



Stable Isotopes as Tools for Evaluating Vitamin Contents and Bioactivity

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Outline



1. Introduction: Stable isotopes, folates
2. Stable isotopes for accurate quantitations: SIDA
3. SIDA in bioavailability studies
4. Double isotope studies: isotopes as tracers
and as standards
5. Visions for stable isotope applications

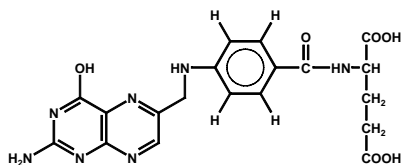


The Atomar View: Natural Stable Isotopes

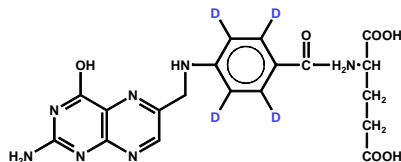
Element	Isotope	%
Hydrogen	H-1	99.985
	H-2 (Deuterium)	0.015
Carbon	C-12	98.89
	C-13	1.11
Oxygen	O-16	99.759
	O-17	0.037
	O-18	0.204
Nitrogen	N-14	99.63
	N-15	0.37
Sulfur	S-32	95.02
	S-33	0.75
	S-34	4.21

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Examples of isotopologically labelled compounds



Analyte:
Folic acid M_r : 441 g/mol



Isotopologic Standard
for Quantitation:
 $[^2\text{H}_4]$ -Folic acid M_r : 445 g/mol

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Prinziple of the Stable Isotope Dilution Assay (SIDA)

Isotopologic Standard

Analyte

Clean-up

Derivatization

MS

RT: 14.47

RT: 14.51

Time (min)

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Folates: Introduction

Folates involved in methylations of amino acids and nucleotides

C1=NC2=C(N1)N=CN=C2CNC3=CC=CC=C3C(=O)NC(C(=O)O)CC(=O)O

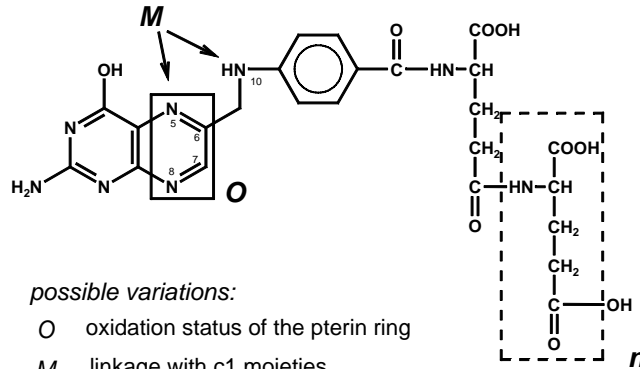
Average intake in Europe covers only 50 % of the recommended dose

Evident / possible consequences of deficiency:

- neural tube defects
- elevated plasma level of homocysteine
⇒ coronary heart disease
- colon cancer ??????
- alzheimer's disease

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Structural Variety of Folates



possible variations:

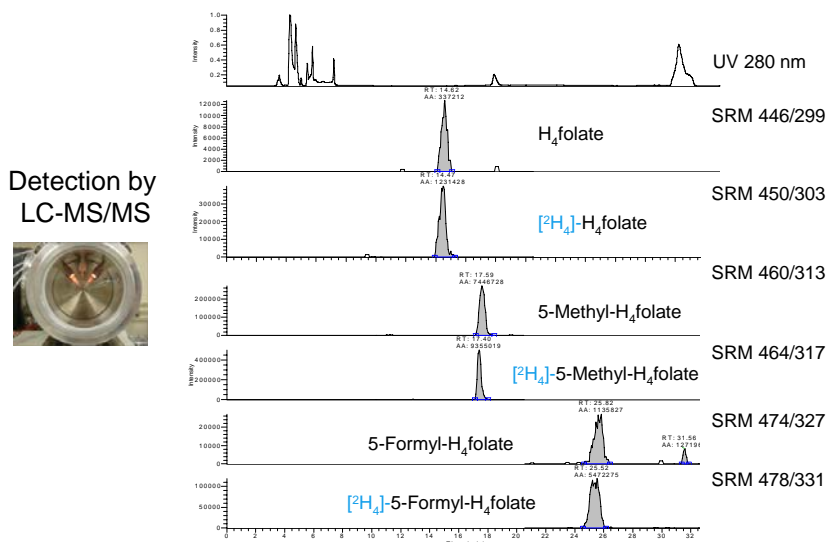
- O oxidation status of the pterin ring
- M linkage with c1 moieties
- n number of glutamate moieties

