9995
Panthothenic acid	220.1>90	5-100	0.9999
Cyanocobalamin	678.5>146.8	50-10000	0.9992
Riboflavin	377.2>242.8	10-1000	0.9999
Biotin	245.1>227.1	5-5000	0.9993
Folic acid	442.2>295.1	10-5000	0.9993

(b)

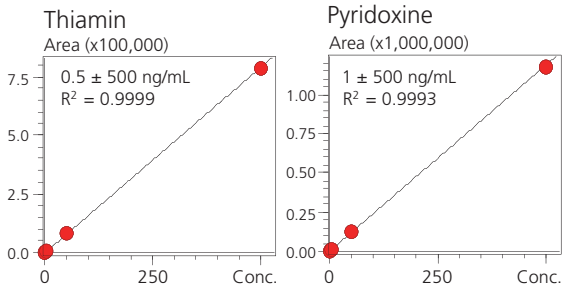

Fig. 6 (a) Linearity evaluation of 9 vitamins
(b) Representative calibration curve (Thiamin, Pyridoxine)

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Quantitative Analysis of Water-soluble Vitamins from Serial

This approach was applied to measurement of commercial vitamin supplements and to meet the needs of regulatory bodies (FDA has implemented a "current good manufacturing practices" policy to ensure dietary supplements produced in a quality manner, do not contain contaminants or impurities, and are accurately labeled). The results of commercial vitamin supplements analysis by mixed mode ODS combined with fast polarity switching, high speed MS/MS data acquisition were in agreement with manufacturers published data.

10 tablets
↓
Add 10mL diluting Solution*1 and mix for 30 seconds then heat (65℃, 5min) *2
↓
Mix for 30 seconds, Centrifuge (12,000rpm, 5min)
↓
Centrifuge of supernatant (3,000rpm, 10min)
↓
Filtrate (0.45um filter) of supernatant

*1:Water/Acetonitrile/acetic acid=94/5/1
*2:2 times for mix & heat

Fig. 7 Sample preparation from serial

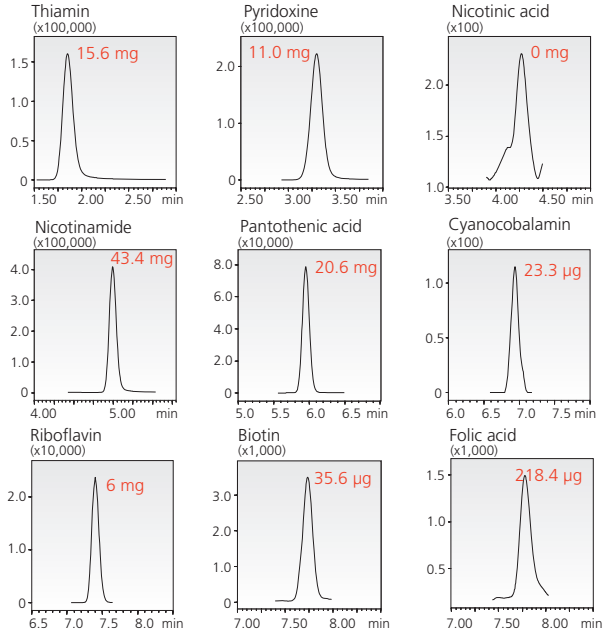

Fig. 8 Quantitation of vitamins in serial

Conclusion

Water-soluble vitamins were analyzed simultaneously and quantitated high sensitively by multi-mode column Scherzo using LC-MS/MS without ion-pair solvent.

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SHIMADZU CORPORATION. International Marketing Division
3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan
Phone: 81(3)3219-5641 Fax. 81(3)3219-5710
URL http://www.shimadzu.com