

INTRODUCTION

- B-vitamins and related metabolites are essential co-factors vital for human health and normal functions.
- Comprehensive profiling better detects functional deficiencies than single-nutrient tests.
- Plasma analysis reflects current population status, guiding the need for interventions or fortifications.

OBJECTIVE

Develop a LC-MS/MS method for analyzing

- Vitamin B1: thiamine (T), thiamine-monophosphate (TMP), thiamine-diphosphate (TDP)
- Vitamin B2: riboflavin (R), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN)
- Vitamin B3: nicotinamide (NAM), nicotinic acid (NAc), nicotinamide mononucleotide (NMN), nicotinamide adenine dinucleotide (NAD), nicotinamide riboside (NR), nudifloramide (2PY),
- Vitamin B6: pyridoxal (PL), pyridoxal-5-phosphate (PLP), pyridoxine (PN), pyridoxamine (PM), 4-pyridoxic acid (4-PAc), 4-deoxypyridoxine (DPN)
- Cholines: choline (Cho), phosphocholine (PC), glycerophosphocholine (GPC)
- Pantothenic acid (B5), biotin (B7), methylmalonic acid (MMA), myo-inositol (myo), betaine (Bet), carnitine (Car), creatinine (CR), methionine (Met), trimethylamine-N-oxide (TMAO).

METHODS

Optimized LC-MS/MS method

UPLC-MS/MS: SCIEX ExionLC-AD → 6500+ QTRAP MS

Columns

- (1) Phenomenex Gemini NX-C18, 100x3mm, 3μm
- (2) Phenomenex Synergi Fusion-RP, 100x3mm, 2.5μm
- (3) Phenomenex Luna Silica (2), 100 x 2mm, 3μm

Solvents

- (1) A: 10mM (NH₄)HCO_{3(aq)} + methanol (MeOH), RP, 5min run time
- (2) A: 0.2% acetic acid_(aq) + acetonitrile, RP, 6min run time
- (3) A: 0.1% propionic acid_(aq) + 0.1% acetic acid_(MeOH), NP, 5min run time