Already known natural vitamers: about 50 compounds
labile, occurring only in traces
all vitamers have to be quantified



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SIDA of Folates in Spinach



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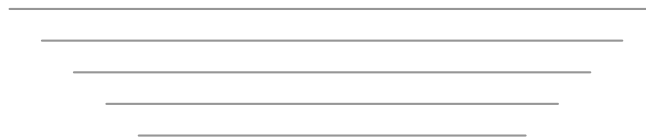
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Foods Rich in Folates

µg/100g	SIDA Own data	LC-FD Lit. data	MA Lit. data
Spinach	96 – 159	100 – 145	143 - 338
Camembert cheese	48 - 65	-	55 - 96
Wheat germs	318	-	190 - 520
Brokkoli	27 - 62	111 - 114	63
Mungo beans	277	-	438 - 625
Whole-meal bread	7 – 58	27 – 33.2	39 - 51

Not only contents, also **bioavailability** of food folates is important for dietary recommendations!
Current assumptions: 50 % ⇒ definition dietary folate equivalents to max 80 % for added folic acid to foods

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Bioavailability: Design of a first human study



- 26 healthy volunteers
- Saturated with folate by gavage of folic acid before the trial
- Design: random cross-over in ranges of 1 week:
11 blood samplings within 24 h

- Meal A: 500 g spinach
- Meal B: 50 g wheat germs
- Meal C: 200 g Camembert cheese
- Meal D: 400 µg folic acid in water



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Design of a first human study



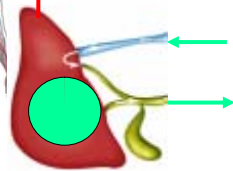
5-Me-THF



[²H₄]-5-Me-THF (IS)

