Analytical Techniques to Detect Adulteration of Vanilla

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Analytical Techniques to Detect Adulteration of Vanilla

- Analysis Methodology to Differentiate Natural versus Synthetic or Biosynthetic Vanillin
- Analytical Techniques to Determine Adulteration of Vanilla with Non-Natural Synergists/Fortifiers or Other Extraneous Compounds

Analysis Methodology to Differentiate Natural versus Synthetic Vanillin

Isotopic Fingerprinting Techniques

- ^{14}C Radiochemical Analysis
- $^{13}C/^{12}C$ Isotope Ratio Analysis
- $^{18}O/^{16}O$ Isotope Ratio Analysis
- $^{2}H/^{1}H$ (D/H) Isotope Ratio Analysis

^{14}C Radiochemical Analysis

- ^{14}C is an unstable, radioactive isotope of carbon (β^- decay)
- ^{14}C β^- decay $\rightarrow$ ^{14}N + antineutrino + e^-
- ^{14}C has a cosmic origin
- In earths upper atmosphere cosmic rays interact with atmospheric molecules to produce a flux of energetic neutrons
- $^{14}N + {}^{1}n \rightarrow {}^{14}C + {}^{1}p^+$

^{14}C Radiochemical Analysis

- Atmospheric production of ^{14}C is relatively constant but spikes have been documented due to above ground nuclear testing and supernovae (an unusual 10 fold spike was noted for AD 774-775)
- ^{14}C is present in CO_2 at exceedingly trace concentration (about one in one trillion molecules)

^{14}C Radiochemical Analysis

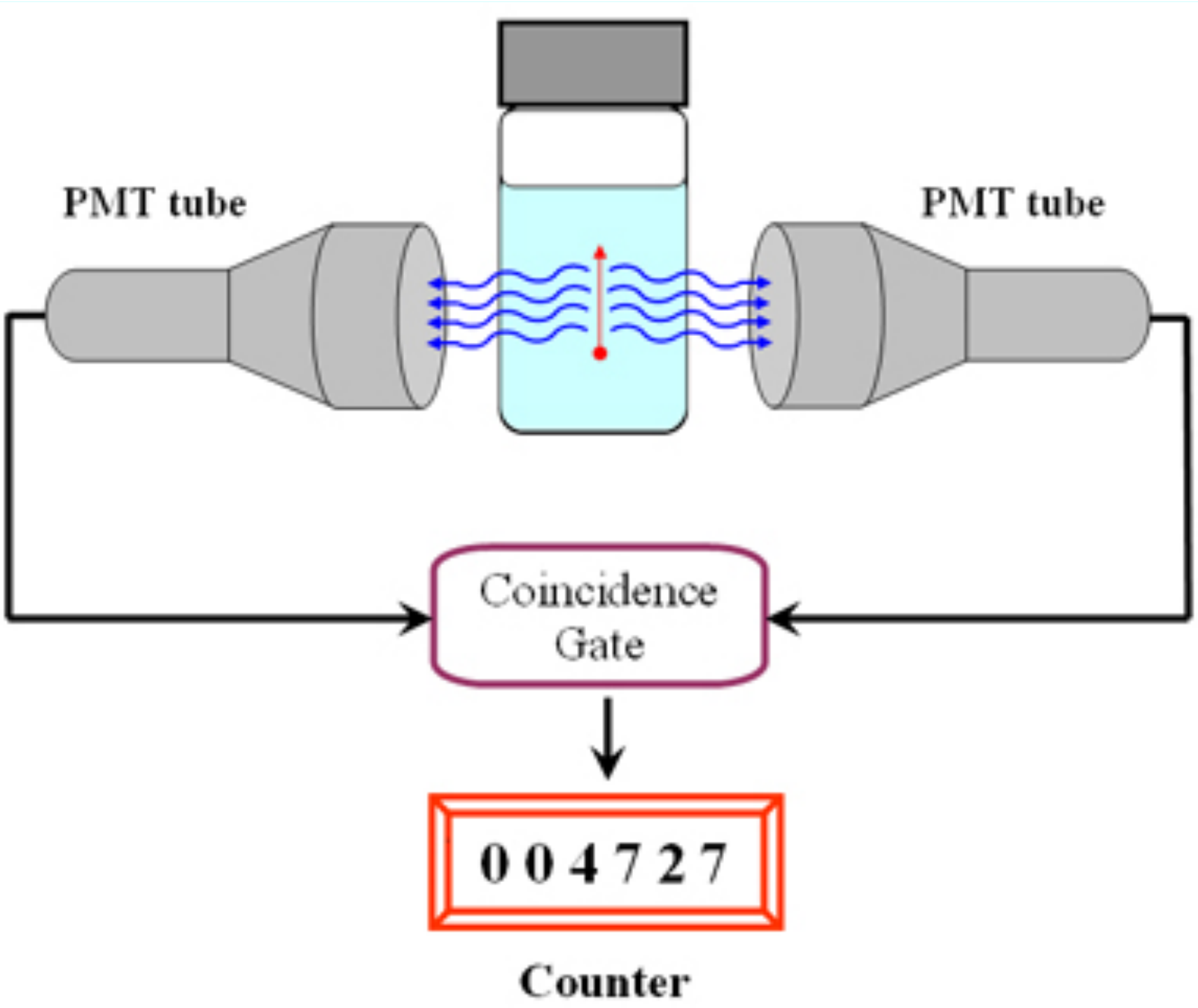
- ^{14}C is the basis for radiocarbon dating
- ^{14}C half life is 5730 years
- $^{14}CO_2$ is incorporated into plants via photosynthesis and hence all living organisms contain^{14}C in their biomass
- When organisms die ^{14}C no longer is incorporated and it begins to decay. Age of fossil carbon can be calculated via residual ^{14}C concentration

^{14}C Radiochemical Analysis

- Natural vanillin contains ^{14}C at an abundance typical of living organisms
- Synthetic vanillin is often made from petrochemicals derived from fossil carbon sources like oil or coal (i.e., guaiacol from coal tar)
- Fossil carbon is so old that ^{14}C is depleted
- ^{14}C radiochemical analysis can differentiate natural vanillin from vanillin synthesized from fossil carbon sources

^{14}C Radiochemical Analysis

- ^{14}C concentration can be measured by liquid scintillation counting (LSC)
- Sample is subject to combustion, evolved CO_2 is trapped in ethanolamine or KOH (as potassium carbonate) and then analyzed by LSC to determine $^{14}CO_2/^{12}CO_2$ ratio
- Relatively large amount of pure sample is required (at least 1-2 grams of pure vanillin)

