Analysis of Chemopreventive Agents in Foods Commonly Consumed in Hawai‘i

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Shared Resources: Analytical Laboratory, Nutrition Support
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www.crch.org

FOOD ANALYSES

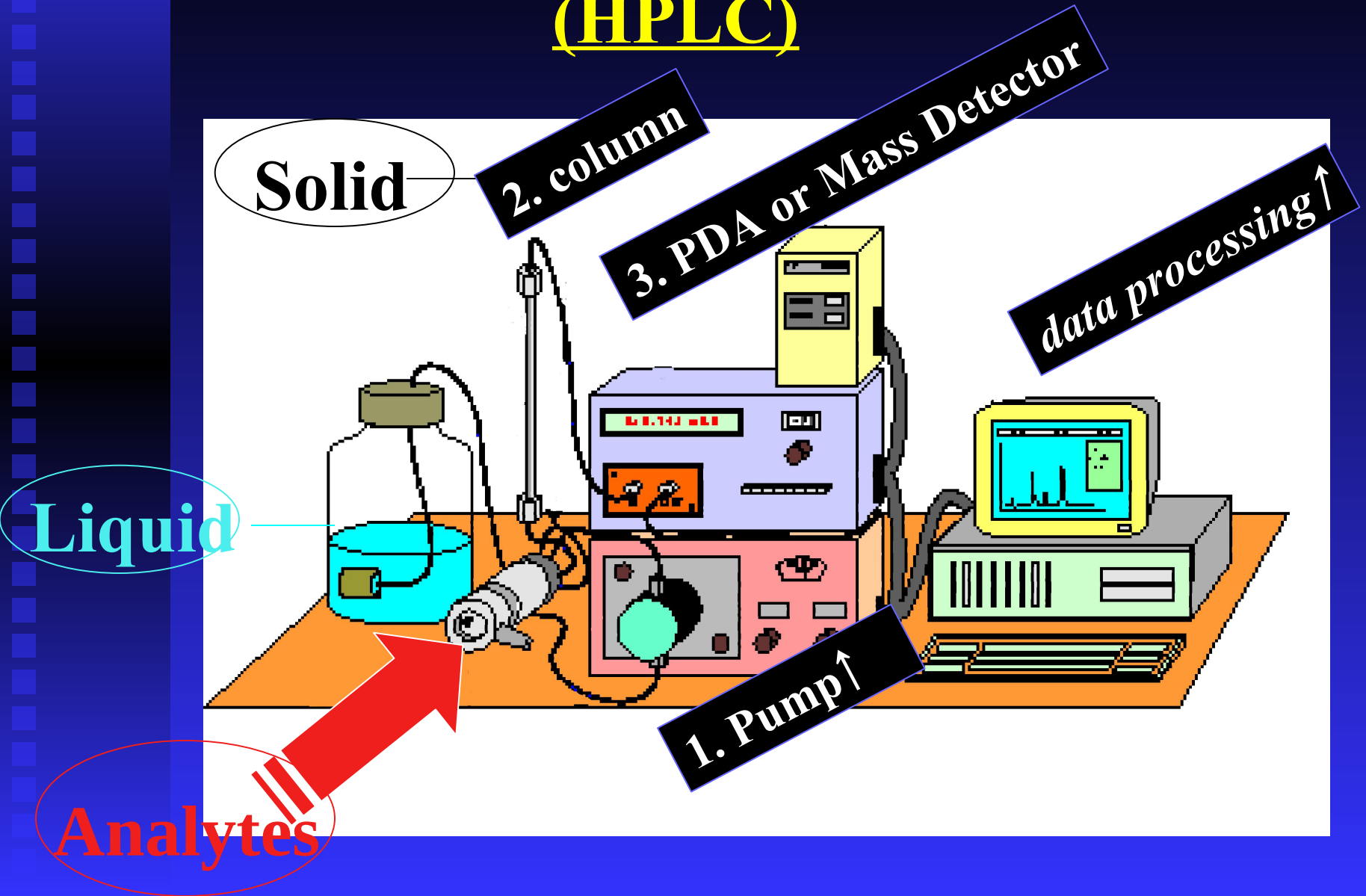
A) Analytical Techniques

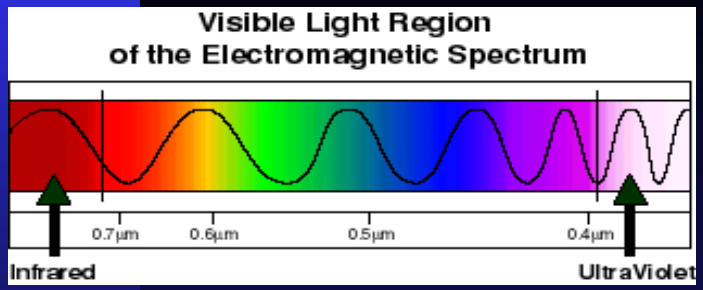
B) Levels in Foods in Hawai‘i

- **Hydrophilic Phytochemicals**
- **Lipophilic Phytochemicals**

due to incomplete data

High Pressure Liquid Chromatography (HPLC)





656 nm
H_2=C-line

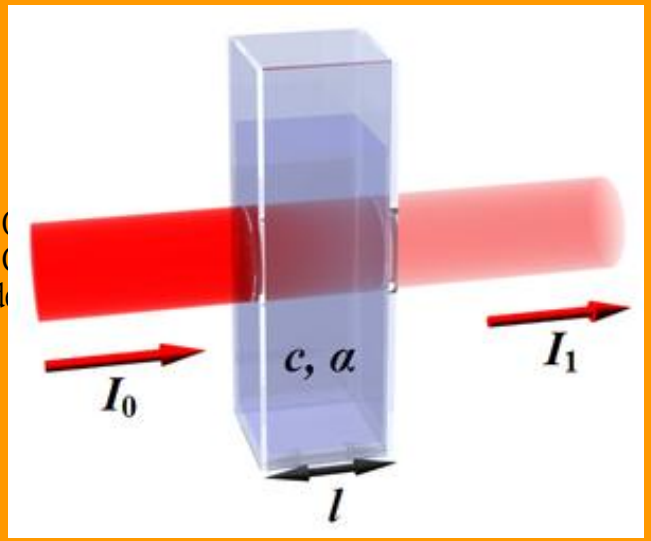
589 nm
Na=D-line

486 nm
H_2=F-line

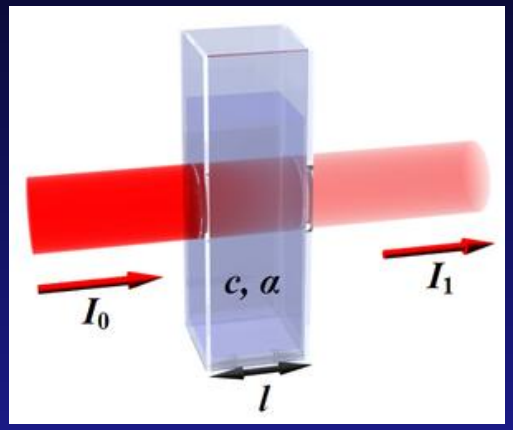
Molecules interact with light

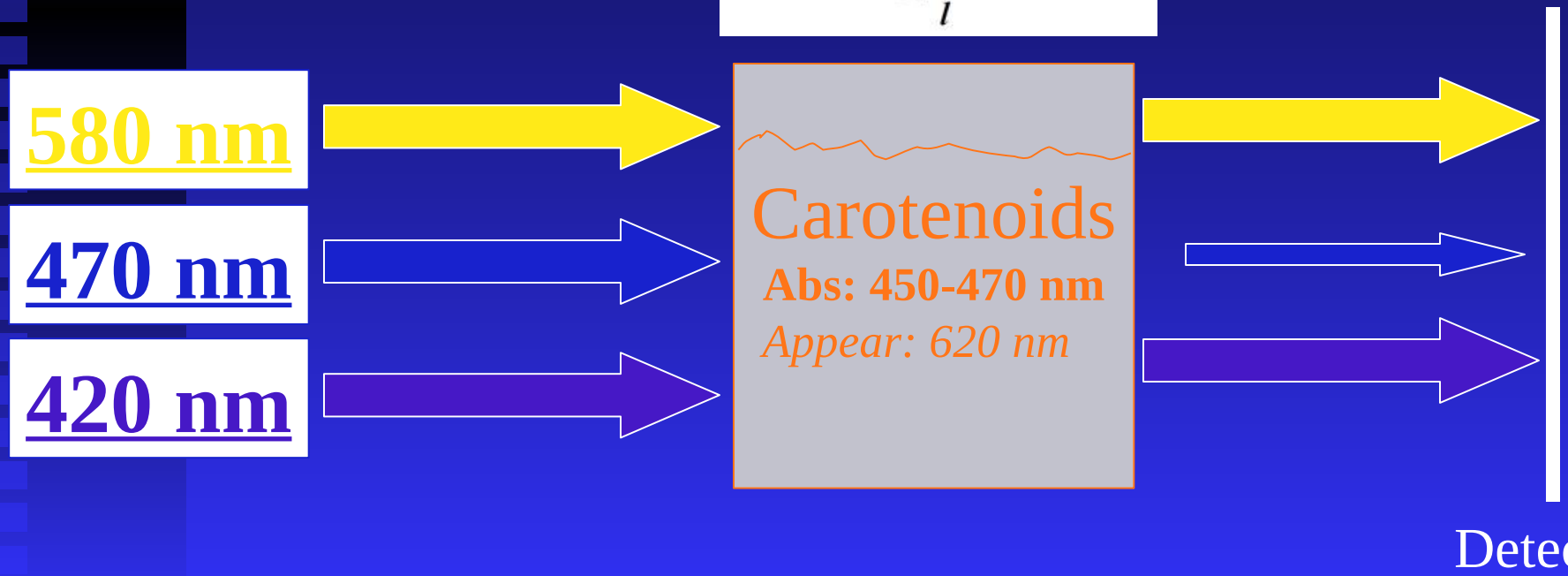
QuickTime[a] and a
Photo - JPEG decompressor
are needed to see this picture.

are needed



Principle of UV/VIS Detection
via photo-diode array (PDA)





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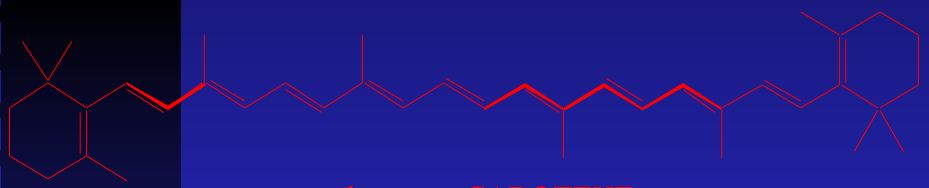
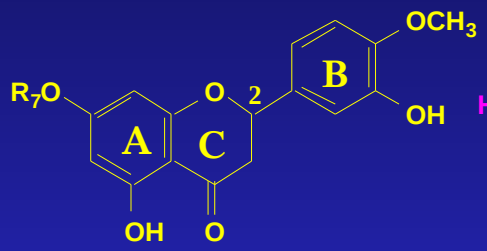
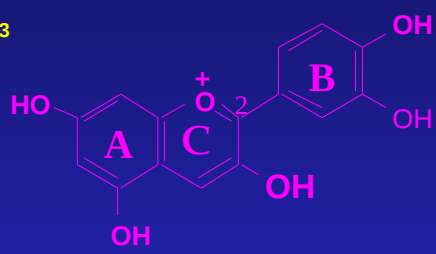


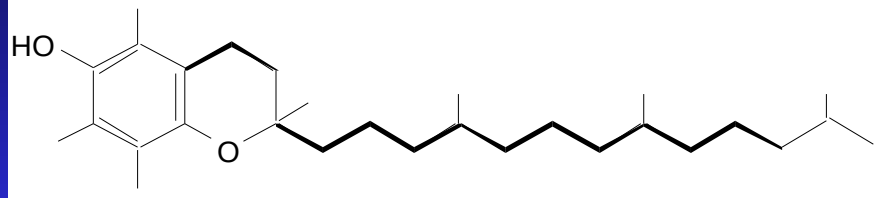
trans beta- CAROTENE
(provitamin A)



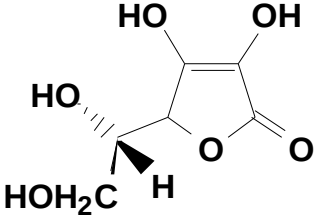
FLAVONOIDS
(vitamin 'P')



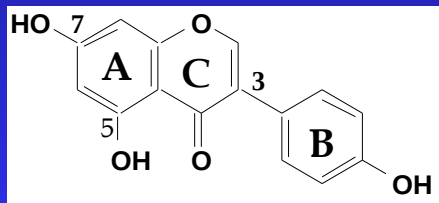
ANTHOCYANIDINS



α-Tocopherol
(a vitamin E)