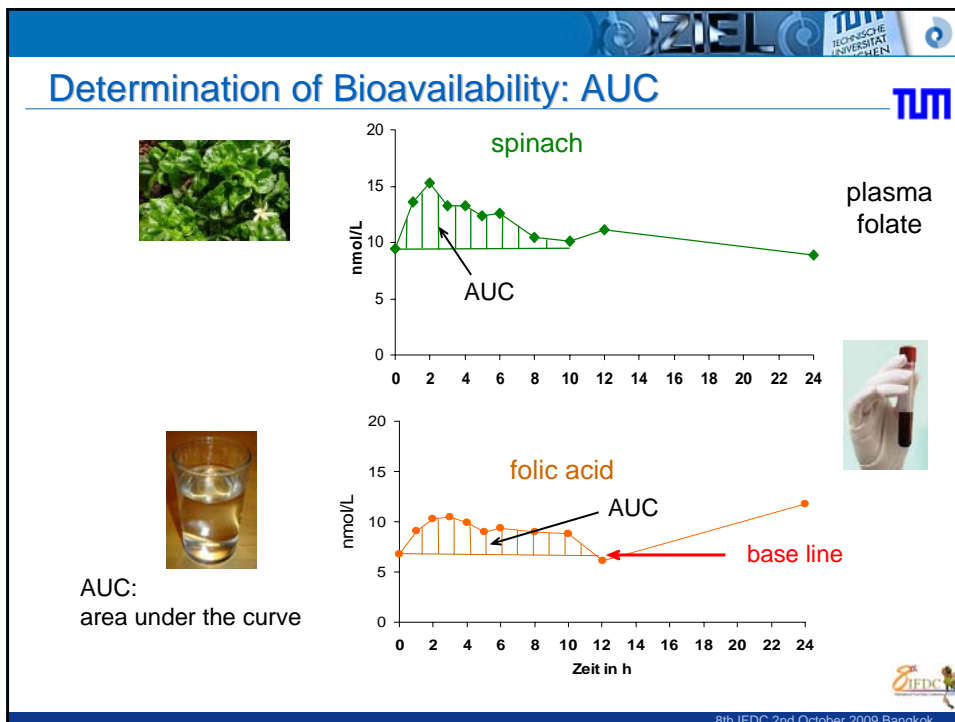


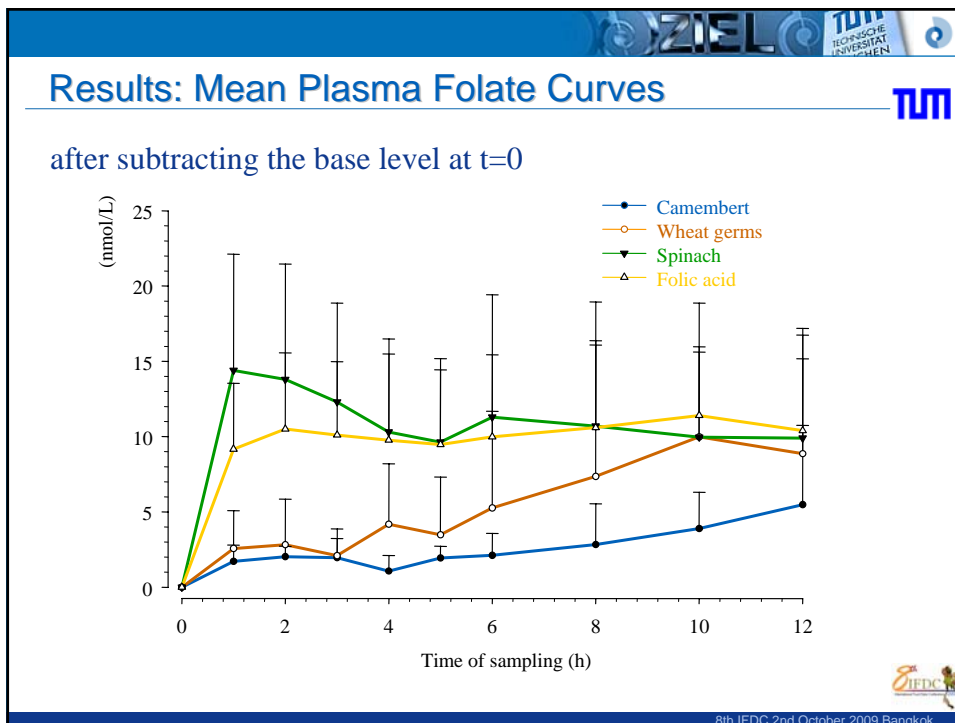
[²H₄]-Folate (IS)






Endogenous Folates

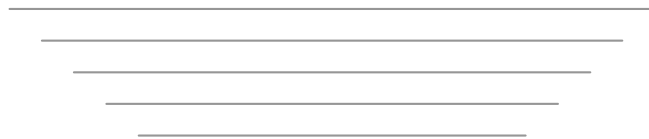
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Results			
			
Bioavailability relative to folic acid = 100 %	Spinach 523 µg	Wheat germs 236 µg	Camembert 254 µg
Lower limit of 90 % confidence interval (AUC)	43.4 %	19.5 %	11.4 %
Mean (AUC)	73.0 %	33.0 %	19.9 %
Upper limit of 90 % confidence interval (AUC)	122.9 %	56.0 %	34.7 %

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Summary of the First Study	
•Bioavailability of certain food folates:	Spinach 70-80 %, wheat germs appr. 30%, camembert 20%
•High inter individual differences (appr. ±100 %)	
•Definition of dietary folate equivalents (50 % bioavailability of food folates) is still justified	

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First Study: Open Questions

5-Me-THF

$[^2\text{H}_4]$ -5-Me-THF (IS)

$[^2\text{H}_4]$ -Folate (IS)

Endogene Folate

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Further isotopologically labelled folates

Analyte:
Folic acid M_r : 441 g/mol

Isotopologic Standard for Quantitation:
 $[^2\text{H}_4]$ -Folic acid M_r : 445 g/mol

Isotopologic Standard for Tracing
 $[^{13}\text{C}_5]$ -Folic acid M_r : 446 g/mol