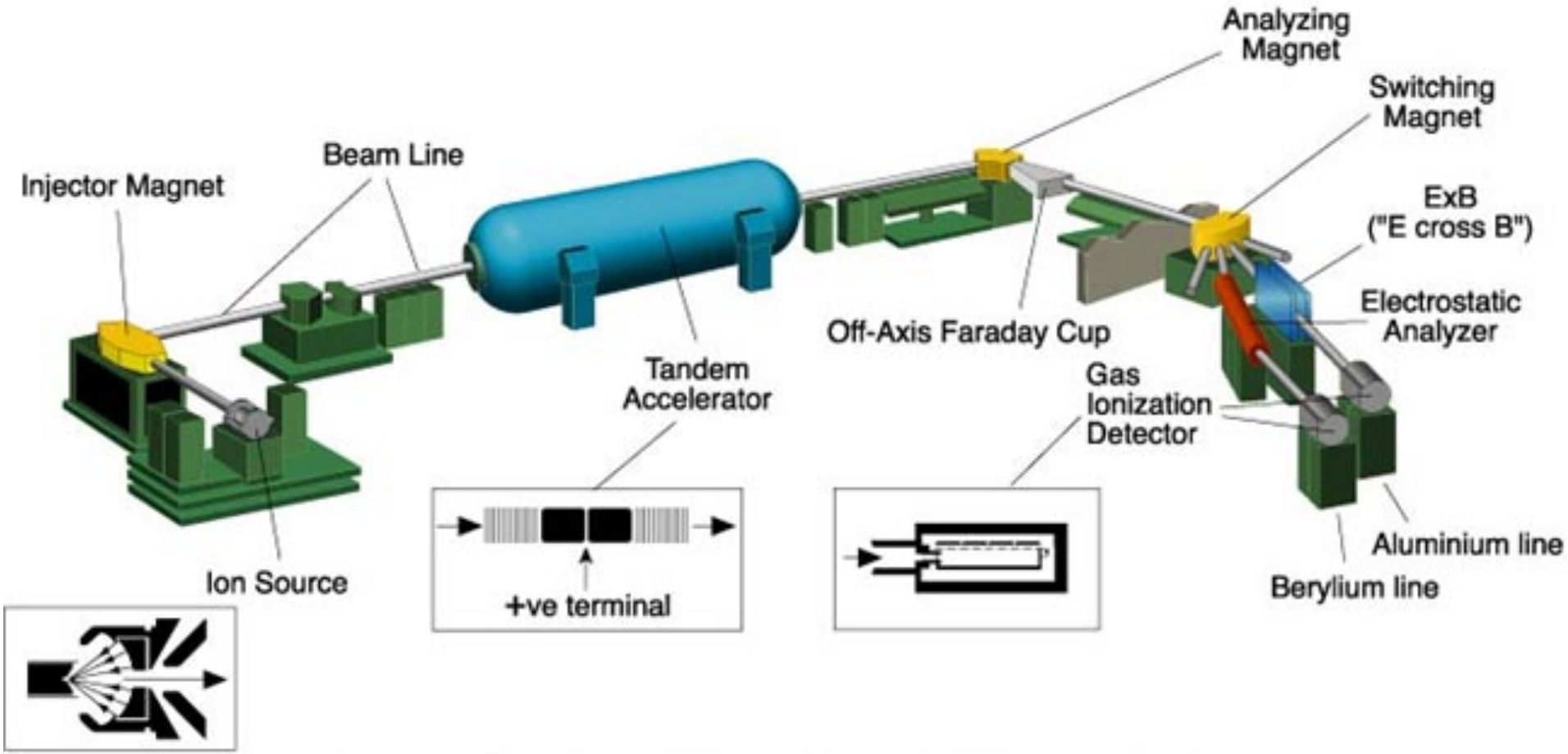

PMT tube
PMT tube
Coincidence Gate
0 0 4 7 2 7
Counter

^{14}C Radiochemical Analysis

- ^{14}C concentration can also be determined on smaller amounts of sample using Accelerator Mass Spectrometry (AMS)
- Sample is subject to combustion, CO_2 is then analyzed by AMS to determine $^{14}CO_2/^{12}CO_2$ ratio
- Smaller sample requirement but still several mg may be required
- AMS instrumentation is complex and costly

Accelerator Mass Spectrometry (AMS)

A method for counting cosmogenic radionuclides at a resolution of 1 atom in 1,000,000,000,000,000 (1×10^{15})



Cosmogenic radionuclides used in Earth Science applications measured by AMS with half-lives in years:

^{14}C (5730) ^{41}Ca (100,000) ^{36}Cl (301,000) ^{26}Al (705,000) ^{10}Be (1,510,000)

^{14}C Radiochemical Analysis

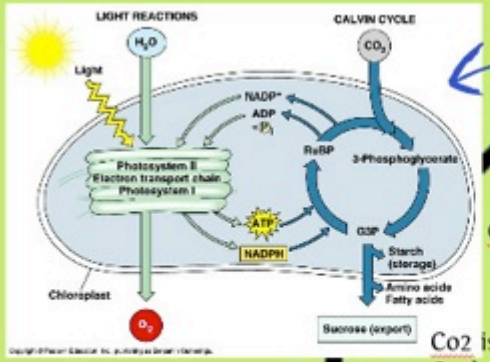
- Limitations
- Sample size and purity requirements for LSC
- Cost and availability of AMS instrumentation
- Can be defeated by using synthesis precursors of natural origin (i.e., guaiacol from *Guaiacum* flowers, lignin from paper pulp waste, biosynthetic vanillin from ferulic acid or eugenol etc.)
- May not be able to differentiate natural bean-derived vanillin from biosynthetic vanillin

$^{13}C/^{12}C$ Isotope Ratio Analysis

- ^{13}C is a stable isotope of carbon
- ^{13}C has a stellar origin – created in a sun by nuclear fusion reactions
- $^{13}C/^{12}C$ natural abundance is 1.11% in our solar system
- $^{13}CO_2/^{12}CO_2$ is incorporated into plants via photosynthesis
- Plants discriminate against incorporation of heavy isotopes in a process called biochemical isotope fractionation

$^{13}C/^{12}C$ Isotope Ratio Analysis

- Plants are classified into categories of C3, C4 and CAM type plants based on carbon fixation characteristics of their photosynthesis
- For instance the first carbon compounds synthesized by C3 and C4 type plants in photosynthesis have C3 or C4 carbon backbones
- Vanilla beans come from CAM plants. CAM is the acronym for Crassulacean acid metabolism. CAM photosynthesis is a carbon fixation pathway that evolved in some plants where the stomata is closed during the day and opened at night. CAM plants are differentiated from C3 and C4 plants in that photorespiration is never used.



C3

called C3 because the CO2 is first incorporated into a 3-carbon compound

CO2 fixation by Rubisco

Co2 is Directly fed into Calvin Cycle

More efficient that c4 and cam in cold moist climates

Photorespiration is used

C4

Called C4 because the CO2 is first incorporated into a 4-carbon compound

Photosynthesizes is faster than C3 plants under high light intensity and high temperatures because the CO2 is delivered directly to rubisco, not allowing it to grab oxygen and undergo photorespiration.

Uses PEP Carboxylase for the enzyme involved in the uptake of CO2, then it delivers the CO2 directly to rubisco for photosynthesis.