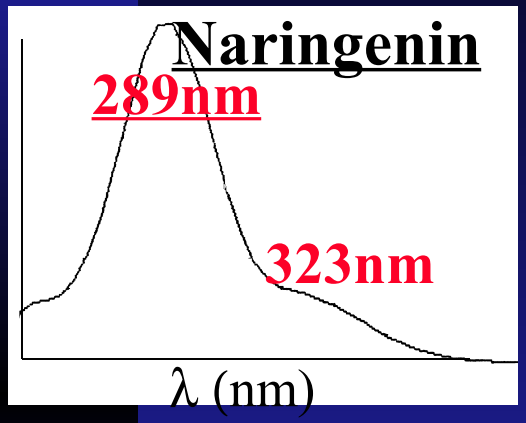


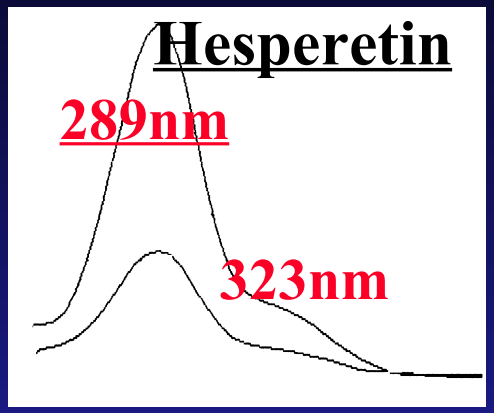
Ascorbic acid
(vitamin C)

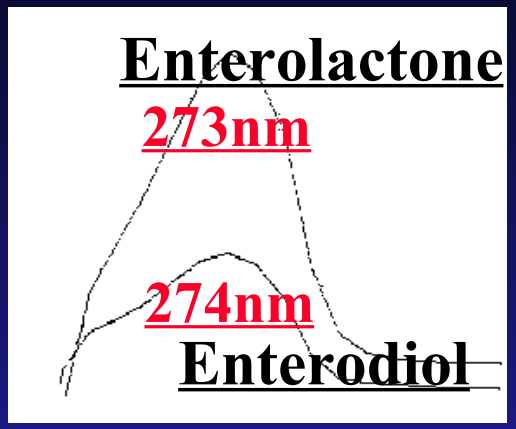


ISOFLAVONOIDS

UV Scan as Diagnostic Tool

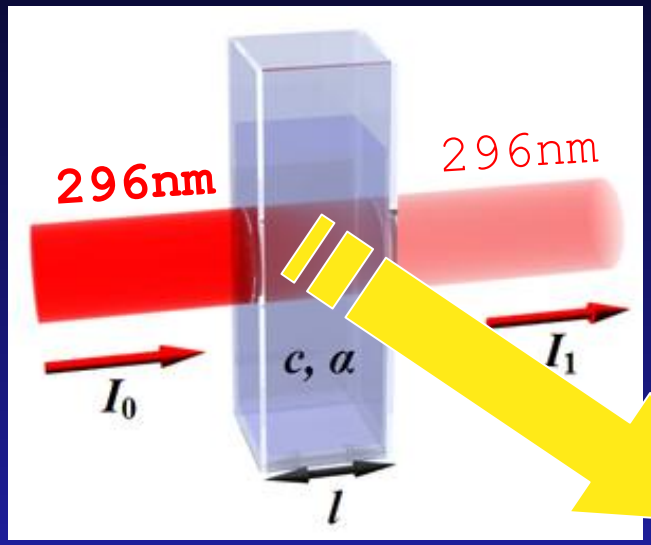






Principle of Fluorescence Detection

excitation by & emission of ***light***



336nm

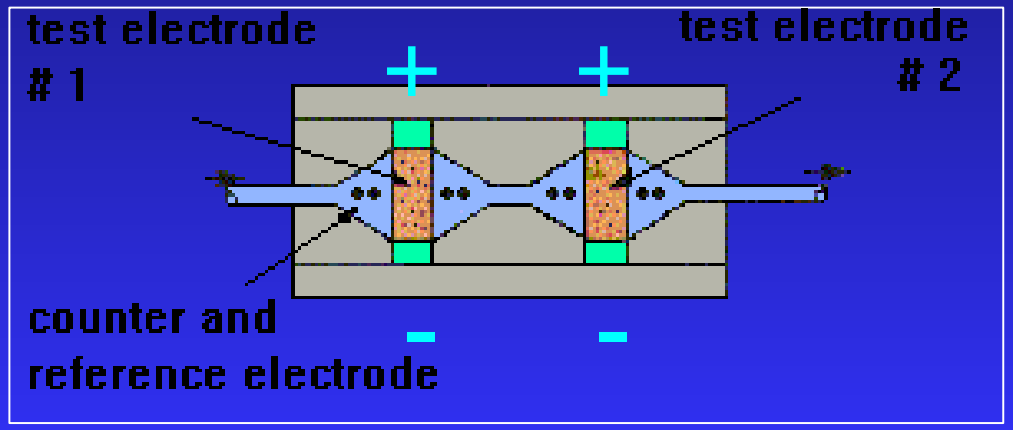
*excitation 296nm → E vitamers → emission 336nm

Principle of Electrochemical Detection

electron flow

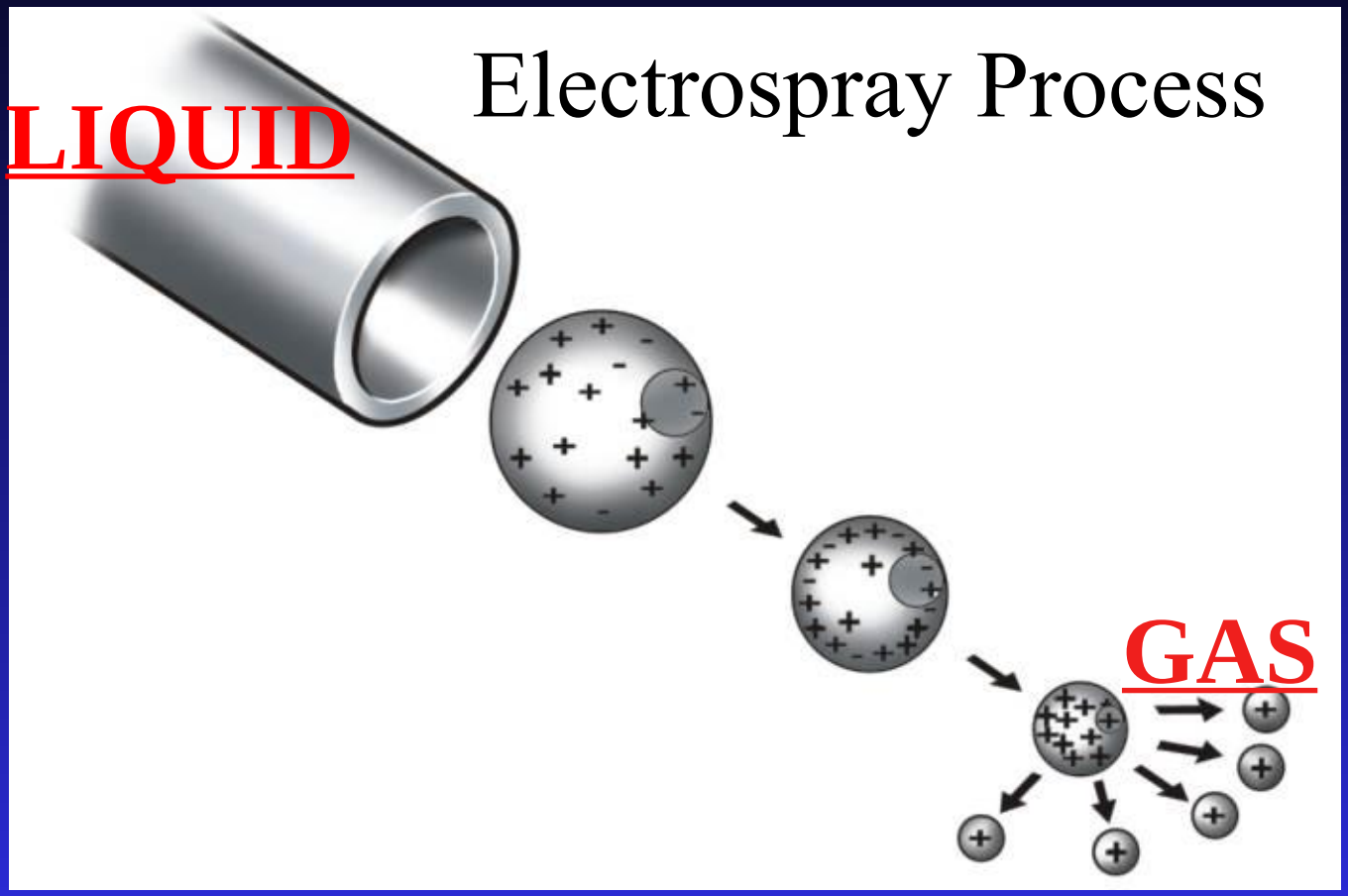
$$AH \longrightarrow A + H^{+}$$

$$e^{-} + DNAox \longrightarrow DNA$$



Principle of Mass Spectrometry (MS)

a) formation of charged ('ionic') species



liquid to gas by electrospray (ESI)

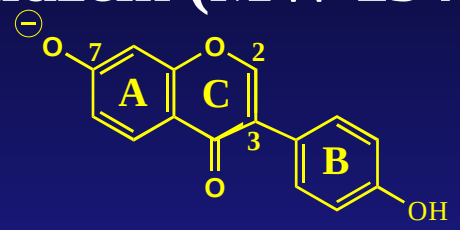
Charged droplet formation, desolvation, disintegration and ion evaporation

Principle of Mass Spectrometry (MS)

b) ion separation in el.magn. field,

c) detection by electron multiplier

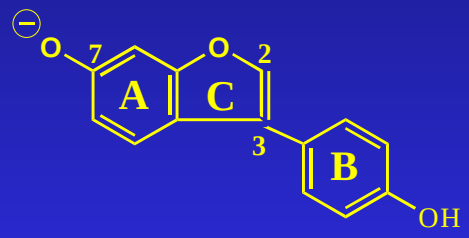
Daidzein (MW 254.1)



m/z 253

-CO

Multiple Reaction Monitoring (MRM)