Detection

- MRM in positive and negative mode electrospray ionization

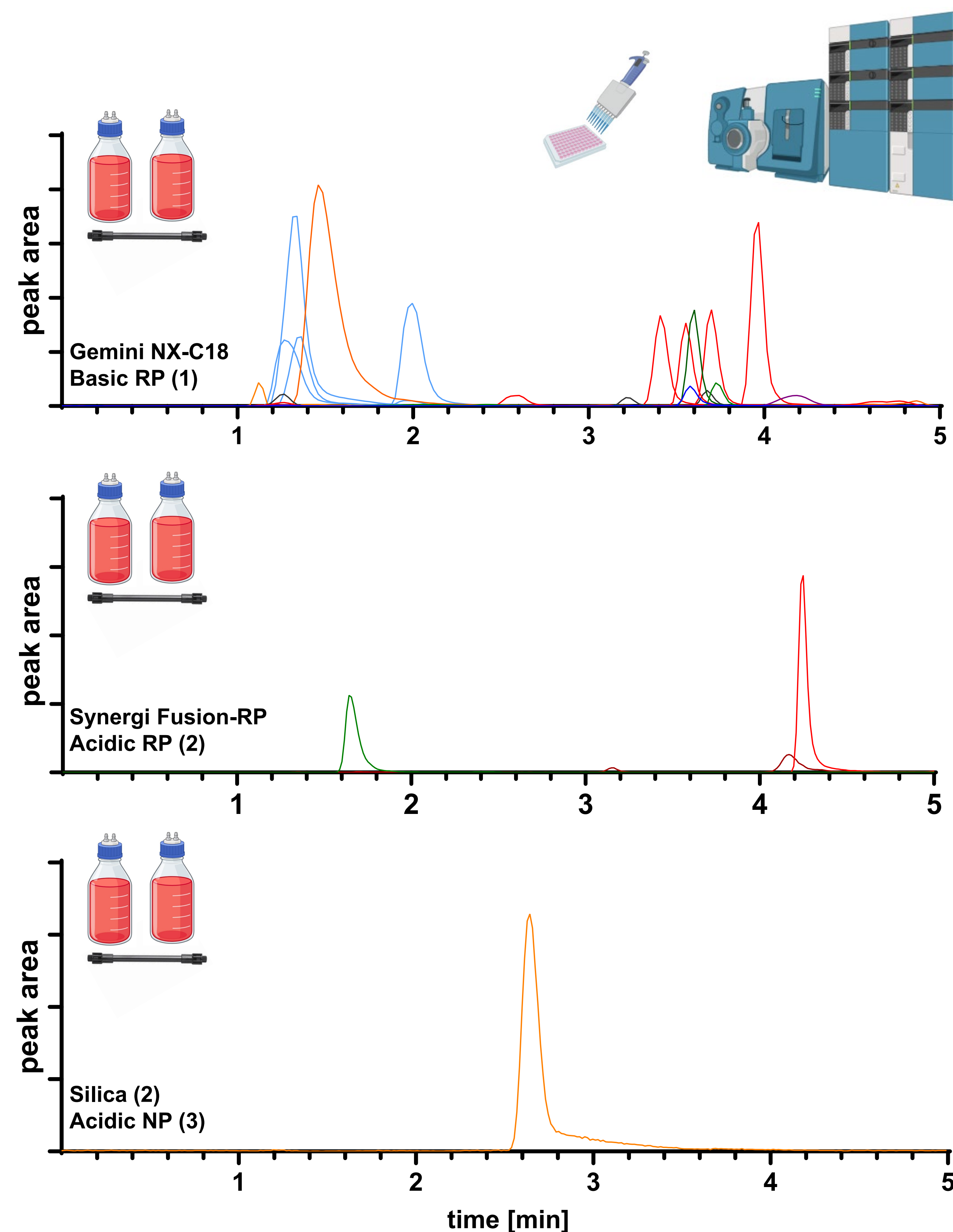
Sample preparation

- Plasma (30μL) was deproteinized with MeOH/internal standard mix
- Dried sample extract was reconstituted in 0.2% acetic acid, filtered, and analyzed

RESULTS

Optimized chromatograms

- Optimized conditions result in 5-6min analytical run time in 3 injections to avoid the use of iron pairing reagents



Total ion chromatograms for B-vitamins, cholines, and related metabolic co-factors. (1): T, TMP, TPP, R, FAD, FMN, NAM, NAc, NAD, NR, 2-PY, PA, PL, PN, PM, DPN, 4-PAc, B7, Cho, PC, Bet, Car, CR, MET, TMAO; myo (2): NMN, MMA, PLP; (3): GPC; (RP: reversed phase, NP: normal phase)

RESULTS

Analyte recovery

- Evaluated on 3 different days at 3 levels and 4 replicates each using a plasma pool (UTAK)
- In lieu of an available certified standard for all analytes, Chromsystems(CS) B6 and MMA plasma standards were used (n =3)

Analyte	Conc. ¹ [μg/L]	Recovery AVG [%]	CV [%]	Analyte	Conc. [μg/L]	Recovery AVG [%]	CV [%]
T	3.8	110.8	8.2	PM	1.8	72.7	15.2
TMP	n.d.	123.5	10.8	PN	n.d.	78.5	9.0
TPP	7.6	85.8	11.4	4-PAc	27.9	100.1	9.7
R	3.6	117.5	8.7	DPN	0.94	89.5	9.4
FAD	19.9	126.1	9.2	B7	1.1	110.5	9.7
FMN	2.3	113.1	5.8	MMA	24.6	101.2	8.4
NAM	6.9	128.6	6.5	Cho	1898	108.1	7.4
NAc	n.d.	119.5	10.5	PC	13.7	116.4	4.5
NAD	3.7	117.9	11.5	GPC	1371	98.8	8.7
NMN	6.6	94.4	8.6	Bet	3887	95.7	8.5
NR	29.2	112.5	10.3	Car	4114	124.9	9.2
2-PY	618	103.7	9.2	CR	6418	104.5	9.6
PA	129	105.9	7.0	Met	3233	94.7	8.9
PL	16.0	107.3	8.7	TMAO	697	108.7	7.6
PLP	27.2	99.3	9.2	myo ²	8.8	45.7	10.4

¹CVs ranged from 0.6-14.7%. ²myo-inositol in mg/L.

- ChromSystems accuracy (CV):
 - PL = 100% (2.6), PLP = 104% (9.0), MMA = 99.9% (3.58)
- This method is currently employed for nutrient assessment in large-scale studies, such as the MILQ/E-MILQ Study and the MOMI Consortium.

CONCLUSIONS

- We successfully developed a LC-MS analysis of 30 B-vitamins/cholines and metabolic co-factors, without the use of ion pairing reagents.
 - Ion pairing may increase analyte coverage in a single run but negatively affects columns and MS sensitivity.
- One sample preparation → 3 analytical runs in the 96-well plate format.
- The wide variety of LC-MS conditions allows to extend the current analyte portfolio for future needs.
- Whole blood and red blood cells are currently validated.
- This platform is effective for assessing interventions, fortifications, and relationships among mother, milk, and infant nutritional status.

REFERENCES

Hampel *et al.* 2022, *Current Developments in Nutrition*, 6, 766

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