Photorespiration is seldom used

produce glucose

Stomata are open during the day.

same amount of CO2 gain

Take in CO2

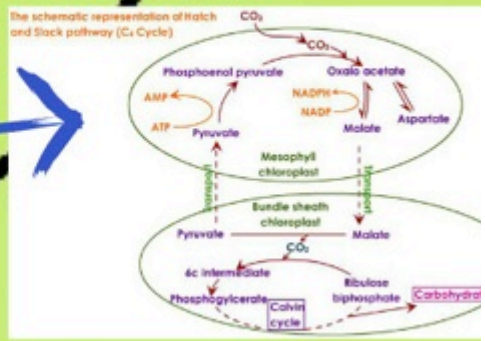
Rubisco present

sight of light reactions mostly mesophyll cells

Calvin cycle occurs in mesophyll cells

Hatch-Slack Pathway

photosynthesis occurs with stomata partially or completely closed on hot, dry days.



Stomata closed during the day and open at night.

CO2 is stored in the form of an acid before use in photosynthesis, the acid is broken down during the day and is released to rubisco

Photorespiration is never used

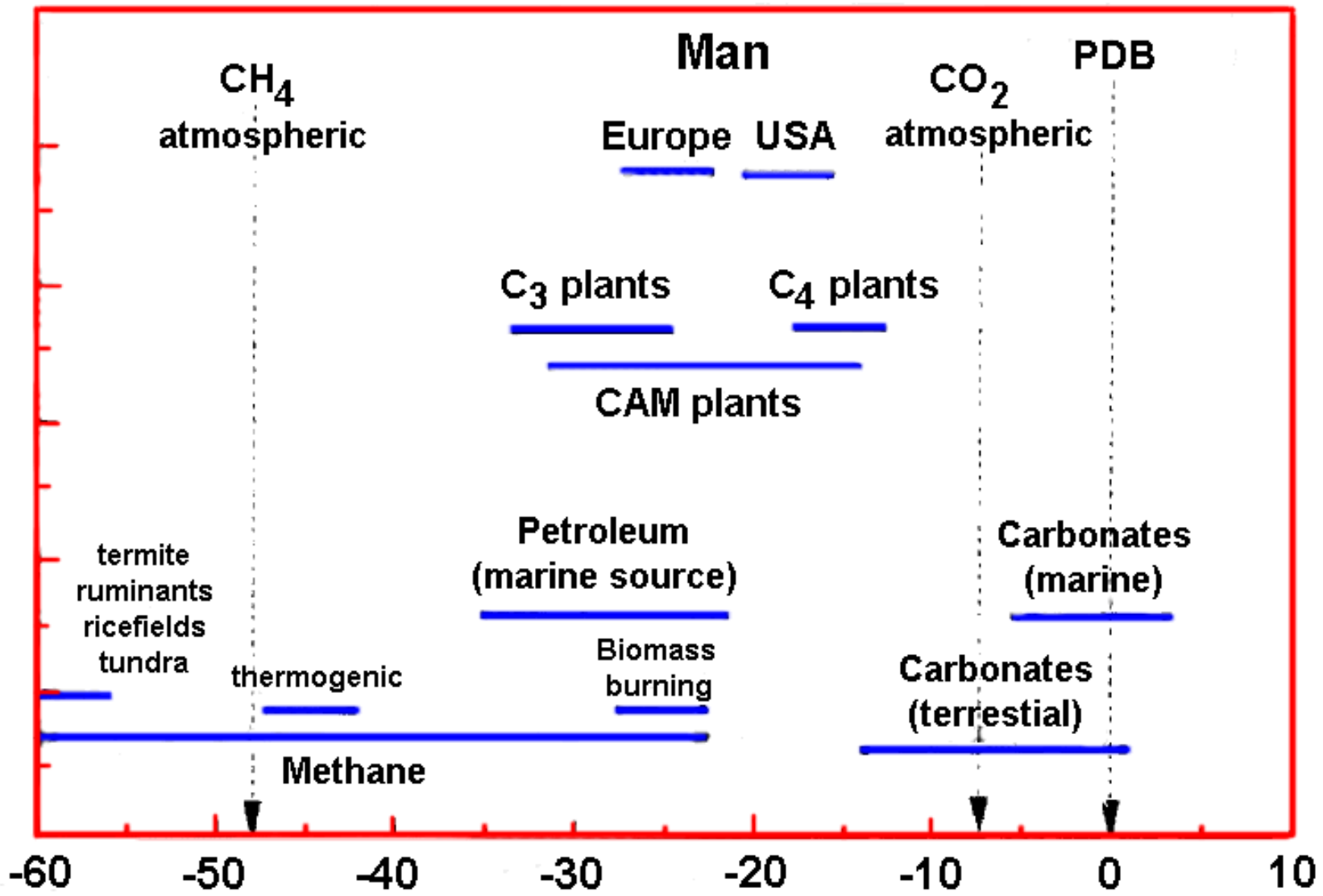
occurs mainly in Crassulacean species

CAM

$^{13}C/^{12}C$ Isotope Ratio Analysis

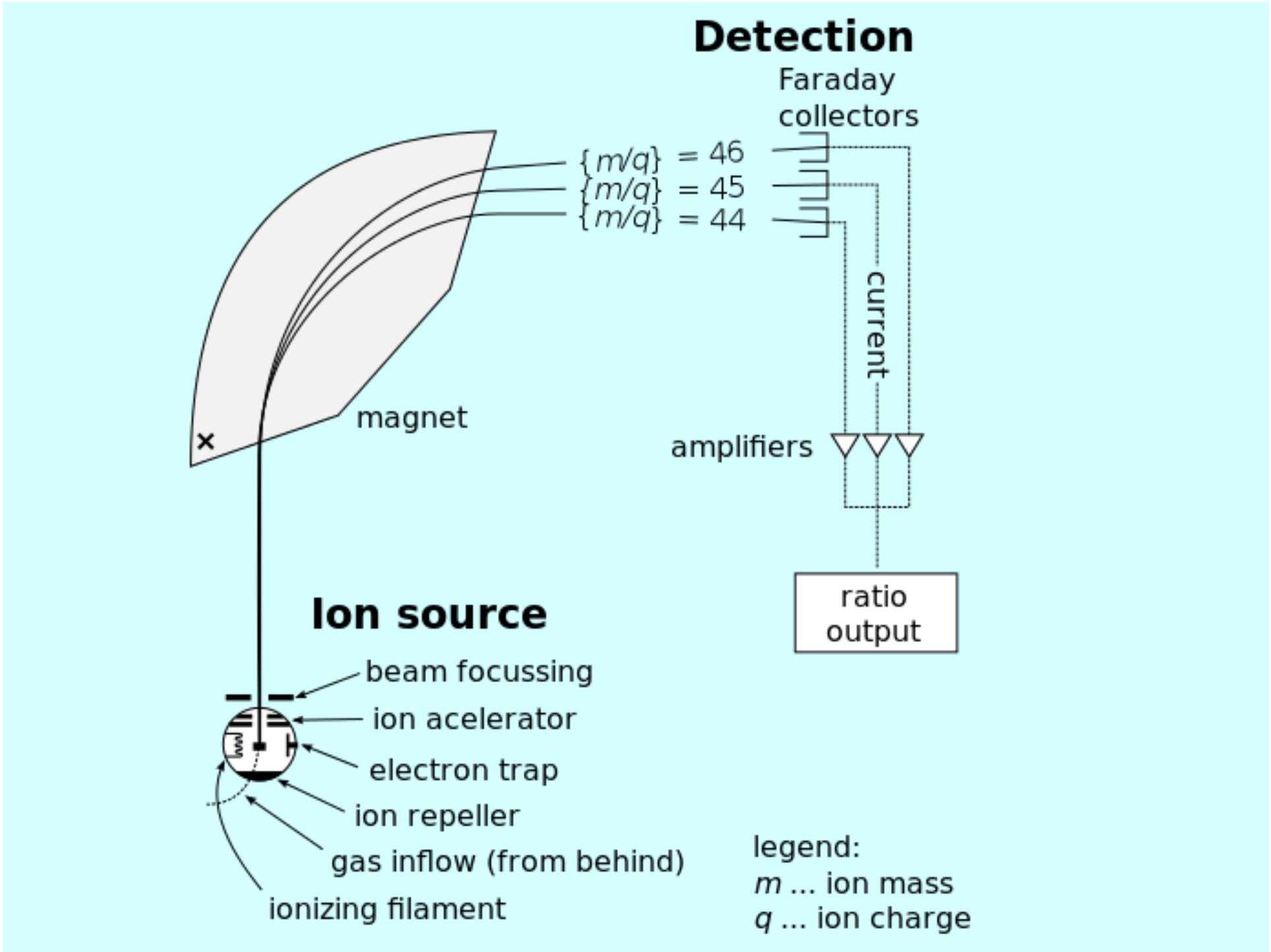