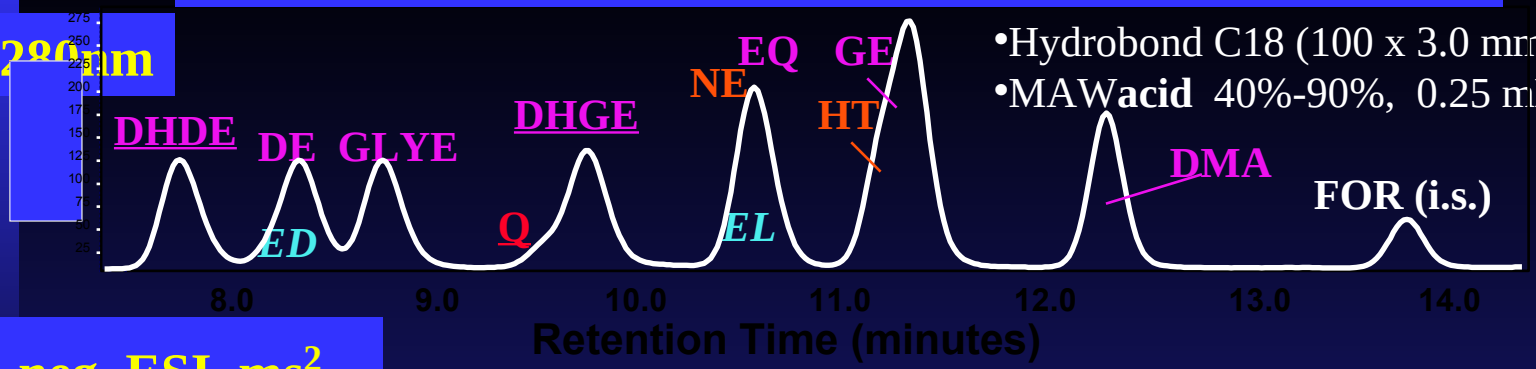


m/z 225 + **m/z 197**

Quantitate diagnostic ions

Analysis of Flavonoids, Isoflavonoids & Lignans

PDA: 280nm

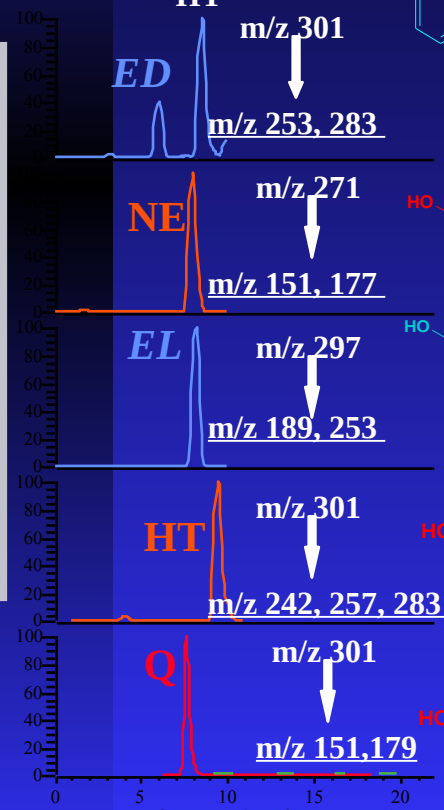


- Hydrobond C18 (100 x 3.0 mm;5µm)
- MAW**acid** 40%-90%, 0.25 mL/min

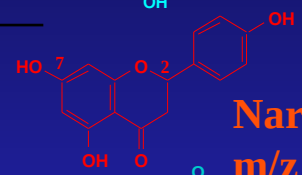
itMS: neg. ESI, ms^2

Run 1

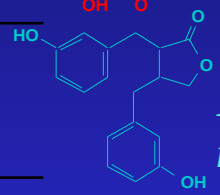
Relative Abundance



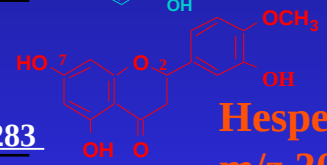
Enterodiol
m/z 302.1



Naringenin
m/z 272.1



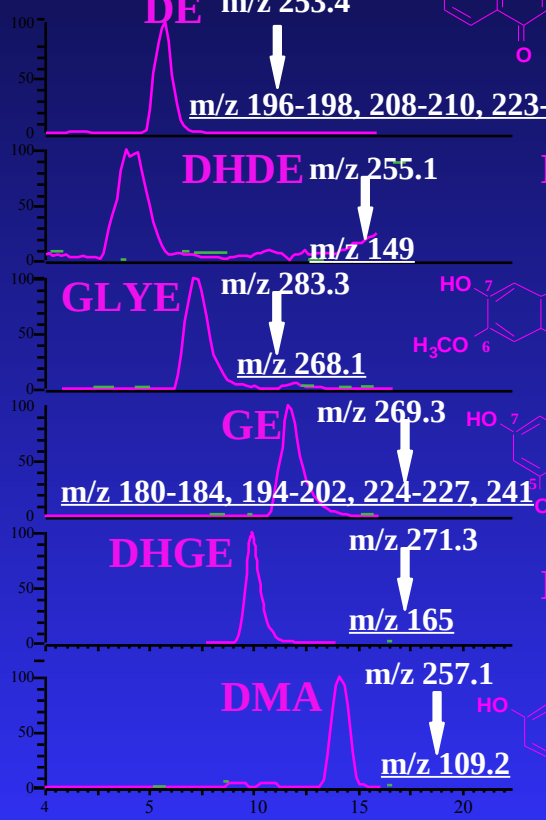
Enterolactone
m/z 298.1

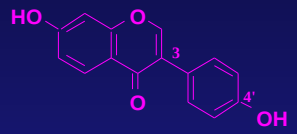


Hesperetin
m/z 302.1

Quercetin
m/z 302.1

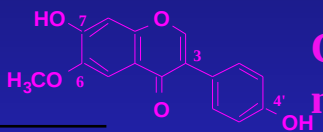
Run 2



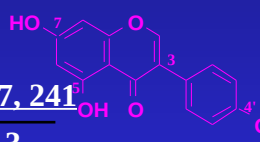


Daidzein
m/z 254.1

Dihydro-Daidzein
m/z 256.1

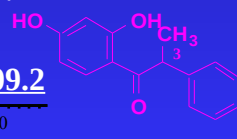


Glycitein
m/z 284.1



Genistein
m/z 270.1

Dihydro-Genistein
m/z 272.1



O-DMA
m/z 258.1

PDA combined with MS

easy , complementary

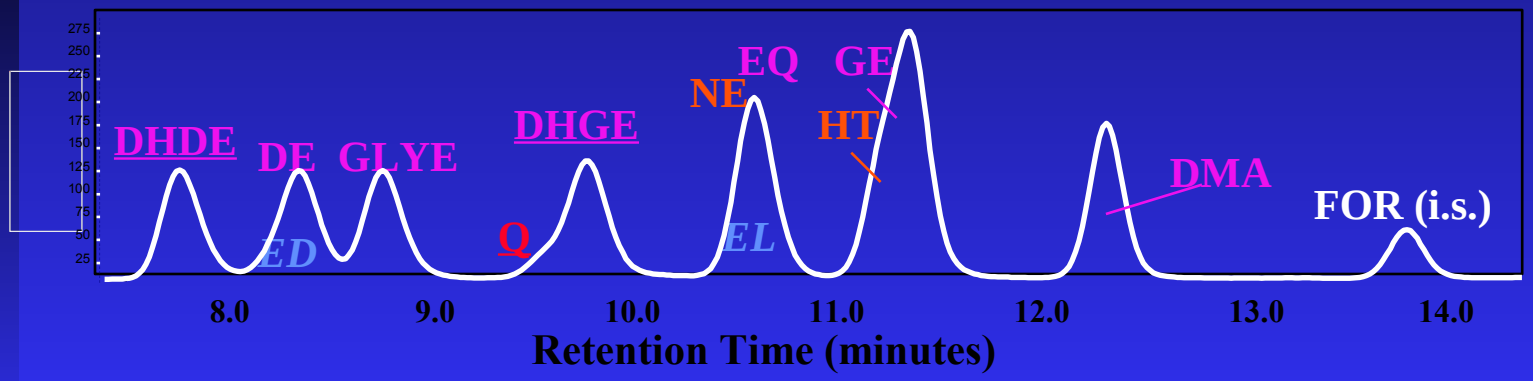
	PDA	MS
Destructive	**no**	**yes**
Analysis	+/-sensitive	sensitive & selective
good at	med.-high levels	low levels
Cost	low	high
Chromatogr.	long	short

Bottom line:

<u>SIGNAL/noise</u> ratio

(interference resolution)

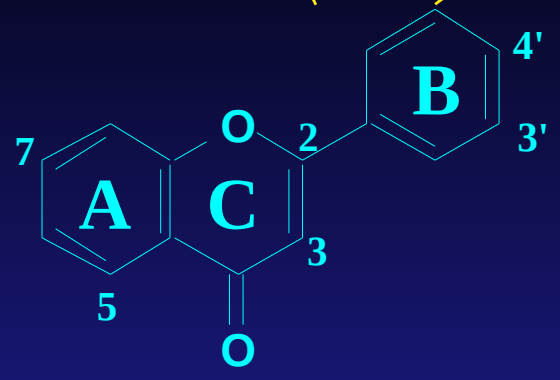
<u>should be sufficient</u>



1. Hydrophilic Phytochemical Analysis from Foods

Flavonoids & Vitamin C

Flavon(ol)s



Flavones

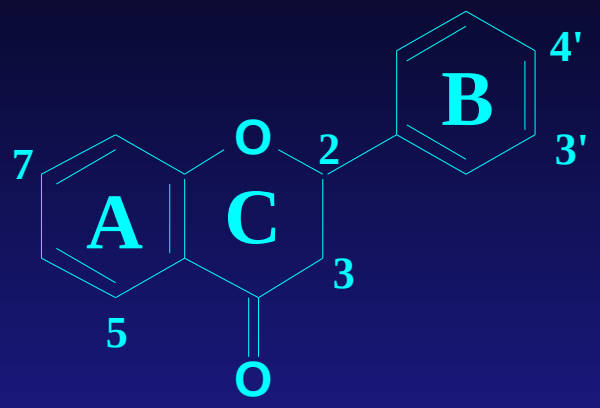
Flavone*	F	
Apigenin	A	OH-4',5,7
Luteolin	L	OH-3',4',5,7

Flavonols (OH-3)

KŠmpferol	K	OH-3,4',5,7
Quercetin	Q	OH-3,3',4',5,7
Myricetin	MYR	OH-3,3',4',5,5',7

* internal standard (i.s.)

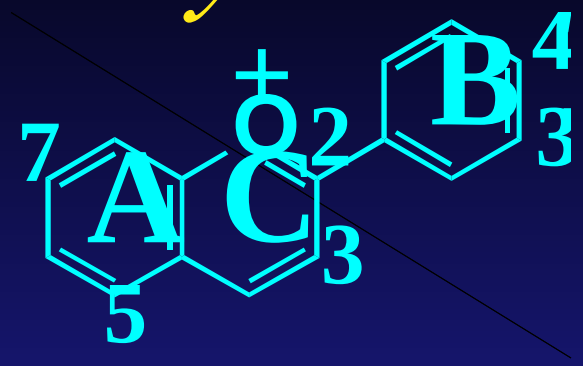
Flavanones



<u>Flavanones (citrus flavonoids)</u>

Naringenin	NE	OH-4',5,7
Naringin	NI	OH-4',5;Neohespridoxy-7
Narirutin	NR	OH-4',5;Rutinoxy-7
Hesperetin	HE	OH-3',5,7;OMe-4'
Hesperidin	HD	OH-4',5;OMe-3';Rutinoxy-7

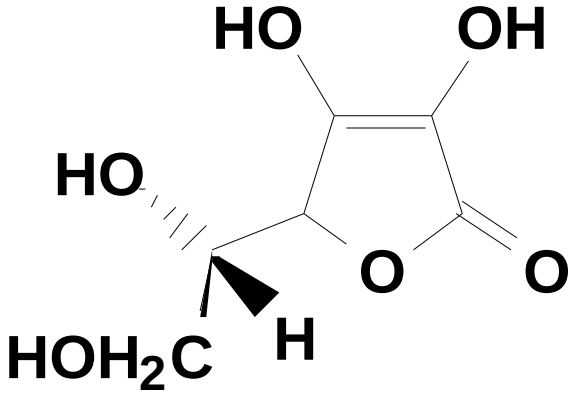
Anthocyanidins



<u>Anthocyanidins</u>

Pelargonidin	PEL	OH-3,3',5,7
Cyanidin	CYA	OH-3,3',4',5,7
Delphinidin	DEL	OH-3,3',4',5,5',7

Vitamin C



Ascorbic acid
(vitamin C)

Food Preparation

- buy at local markets
- store according to household habits
- prepare according to local recipes
- freeze-dry
- store in N_2-flushed zip-loc bags
- take representable aliquot
- homogenize

Food Extraction

Flavon(ols) & Anthocyanins

0.5 g powdered dry food +flavone(i.s.)

- **acid hydrolysis**
  22.5 mL MeOH/$H_2$0 (5:4)
  +2.5 mL HCl (12M)
  saturated with BHT
- **sonicate**
- **reflux 1h, 2h**

Aglycons

HPLC/PDA/MS

Food Extraction

Flavanols (citrus flavonoids)

0.5 g powdered dry food +flavone (i.s.)

↓

- **80% aq MeOH**
- **shake 2 h**

HPLC/PDA/(MS)

Food Extraction

Vit.C

0.1-0.5 g powdered dry food

- **3 x Meta Phosphoric Acid (5%),**
  Acetic Acid (10%),
  Sulfuric Acid (3N),
  EDTA (1mM)
  -on ice-
- **centrifuge**

HPLC/ECD/PDA

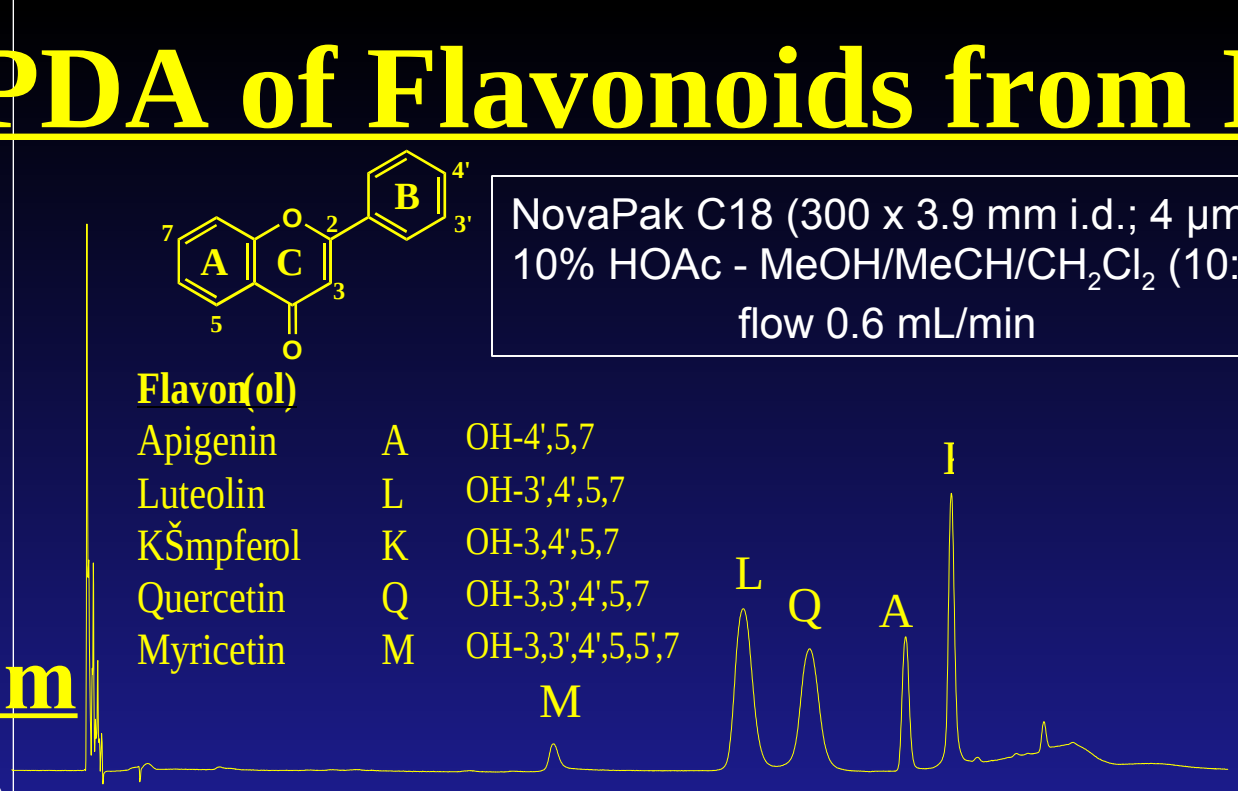
LC/PDA of Flavonoids from Foods

NovaPak C18 (300 x 3.9 mm i.d.; 4 µm)
10% HOAc - MeOH/MeCH/CH_2Cl_2 (10:5:1) Gradient
flow 0.6 mL/min