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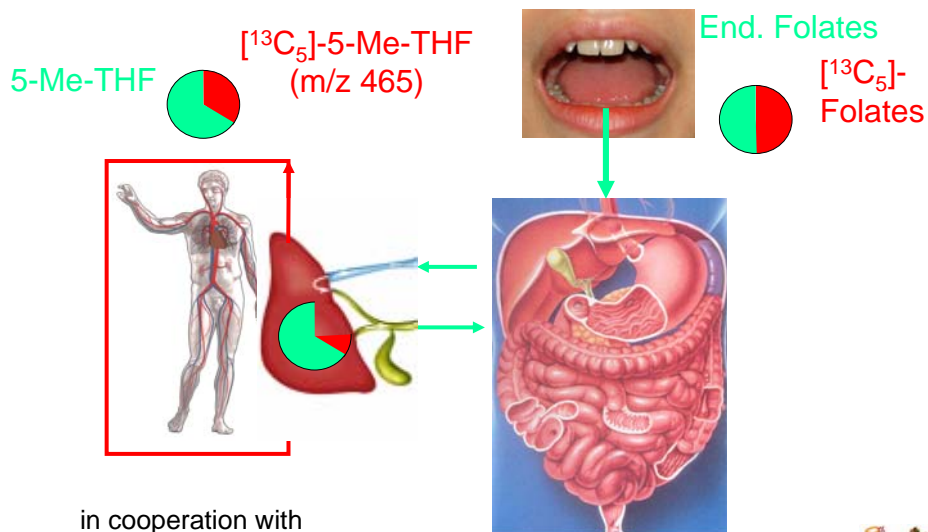


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Next Study: Stable Isotope as Tracer



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Next Study: Double Isotope Model

5-Me-THF

$[^{13}\text{C}_5]$ -5-Me-THF (m/z 465)

$[^2\text{H}_4]$ -5-Me-THF (IS, m/z 464)

End. Folates

$[^{13}\text{C}_5]$ -Folates

$[^2\text{H}_4]$ -Folates (IS)

in cooperation with C. Withhöft, Uppsala

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Differentiation of Isotopologues in TripleQuadMS