- $^{13}C/^{12}C$ isotope incorporation differs in C3, C4 and CAM plants
- $^{13}C/^{12}C$ ratios are typically expressed as a difference or delta ($\delta^{13}C$) from natural $^{13}C/^{12}C$ abundance or more accurately relative to a reference standard of fossil carbon that is close to natural abundance (i.e., a marine limestone sediment). $\delta^{13}C$ values are very slight in concentration and are measured in units of parts per thousand or “per mil” (‰).
- The international standard for determining $\delta^{13}C$ values is a fossil belemnite limestone sediment formed in the Cretaceous period in South Carolina, USA and is called the Pee Dee formation (Vienna Pee Dee Belemnite).

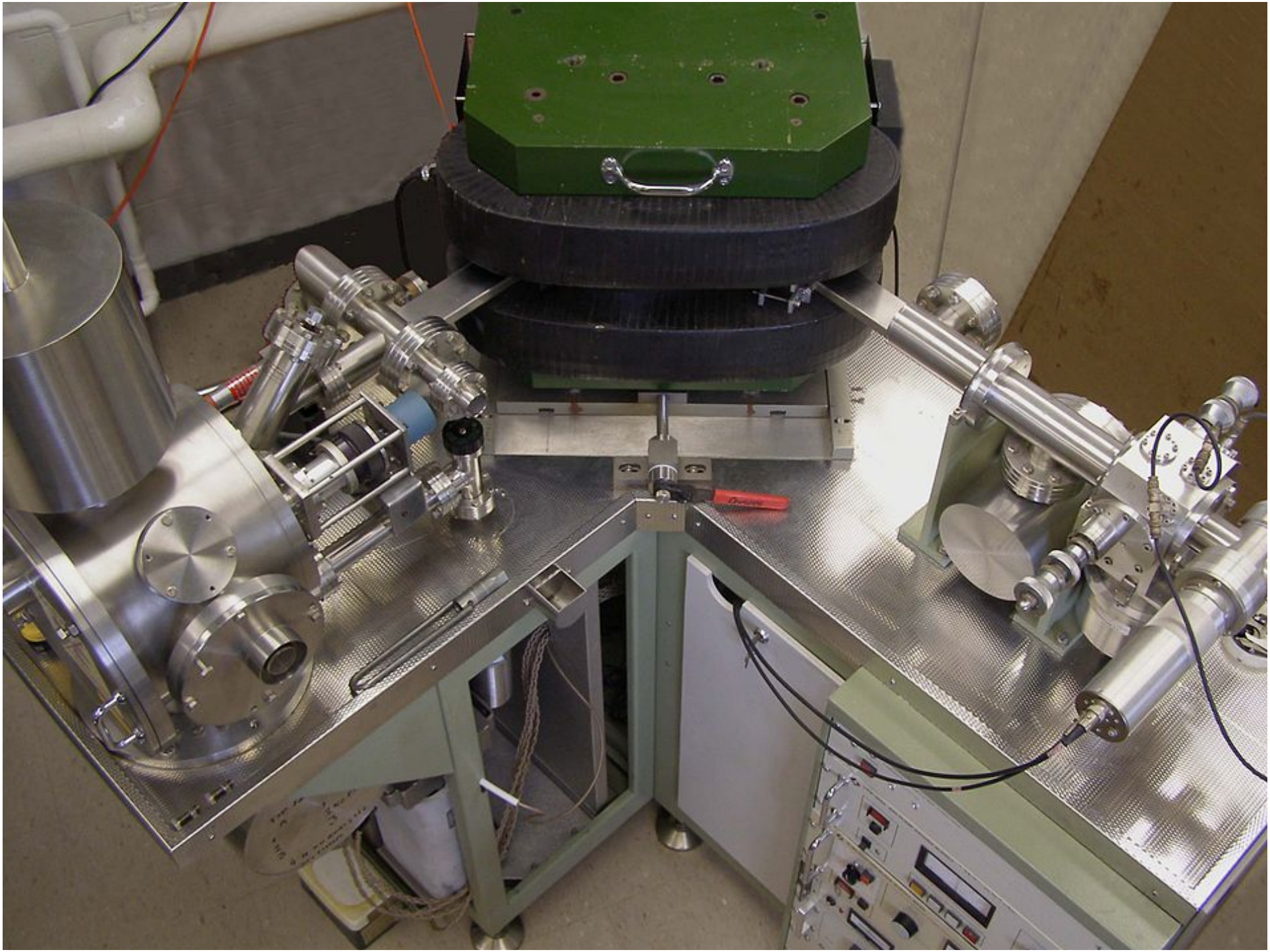
$\delta^{13}C$ Values



How are $^{13}C/^{12}C$ Isotope Ratios Measured?