Flavon(ol)

Apigenin	A	OH-4',5,7
Luteolin	L	OH-3',4',5,7
KŠmpferol	K	OH-3,4',5,7
Quercetin	Q	OH-3,3',4',5,7
Myricetin	M	OH-3,3',4',5,5',7

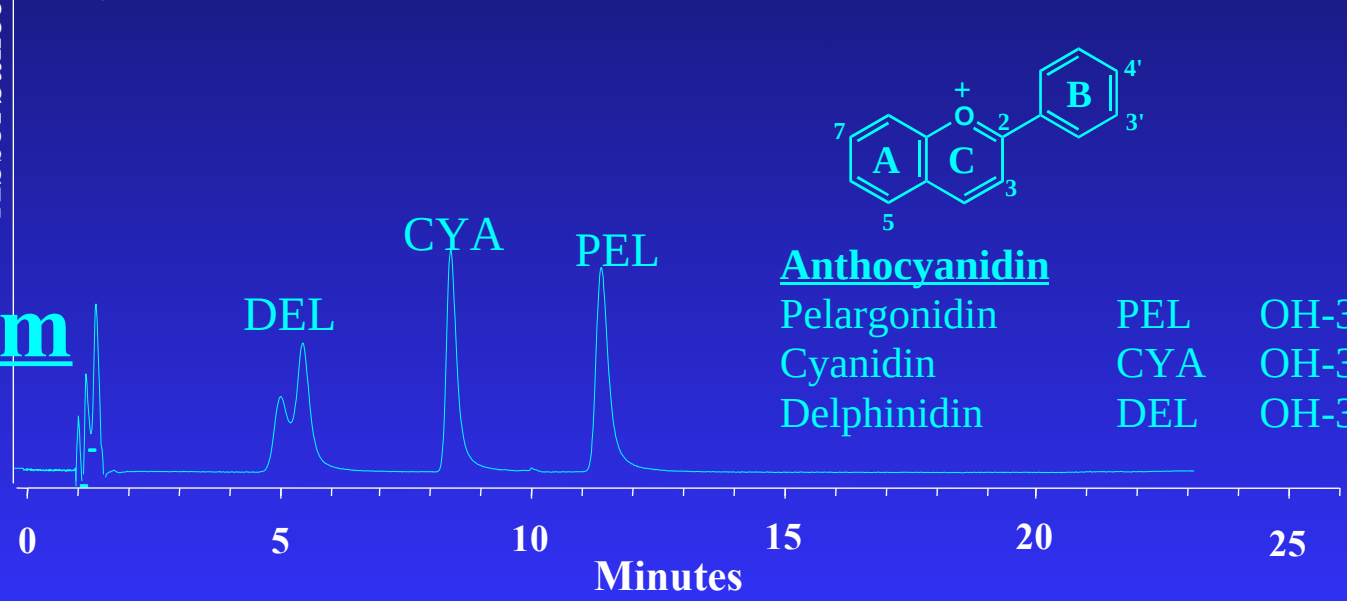
at 370 nm



Anthocyanidin

Pelargonidin	PEL	OH-3,3',5,7
Cyanidin	CYA	OH-3,3',4',5,7
Delphinidin	DEL	OH-3,3',4',5,5',7

at 525 nm



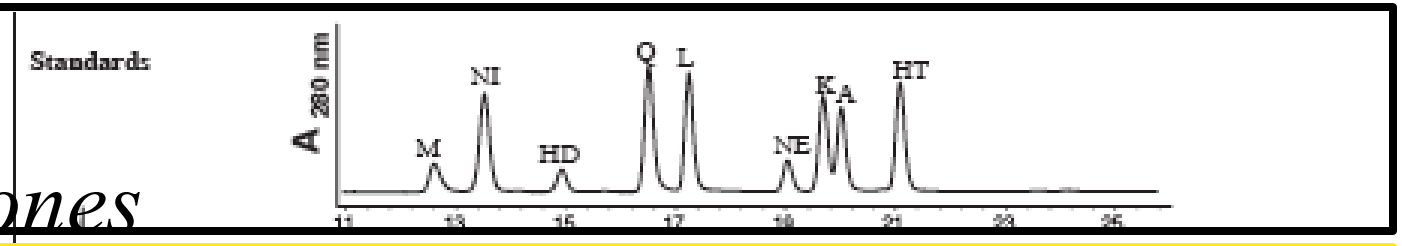
Franke 2004

LC/PDA of Flavonoids from Foods

NovaPak C18 (300 x 3.9 mm i.d.; 4 µm)
10% HOAc - MeOH/MeCH/CH_2Cl_2 (10:5:1) Gradient
flow 0.6 mL/min