Multiple Reaction Monitoring

A

m/z 460

m/z 313

B

m/z 464

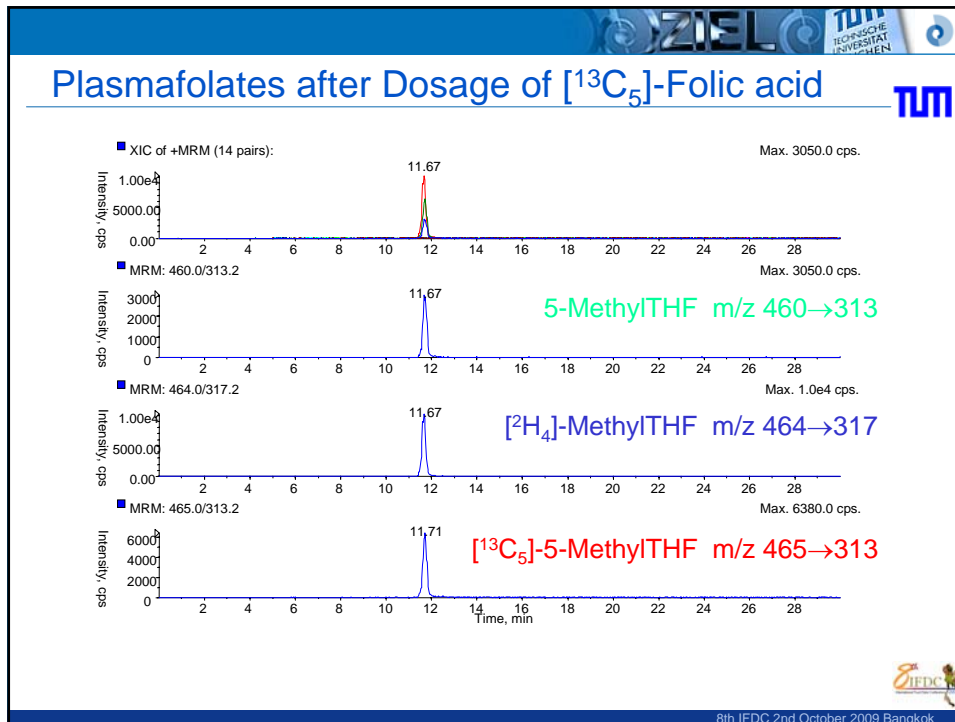
m/z 317

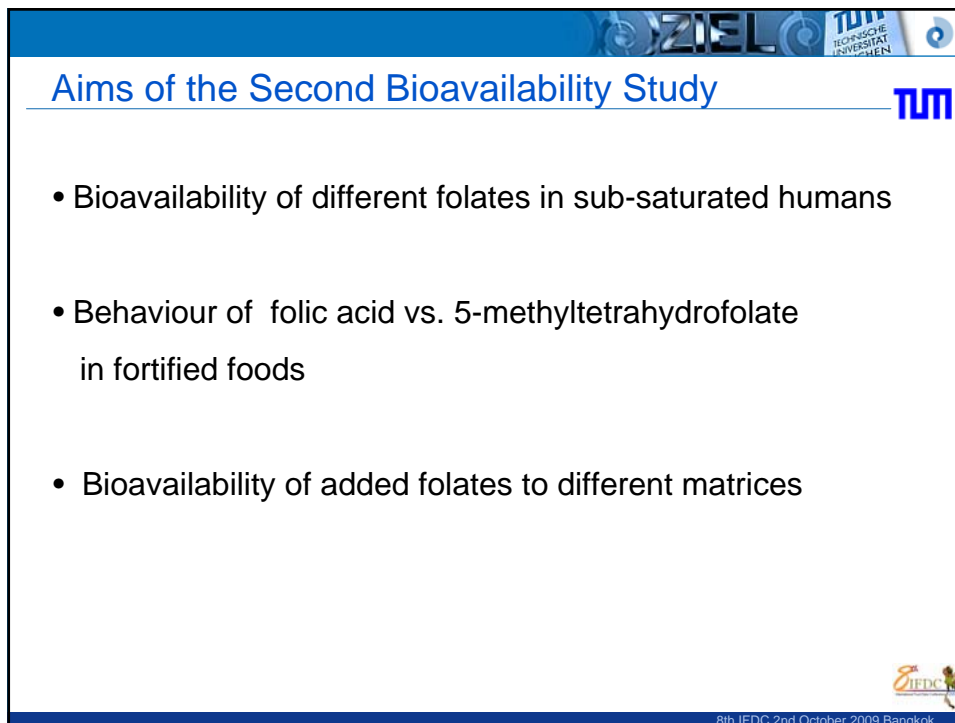
C

m/z 465

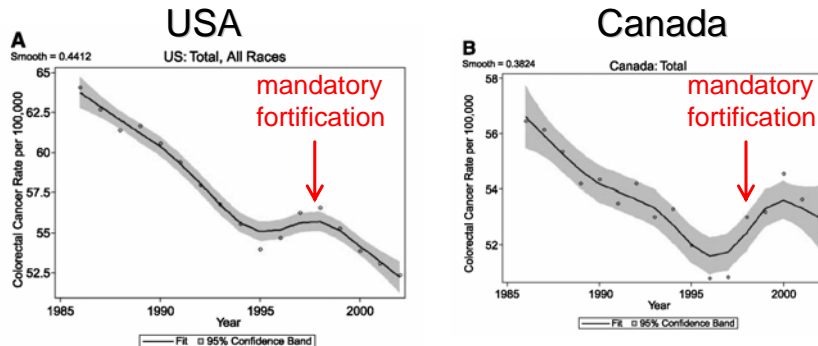
m/z 313

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Hazard from Fortification with Folic Acid?



Colorectal Cancer incidence pre- and post-mandatory fortification

Source: Mason et al., Cancer Epidemiology, Biomarkers & Prevention (2007) 16: 1325



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Visions for the Use of Isotopes in Vitamin Research



- Tracing vitamins from fortified foods
Bioavailability (fortification efficiency)
Metabolism (benefit or hazard)



- Multivitamin assays
Need for different labels, differentiation possible?

- Intrinsic labelling

for tracing of endogenous vitamins

for production of complex vitamins



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