- Vanillin is combusted into CO_2 in the presence of oxygen in a cupric oxide/Pt furnace at about 800C.
- CO_2 is analyzed for $^{13}CO_2/^{12}CO_2$ ratio in an Isotope Ratio Mass Spectrometer (IRMS) with a gas inlet ion source
- First generation IRMS instruments that are still widely in use combust off-line and then feed the CO_2 into the MS ion source


Detection
Faraday collectors
{m/q} = 46
{m/q} = 45
{m/q} = 44
current
magnet
amplifiers
ratio output
Ion source
beam focussing
ion acelerator
electron trap
ion repeller
gas inflow (from behind)
ionizing filament
legend:
m ... ion mass
q ... ion charge



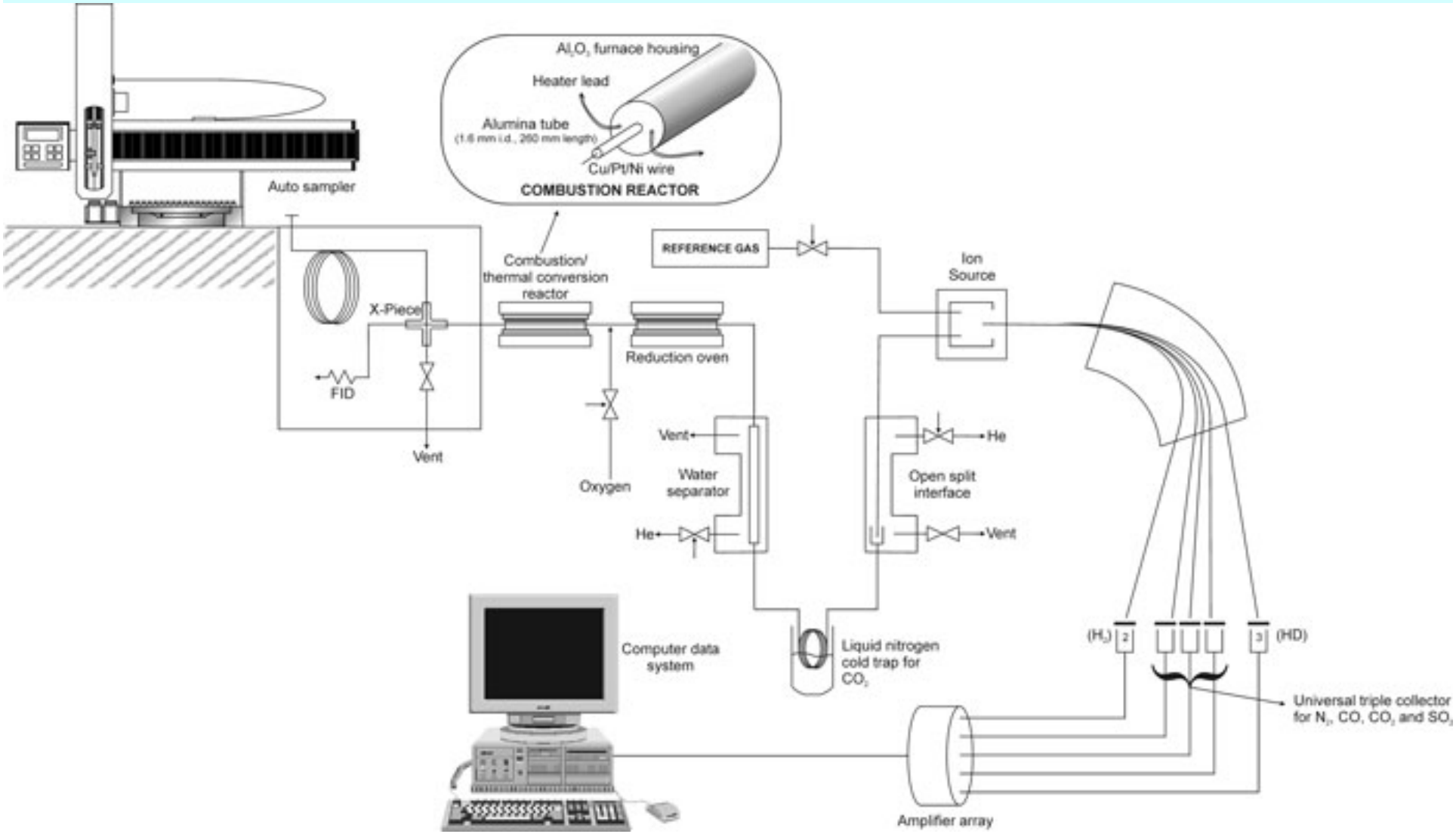
How are $^{13}C/^{12}C$ Isotope Ratios Measured?

- First generation IRMS instruments have relatively large sample requirement (milligrams-grams)
- Vanillin would need to be purified out of samples such as vanilla extract or food products (i.e., ice cream, yogurt etc.) and concentrated prior to analysis
- Not suitable for trace analysis or direct analyses of vanillin in complex mixtures such as vanilla extract or compounded flavors

How are $^{13}C/^{12}C$ Isotope Ratios Measured?

- IRMS instruments coupled to Gas Chromatographs called GC-C-IRMS became commercialized in the late 1990's
- GC-C-IRMS instruments enable $^{13}C/^{12}C$ isotope ratio measurements of individual GC peaks at high sensitivity
- Vanillin in mixtures such as vanilla extract and compounded flavors can now be analyzed directly in microgram and even sub-microgram concentration
- Other isotope ratios such as $^{18}O/^{16}O$ can be analyzed as well using a technique called GC-P-IRMS but tend to be less useful for vanillin as the aldehydic oxygen is exchangeable and leads to more variability in the data