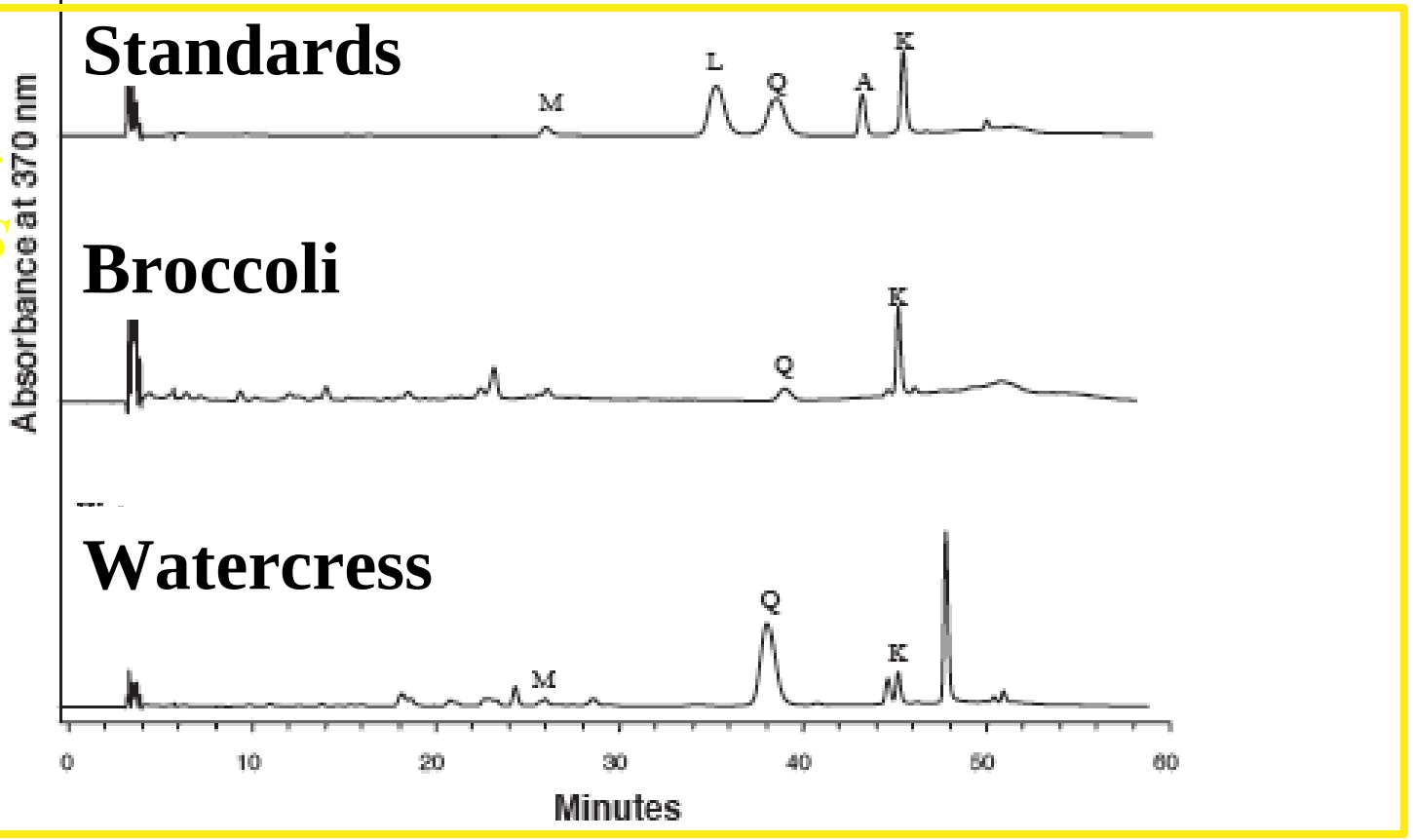
at 280 nm

also flavanones



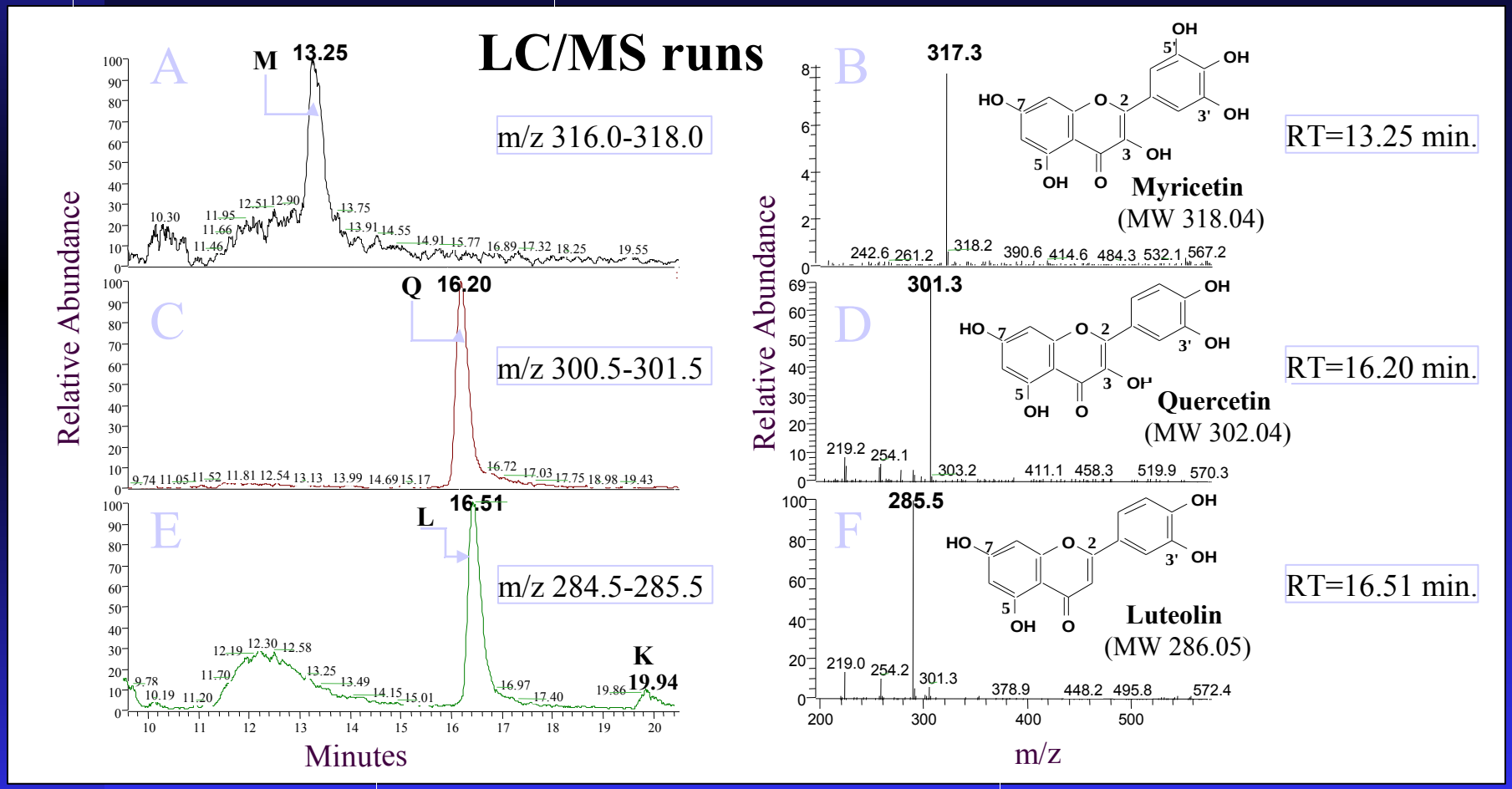
at 370 nm

flavon(ol)s



Confirmation by LC/MS

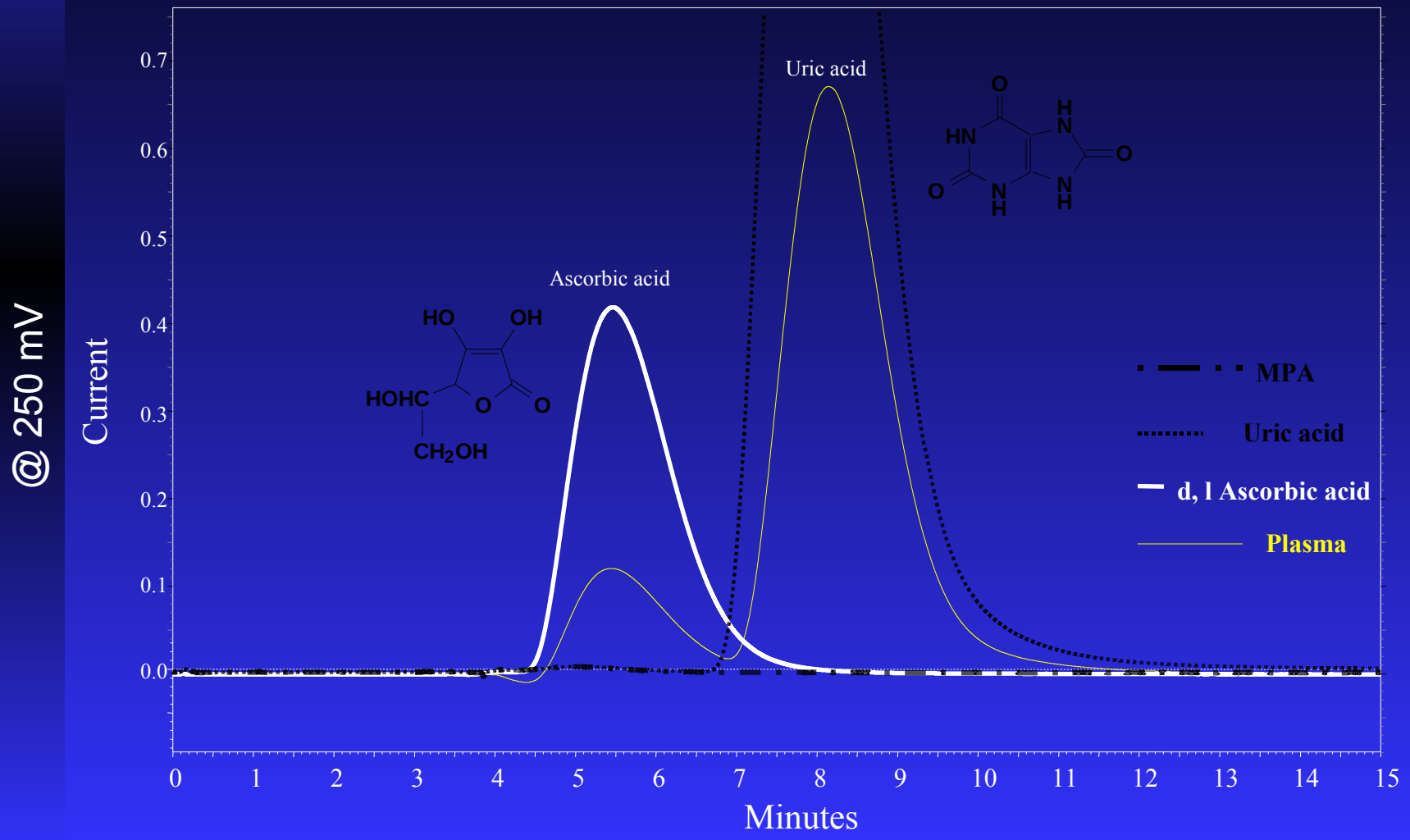
for low levels



Franke 2004

LC/ECD of Vitamin C from Foods

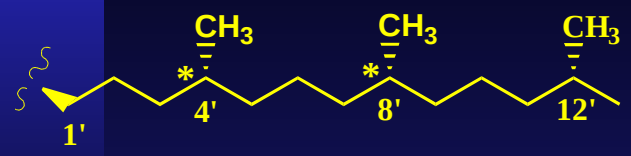
C8 'AQ' (3.0x100 mm, 5 µm)
KH_2PO_4 and 1mM Na_2 EDTA (pH 3),
flow 0.3 mL/min

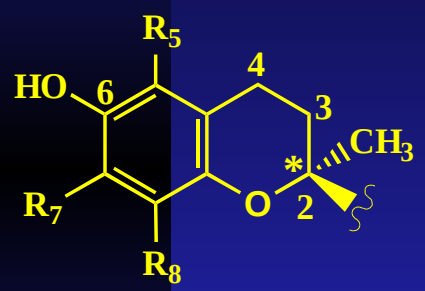


2. Lipophilic Phytochemical Analysis from Foods

Tocopherols & Tocotrienols

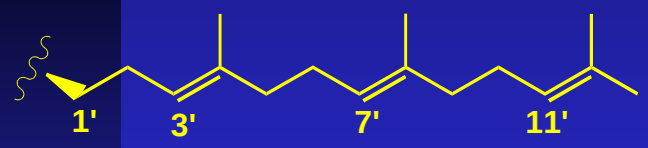
E vitamers





Tocopherols

R_5	R_7	R_8		
CH_3	CH_3	CH_3	‘*vitamin E*’ **alpha-tocopherol**	**aT**
CH_3	H	CH_3	beta-tocopherol	bT
H	CH_3	CH_3	gamma-tocopherol	gT
H	H	CH_3	delta-tocopherol	dT
H	H	H	tocol	T



Tocotrienols

R_5	R_7	R_8		
CH_3	CH_3	CH_3	alpha-tocotrienol	aT3
CH_3	H	CH_3	beta-tocotrienol	bT3
H	CH_3	CH_3	gamma-tocotrienol	gT3
H	H	CH_3	delta-tocotrienol	dT3

Food Extraction

Tocopherols & Tocotrienols (E vitamers)

1.0 g powdered dry food +tocol (i.s.)

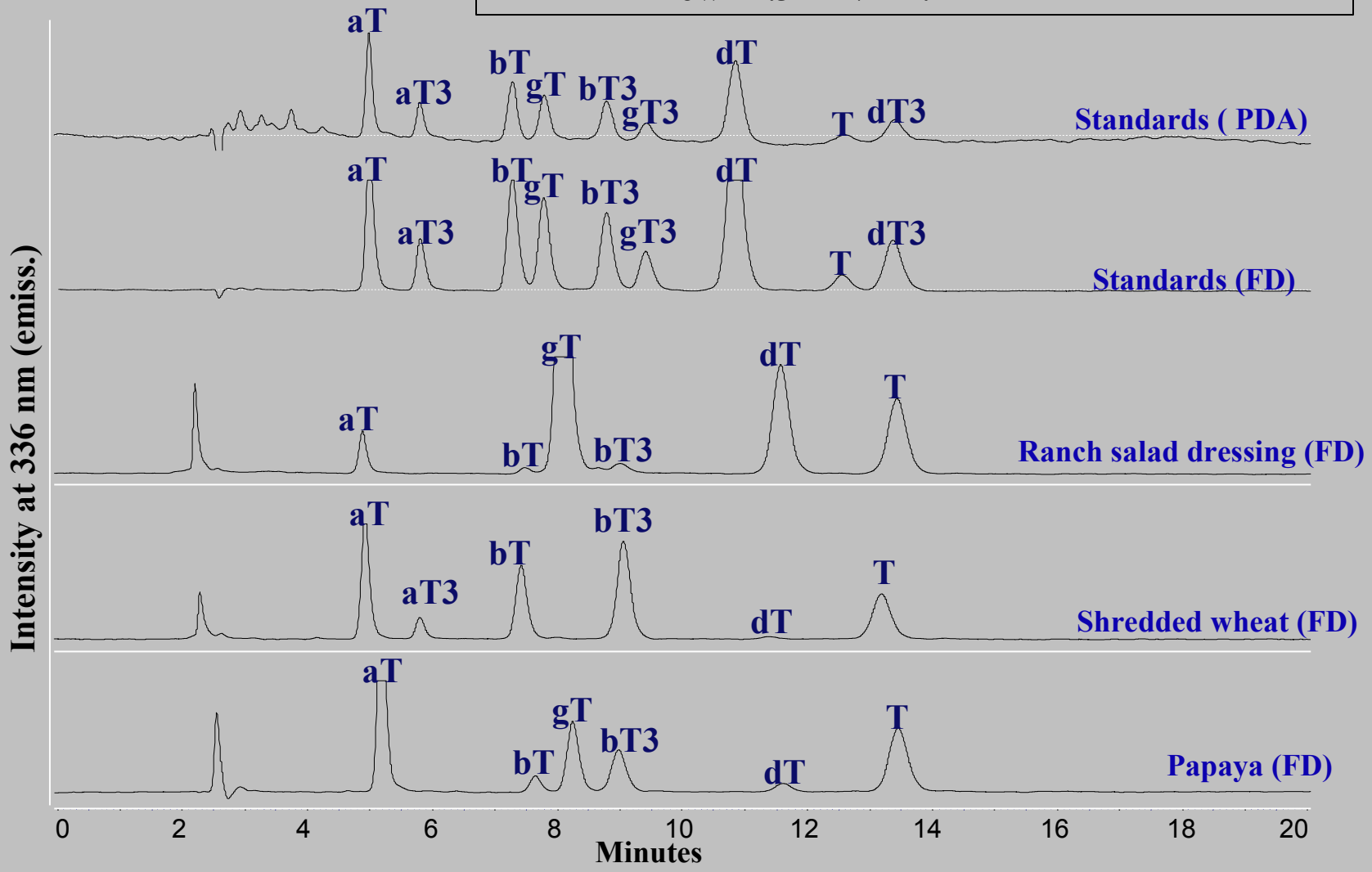
- **4 x 25 mL hexane**
- **centrifuge**
- **concentrate by reduced pressure**

HPLC/FD*/PDA

*exc.296nm; emiss.336nm

LC/FD/PDA of E-vitamers from Foods

Spherex 5 OH (diol, 250 x 4.6 mm, 5µm)
hexane:dioxane (95:5; v/v) with 0.025% BHT (250 mg/L)
flow =1.3 mL /min.



RESULTS

Flavon/ol Food Levels

50 foods, 117 items

<1 mg/kg (dried fruit, juices, jams) **up to 259 mg/kg** (onions)

- mostly quercetin
- apigenin <0.1 mg/kg
- onions, blueberries >watercress >cabbages > sweet orange potatoes >spinach

Flavanone Food Levels

7 citrus foods, 16 items

172 mg/kg (tangerines) **up to 905 mg/kg** (grapefruit)

- analysis of unhydrolyzed glycosides, otherwise degradation
- grapefruit (naringenin 689 mg/kg)
- oranges (hesperidin 515 mg/kg)
- supplements have low levels (0.1-4.4 mg/kg)

Anthocyanine Food Levels

30 foods, 50 items

<0.3 mg/kg (cabbage) **up to 1168 mg/kg** (blueberries)

- acid hydrolysis does not lead to degradation
- cyanidin predominant
- unknown anthos measurable by monitoring at 525 nm
- blueberries>raspberries>>sweet purple potatoes >red cabbage>black>red plums>red grapes>non-skinned red apples.

Vitamin C Food Levels

44 foods, 93 items

4 mg/kg (sweet potato leaves)
up to 801 mg/kg (fresh strawberries)

- boiling loses 28% to 63%
- canning (tomatoes) has a preserving effect! (+7%)
- skinned fruits lower (34–46%) than fruits with the skin

E vitamer Food Levels

63 foods, 127 items

0.6 mg/kg (tamarind) **up to 828 mg/kg** (corn oil)

- T ≤542 mg/kg, T3 ≤432 mg/kg
- oils <828 mg/kg (aT&gT); margarines 359-457 mg/kg (gT); salad dressings 20-291 mg/kg (gT); cookies 54-243 mg/kg (gT); snacks 135-220 mg/kg (gT); nuts 22-201 mg/kg (aT); vegetables 2-152 mg/kg (aT);
  … fruits 2-16 mg/kg (aT).

Ranking according to aT and TEV levels

	Food	aT (mg/kg)	aT/TEV	RDA (g)[a]	Food name	TEV (mg/kg)	aT/TEV
1	Sunflower oil	542	0.85	28	Corn oil*	828	0.27
2	Safflower oil*	391	0.79	38	Sunflower oil	640	0.85
3	Cereals*[b]	261	0.94	58	Sesame oil*	525	0.12
4	Corn oil*	222	0.27	68	Safflower oil*	497	0.79
5	Honey Roasted Almonds	185	0.92	81	Margarine, 70% fat	457	0.14
6	Potato chips*	77	0.50	196	Margarine, 60% fat*	359	0.09
7	Mixed nuts, oil roasted*	70	0.50	216	Italian salad dressing*	291	0.14
8	Dry roasted peanuts*	69	0.43	217	Cereals*	276	0.94
9	Sesame oil*	65	0.12	231	Microwave popcorn*	220	0.12
10	Margarine, 70% fat	63	0.14	237	Honey Roasted Almonds	201	0.92
11	Sesame & miso salad dressing	58	0.62	259	Oriental salad dressing*	176	0.13

* mean levels for foods with multiple samples; [a] grams of food needed to obtain the recommended dietary allowance of 15 mg aT per day; [b] presence of aTac determined by RP-HPLC; **we assumed rac. aTac and used a multiplication factor of 0.45 to convert to natural aT. aT-alpha Tocopherol; TEV=Total E Vitamers**.