GC-C-IRMS Instrumentation



$^{13}C/^{12}C$ Isotope Ratio Analysis

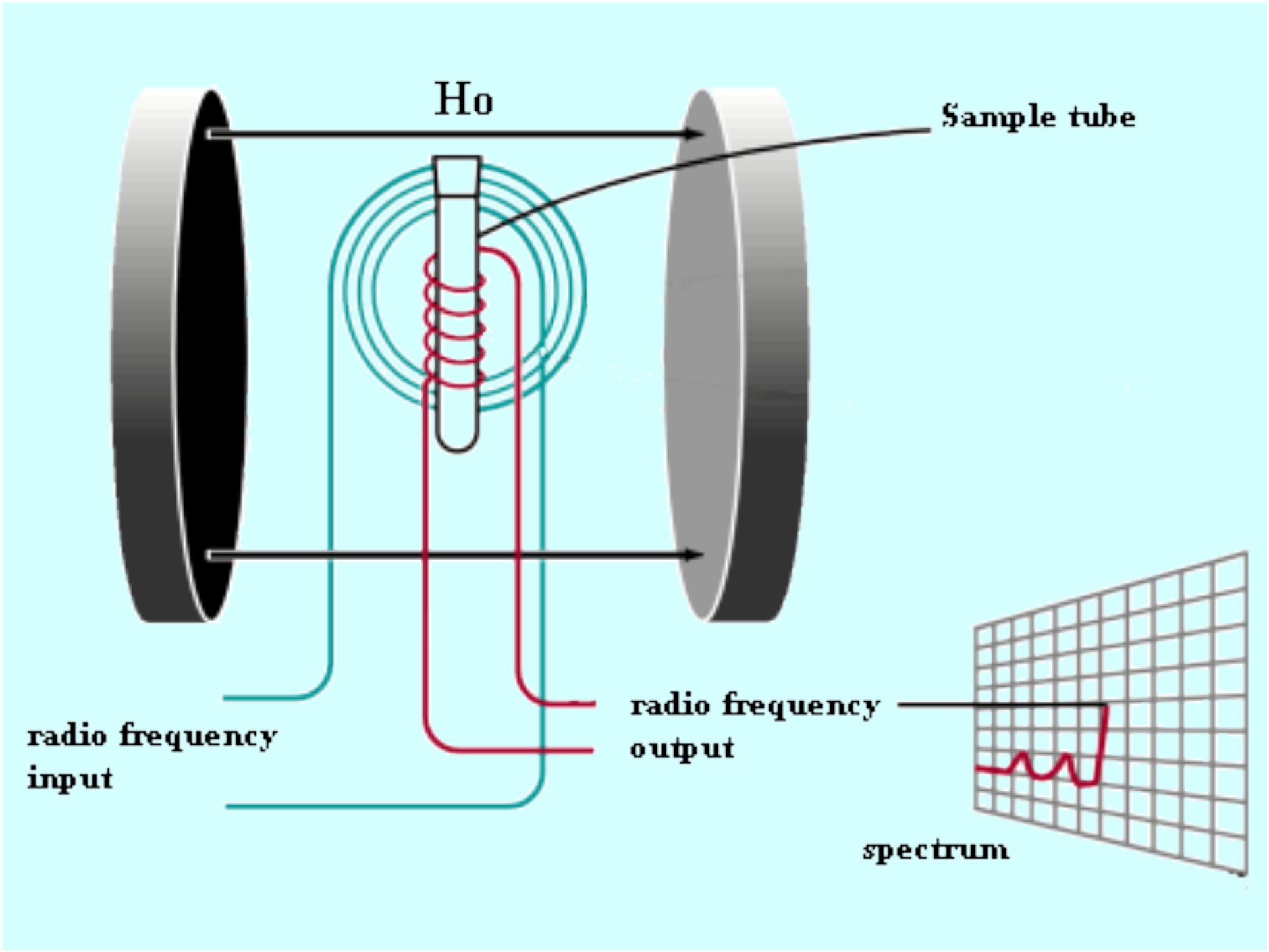
- $\delta^{13}C$ values for bean-derived vanillin typically range from -14 to -22 depending on production region
- $\delta^{13}C$ values for synthetic vanillin typically range from -26 to -33 and biosynthetic values are often greater than -35
- However, many outliers have been reported in the scientific literature
- Methods of adulteration have been reported in which the methyl (methoxy) group of synthetic vanillin was enriched in ^{13}C to bring the molecule within specification. This resulted in development of analytical methods involving cleavage of the methyl prior to IRMS.
- Reportedly, bean-derived vanillin can be differentiated from synthetic vanillin and biosynthetic vanillin by $^{13}C/^{12}C$ IRMS. However, large databases are required for reliable measurements and conclusions
- It is recommended that ancillary techniques be utilized along with $^{13}C/^{12}C$ IRMS to increase reliability

$^{2}H/^{1}H$ (D/H) Isotope Ratio Analysis

- ^{2}H (D) is a stable isotope of hydrogen. It has a natural abundance of 0.01%.
- $^{2}H/^{1}H$ (D/H) Isotope Ratio Analysis can be performed on a GC-IRMS or GC-C-IRMS instruments. However, the vanillin molecule is destroyed during combustion in conversion to CO_2 so the measured D/H ratio is the average for the whole molecule.
- More useful data can be obtained via site-specific natural isotope fractionation (SNIF™) Nuclear Magnetic Resonance spectroscopy (SNIF-NMR®)
- Pioneered by Martin *et al.* (1981) for wine authenticity testing, SNIF-NMR® can measure the ^{2}H concentration at each of the five isotopomers on the vanillin molecule

$^{2}H/^{1}H$ (D/H) Isotope Ratio Analysis

- SNIF-NMR® vanillin analysis was further refined/standardized by Eurofins
- SNIF-NMR® can reliably differentiate natural bean-derived vanillin from synthetic vanillin (i.e., guaiacol or lignin derived) and even biosynthesized vanillin
- Technique is State of the Art with respect to vanillin authenticity testing
- AOAC Official Method 2006.05 Vanillin


Ho
Sample tube
radio frequency
input
radio frequency
output
spectrum


BRUKER
600
UltraShield™

$^{2}H/^{1}H$ (D/H) Isotope Ratio Analysis

- Limitations of SNIFF-NMR®
- Vanillin needs to be isolated and purified from mixtures prior to analysis. NMR technique is not hyphenated as in GC-IRMS
- Isolation and purification techniques utilized must not induce isotope fractionation
- Relatively large samples are required (milligrams)
- Need large and up-to-date reference databases for comparison

Analytical Techniques to Determine Adulteration of Vanilla with Non-Natural Synergists/Fortifier or Other Extraneous Compounds

- GC or GC-MS techniques
- Direct Injection GC-MS (may require extensive sample extraction, cleanup & preparation)
- Direct Thermal Desorption-GC-MS (rapid analysis technique)
- Solid Phase Microextraction (SPME) – rapid analysis technique
- HPLC or HPLC-MS techniques
- New developments in Ultra High Pressure HPLC (UHPLC) offers rapid analysis times

Aroma extraction by vacuum distillation followed by SPE

Grounded cured beans of vanilla pompona (50g)
mixed with water (900 mL)

↓

T: 50°C
P: 50 Torr
Time: 2.5 h

Vacuum Hydro distillation process

↓

Solid Phase Extraction
Oasis® HLB cartridges (20cc/200 mg, 30 µm)

N-vinylpyrrolidone & m-divinylbenzene

↓

Sample elution (Methanol/DCM; 90/10)

↓

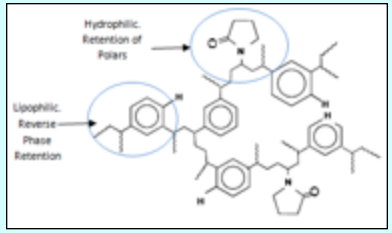
Fractions dried out with anhydrous $MgS0_4$ and Filtered

↓

Combined, Concentrated to 5 mL and evaluated

↓

Concentrated to 0.25 mL and Injected into GC (1 µL)



Aroma extraction by CH_2Cl_2 with GPC cleanup

Grounded cured beans of *Vanilla pompona* (50g)

Extraction with DCM (80 mL) in Vortex apparatus (20 min)

Extract poured onto paper filter (Whatman 12.5 cm/2.7µm)

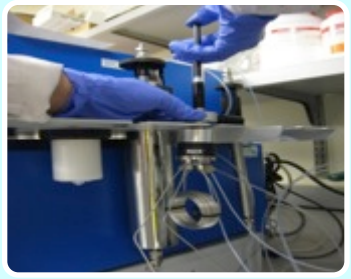
Concentrated extract with stream of Nitrogen

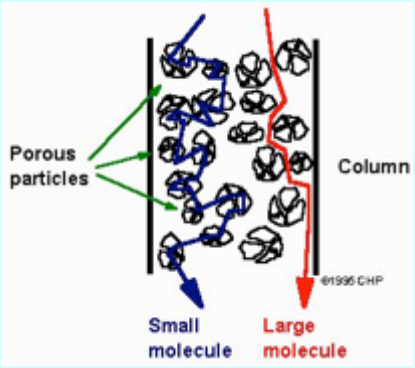
Extract (2.5 mL) transferred to a Syringe and injected into GPC system