Correlations among E vitamers and between E vitamers and fat

	TEV	aT	bT	gT	dT	aT3	bT3	gT3	dT3
aT	**0.68**								
bT	0.60	0.40							
gT	**0.87**	0.25	0.59						
dT	0.73	0.17	0.17	**0.75**					
aT3	0.08	0.01	0.00	0.06	-0.01				
bT3	0.65	0.38	0.22	0.56	0.54	0.18			
gT3	0.51	0.26	0.16	0.43	0.42	0.30	0.40		
dT3	0.32	0.44	0.14	0.06	0.17	0.10	0.08	0.31	
Total fat	**0.87**	0.62	0.60	0.74	0.58	0.16	0.50	0.37	0.31
SAT	**0.69**	0.39	0.50	**0.67**	0.49	0.18	0.38	0.33	0.14
MU	**0.67**	0.35	0.59	**0.68**	0.45	0.17	0.35	0.27	0.07
PU	**0.83**	**0.78**	0.51	0.58	0.50	-0.03	0.46	0.35	0.51

TEV=Total E Vitamers

SAT=saturated fat; MU=monounsaturated fat; PU=polyunsaturated fat

SUMMARY

HPLC/PDA +ECD, FD, or MS

reliable, easy, affordable, fast

FLAVONOIDS

- **Flavon/ol *(<260 mg/kg onions)***
- **Citrus FLAVs *(330-630 mg/kg)***
- **Anthos *(<1200 mg/kg blueberry)***

MS is helpful at low levels

Vit C:

wide range (4-800 mg/kg);

lyophiliz. ok; careful extraction!

SUMMARY -cont'd

E vitamer:

- ***(plant) foods high in seed oils (<1200 mg/kg)***
  FD=selective, PDA at high levels!

every E vitamer deserves individual consideration due to specific bioactivities

CONCLUSIONS

Degradation likely by typical household behaviors in purchasing, storing, and preparing foods

High variability warrants monitoring of food levels in human diet studies

Acknowledgements

all collaborators
Leslie Ashburn

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NCRR #S10 RR020890
CRCH funds

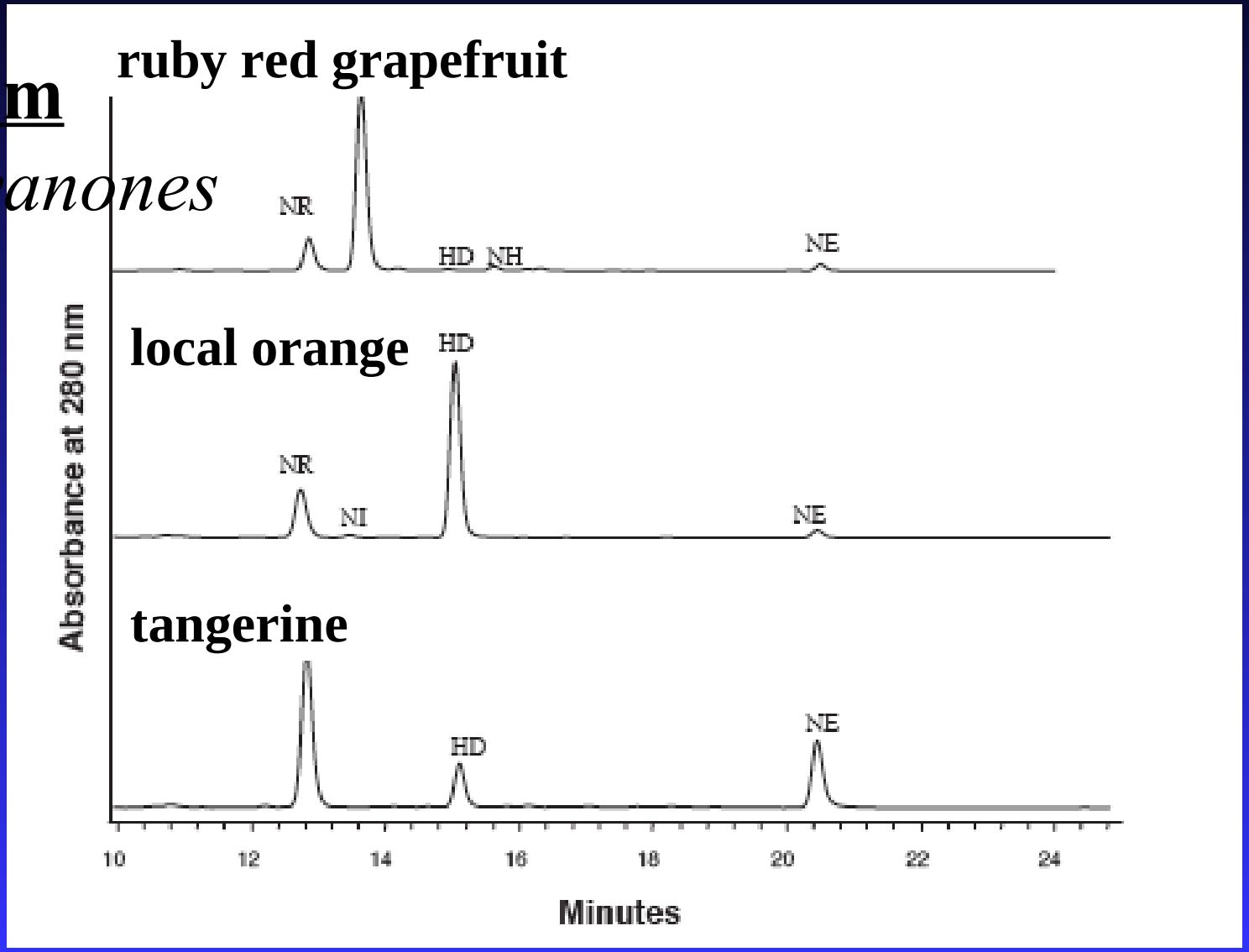
Flavs, Anthos, Vit C: ***Franke et al., J. Food Comp. Anal. 17, 1 (2004).***
E vitamers: ***Franke et al., J. Agr. Food Chem. (submitted 2006)***

LC/PDA of Flavanons from Foods

NovaPak C18 (300 x 3.9 mm i.d.; 4 µm)
10% HOAc - MeOH/MeCH/CH_2Cl_2 (10:5:1) Gradient
flow 0.6 mL/min

at 280 nm

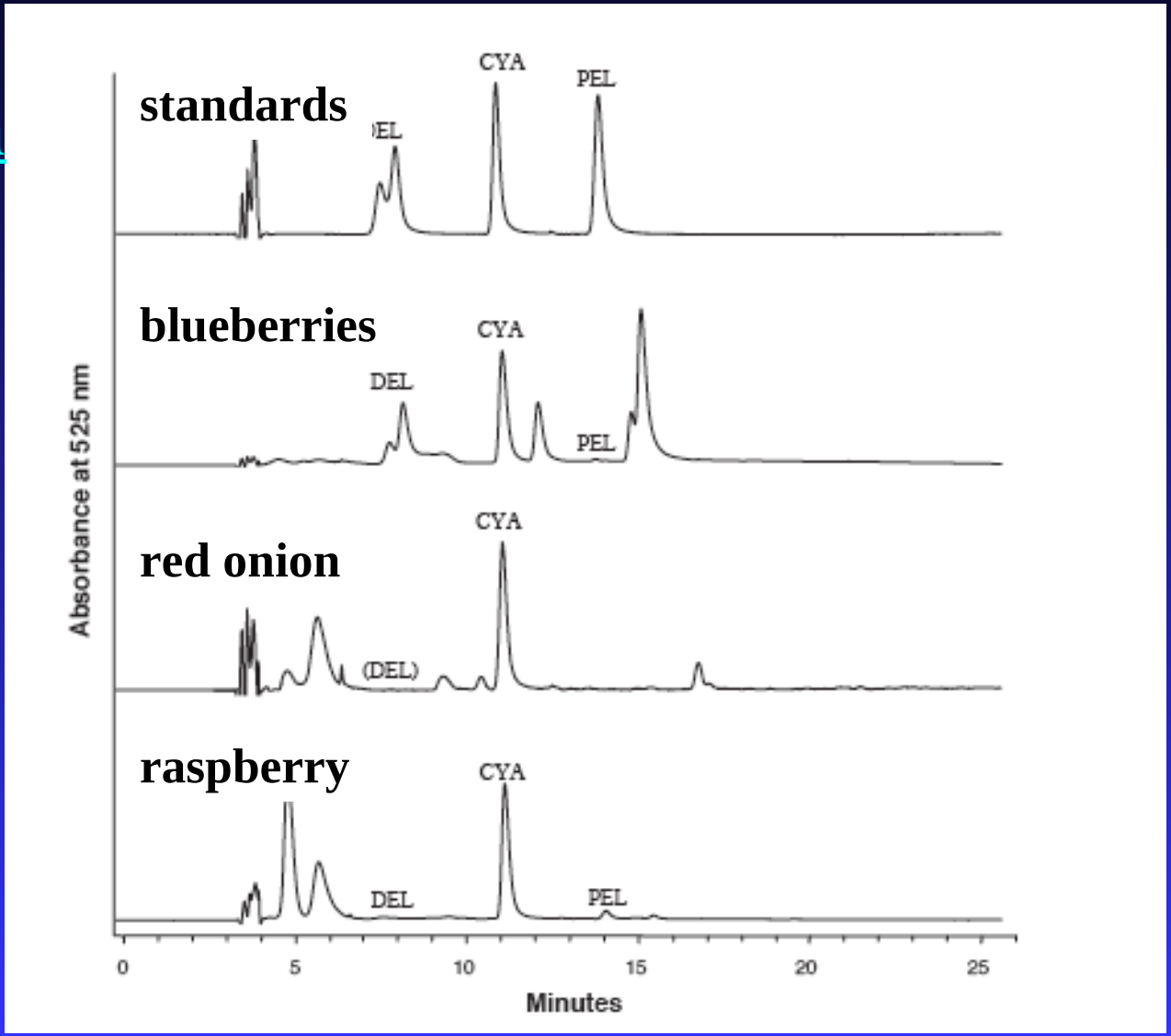
also flavanones



LC/PDA of Anthocyanins from Foods

NovaPak C18 (300 x 3.9 mm i.d.; 4 µm)
10% HOAc - MeOH/MeCH/CH_2Cl_2 (10:5:1) Gradient
flow 0.6 mL/min

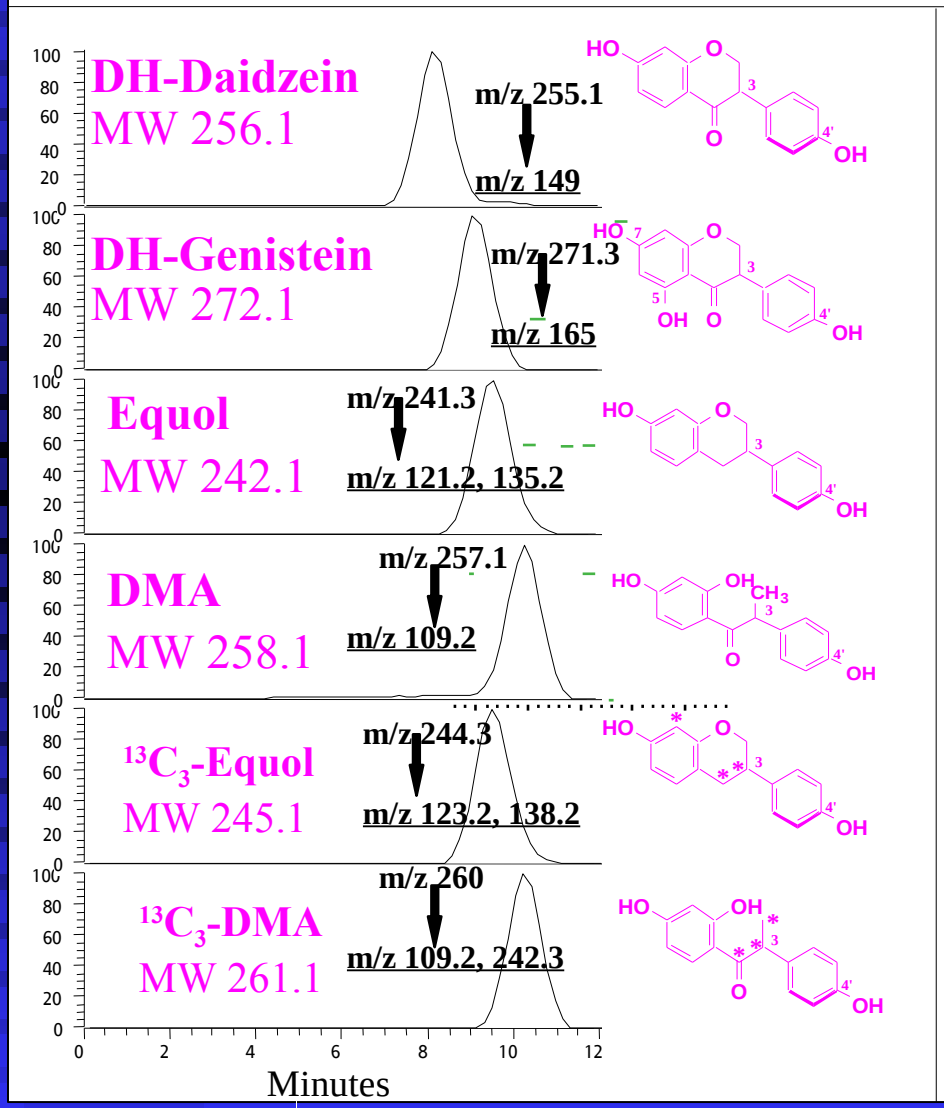
at 525 nm

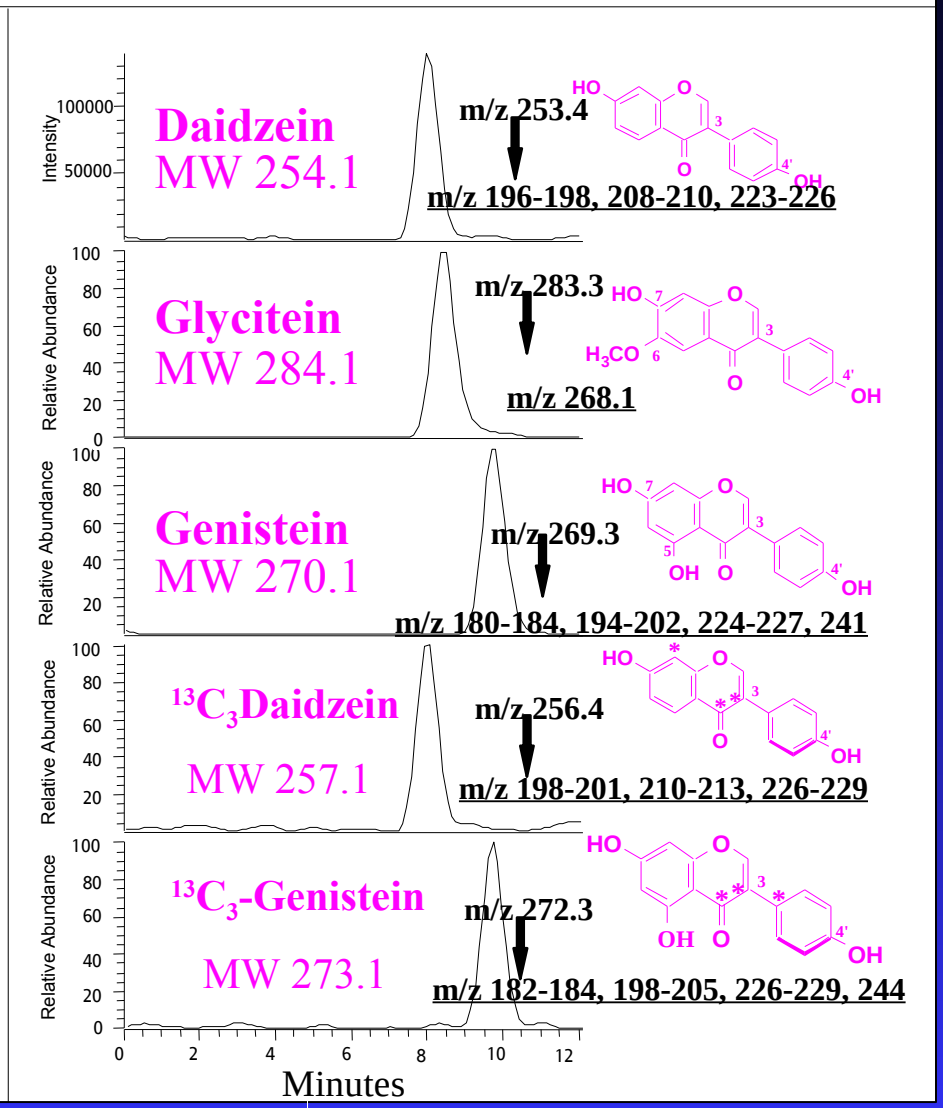


IFL by neg.ESI-itMS (MRM)

Run 1 (metabolites)

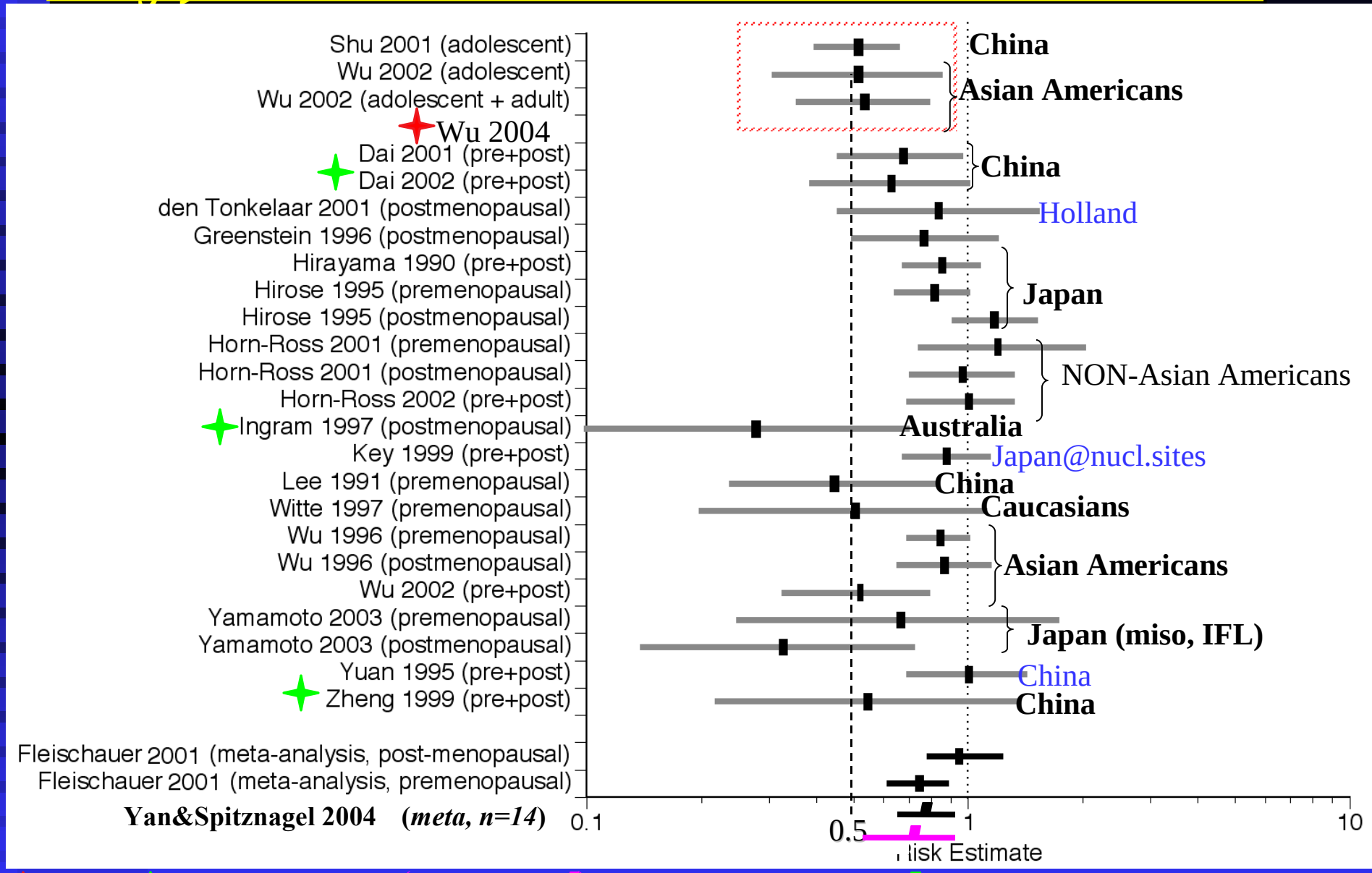
Run 2 (non-metabolites, parents)





Franke 2002; Blair 2003

Soy, Isoflavones & Breast Cancer



Yan&Spitznagel 2004 (***meta, n=14***)

(***meta-post, n=7***)

(***meta-urine, n=4***)

plasma or urinary IFL as biomarker

Cline 2004 pers.commun.

Lipophilic Food Components

Tocopherols & Tocotrienols ('vitamin E')

Food Extraction

Tocopherols & Tocotrienols

1.0 g powdered dry food +tocol (i.s.)

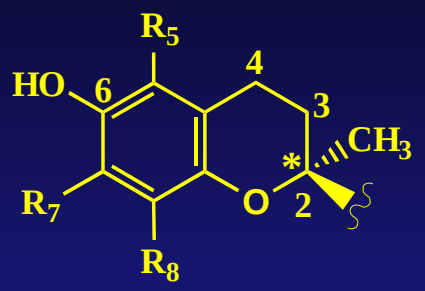
- **25 mL hexane x 4**
- **centrifuge**
- **concentrate under reduced press**

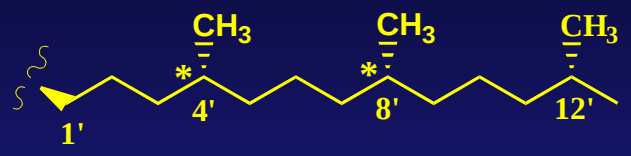
HPLC/PDA/FD*

*296nm exc.;336nm em.

Stationary Phase= **Spherex 5 OH (diol, 250 x 4.6 mm, 5µm)**
Mobile Phase = **hexane:dioxane (95:5; v/v) containing 0.025% BHT (250 mg/L); 1.3 mL per minute**

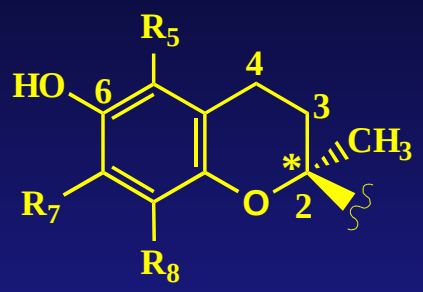
Analytes

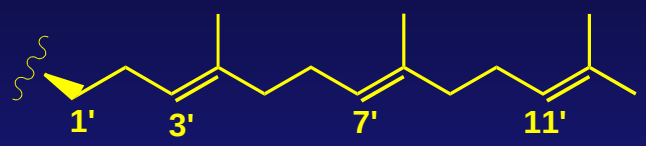




R_5	R_7	R_8	vitamer	code
CH_3	CH_3	CH_3	alpha-tocopherol	aT
CH_3	H	CH_3	beta-tocopherol	bT
H	CH_3	CH_3	gamma-tocopherol	gT
H	H	CH_3	delta-tocopherol	dT
H	H	H	tocol	T