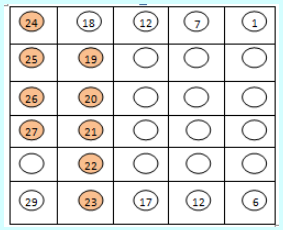
GPC column (25 mm i.d. x 500 mm l. with 50 g of Biobeads® S-X3)

Fractions (6 mL) evaluated one by one for presence of aroma

Combined fractions concentrated to 0.25 mL and injected into GC (1 µL)







Aroma extraction by EtOH with CH_2Cl_2 back extraction

Grounded cured beans of *Vanilla pompona* (50g)

↓

Mixed with Ethanol (95%w/w) in vortex (20min) and filtered

↓

Ethanolic extract (114 mL) + Dichloromethane (100 mL) + Water (200 mL)

↓

Separatory funnel / Centrifugation

↓

Dichloromethane fraction collected twice

↓

Filtrated and dried out with anhydrous $MgS0_4$

↓

Concentrated in TurboVap II evaporator (5 mL)

↓

Concentrated nitrogen stream (0.25 mL)

↓

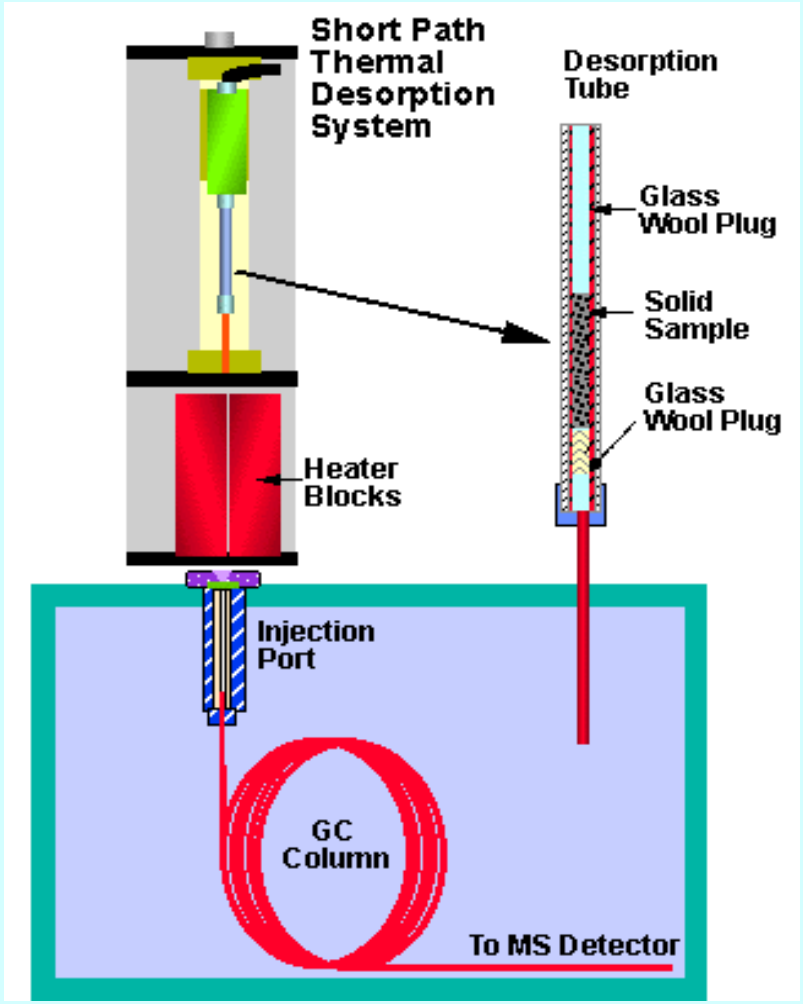
Injected into GC (1 µg)

GC or GC-MS techniques

- Direct Thermal Desorption-GC-MS (DTD-GC-MS) analysis technique for vanilla beans
- DTD-GC-MS technique pioneered by Hartman *et al*. 1992. Vanilla bean analysis was first published application in Food Science
- DTD-GC-MS technique first developed using patented Short Path™ Thermal Desorption jointly developed by Hartman laboratory at Rutgers University and Scientific Instrument Services Inc. (SIS, Ringoes, NJ).
- Short Path™ Thermal Desorption instrumentation marketed worldwide
- Automated analysis with introduction of Autodesorb™ instrument

Measuring Flavor Compounds in Vanilla Beans by DTD-GC-MS

- Vanilla beans ground and composited on inert support.
- Composites transferred to desorption tubes and spiked w/ internal standard.
- Desorption tubes connected to Short Path Thermal Desorber and thermally desorbed into GC-MS for final analysis.



Advantages of DTD-GC-MS Analysis of Vanilla Beans

- Small Sample Requirement
- Individual Beans Can be Analyzed or Composite Samples Can be Prepared
- Rapid Analysis Time (about 1 hour from start to finish including prep)
- Provides Detailed Data on Flavor/Aroma Composition
- Affords Accurate Quantification of Vanillin and other Vanilla Components such as Vanillin/p-Hydroxybenzaldehyde Ratios
- Fingerprinting of Samples from Different Geographical Origins
- Detection of Adulteration or Contaminants
- Gives Data on Chemical Composition Prior to Alcohol Exposure in Vanilla Extract