Analytes

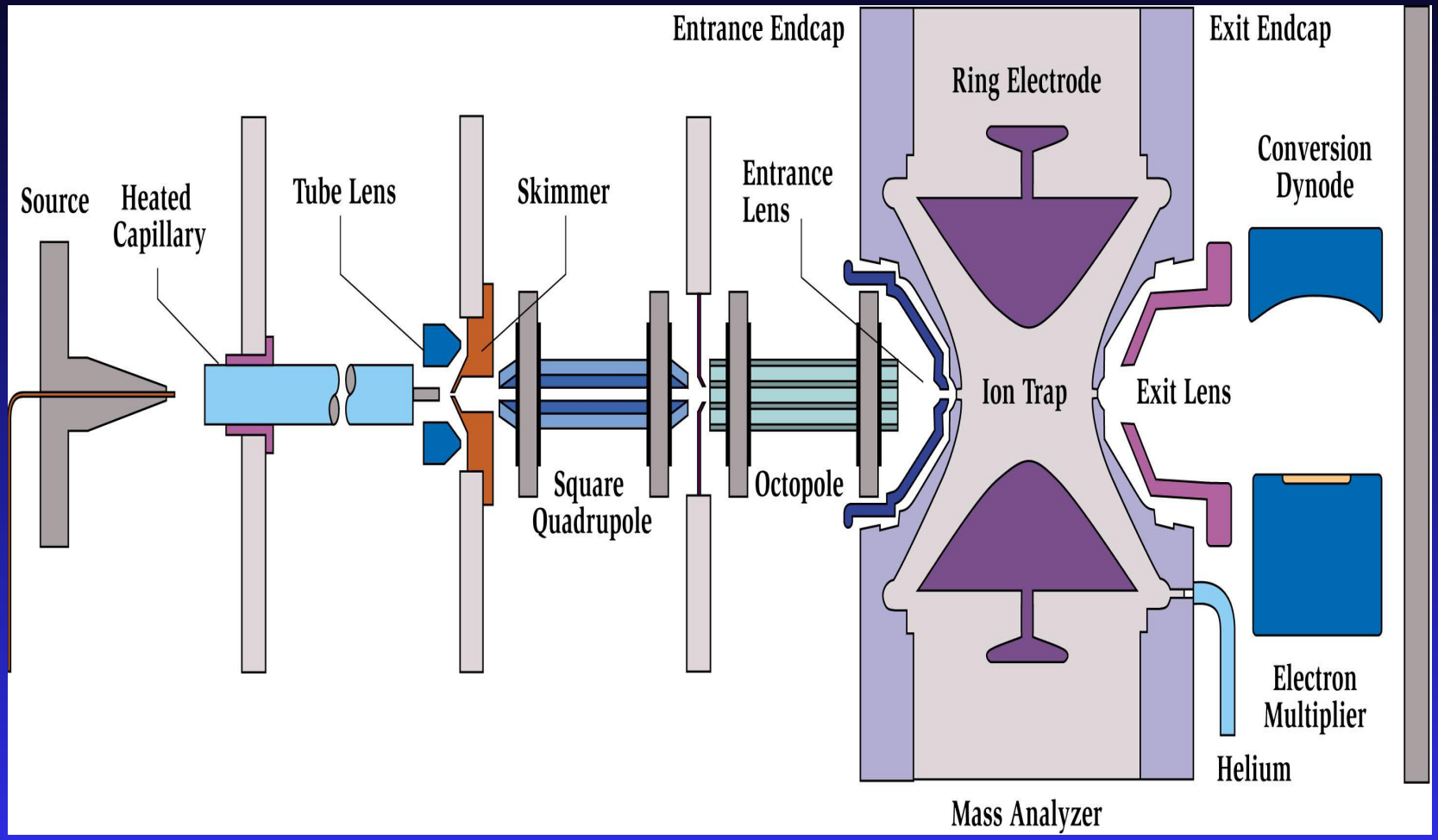




R_5	R_7	R_8	vitamer	code
CH_3	CH_3	CH_3	alpha-tocotrienol	aT3
CH_3	H	CH_3	beta-tocotrienol	bT3
H	CH_3	CH_3	gamma-tocotrienol	gT3
H	H	CH_3	delta-tocotrienol	dT3

Principle of mass detection

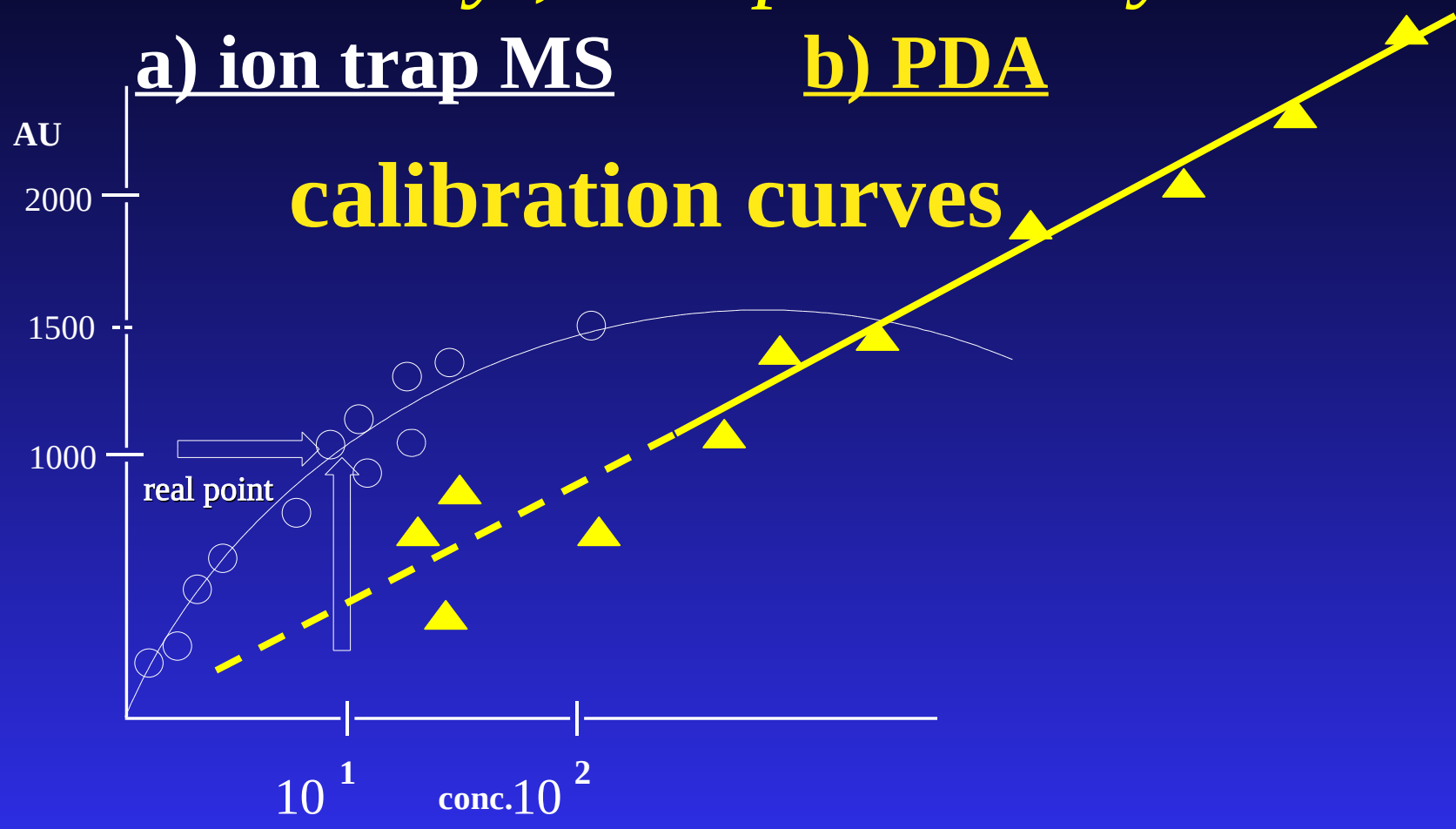
b) ion trap mass spectrometry (itMS)



PDA combined with MS

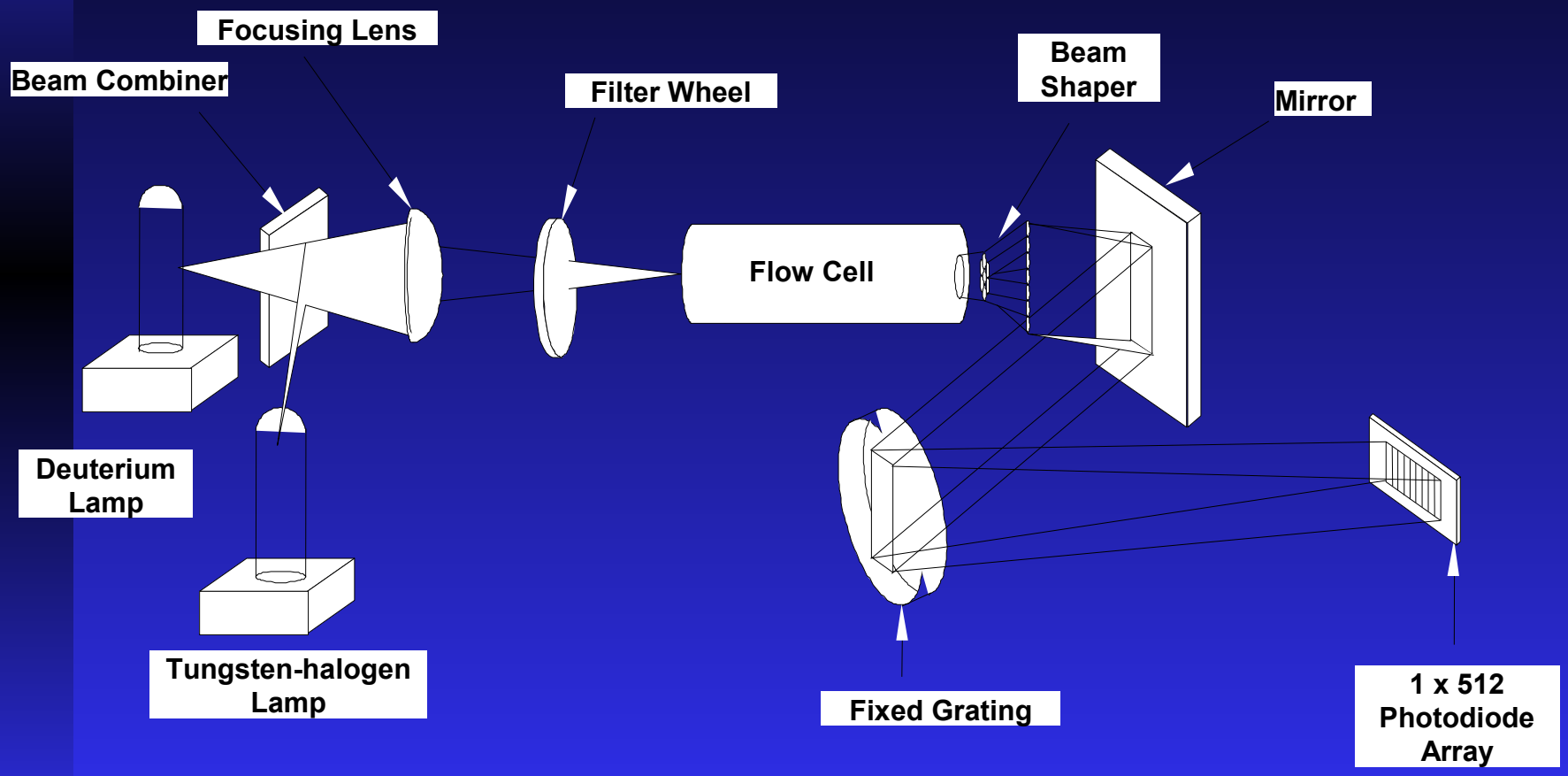
easy , complementary

a) ion trap MS **b) PDA**

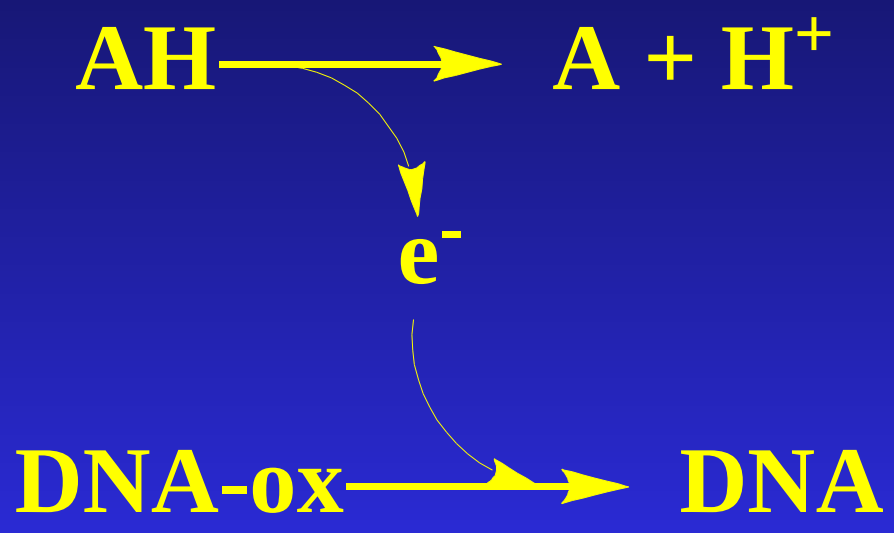


good at: low levels **medium-high levels**

PDA Optical Diagram



Antioxidants



Typical HPLC conditions for (I)FL

•**SOLID PHASE**	Hydrobond C18 (100 x 3.0 mm; 5µm)
	Beta Basic-8 (100 x 2.1 mm, 3 µm)
•**LIQUID PHASE**	MeOH/MeCN/aq HForm or HOAc
gradient	40%-90% organic
flow	~0.25 mL/min

E vitamer Food Levels

63 foods, 127 items

0.6 mg/kg (tamarind)
up to 828 mg/kg (corn oil)

- T ≤542 mg/kg, T3 ≤432 mg/kg
- oils >margarines >salad dressings >cookies> snacks >nuts >vegetables >…fruits.

Text of oil cartoon?

Analytical Techniques

FOOD ANALYSES

- Hydrophilic Phytochemicals
- Lipophilic Phytochemicals

BIOACTIVITY

How potent?

Value of Dietary Phytochemical

EXPOSURE

How much consumed?

BIOAVAILABILTY

How much absorbed & in tissue?

NUTRITION DATABASE

Masses as Diagnostic Tool

some uncertainty

	MW
Genistein	270.0528
Apigenin	270.0528
Estrone	270.1620
Estriol-water	270.1620
DHGE	272.0685
Naringenin	272.0685
Estradiol	272.1776