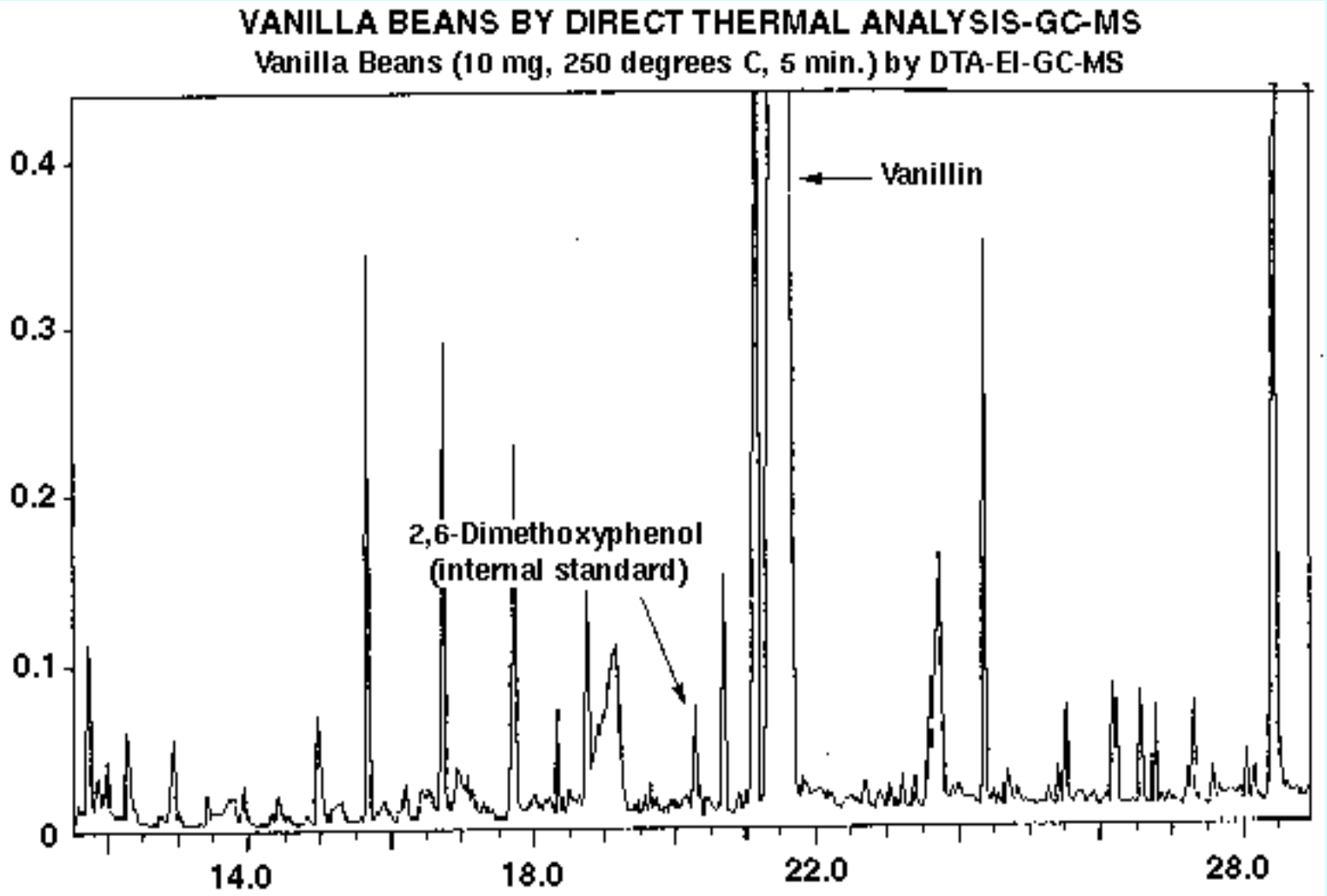
Limitations of DTD-GC-MS Analysis

- Small sample size analyzed may not be representative
- Problem can be solved by compositing prior to analysis
- Thermal decomposition artifacts
- Must be able to interpret precursor chemistry of potential thermal decomposition artifacts

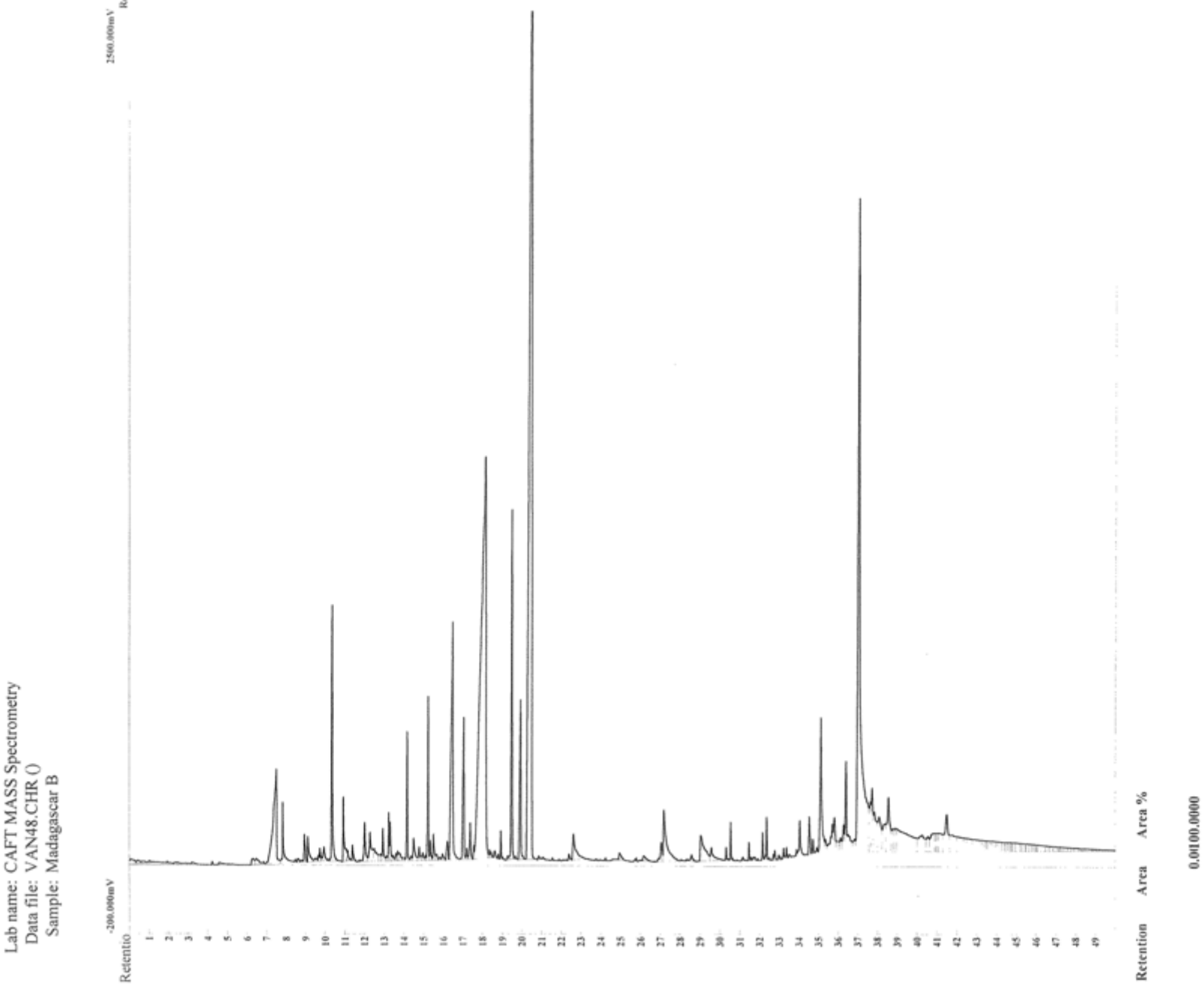
Adulterants that can be Detected by DTD-GC-MS

- Vanilla synergists/fortifiers not natural to vanilla beans
- Compounds such as piperonal (heliotropin), ethyl vanillin, coumarin (from Tonka beans), veratraldehyde (methyl vanillin)
- Glycols such as glycerol, propylene glycol, butylene glycol etc. that occur naturally in vanilla beans but if present in unusually high concentration may indicate spraying of beans to increase weight
- Possibly “boosting” with synthetic vanillin if unusually high vanillin concentrations are detected or if atypical vanillin/p-hydroxybenzaldehyde ratios are observed

Typical Gas Chromatogram of Flavor Compounds in Vanilla Beans



DTD-GC-FID Chromatogram of a Vanilla Bean Sample from Madagascar

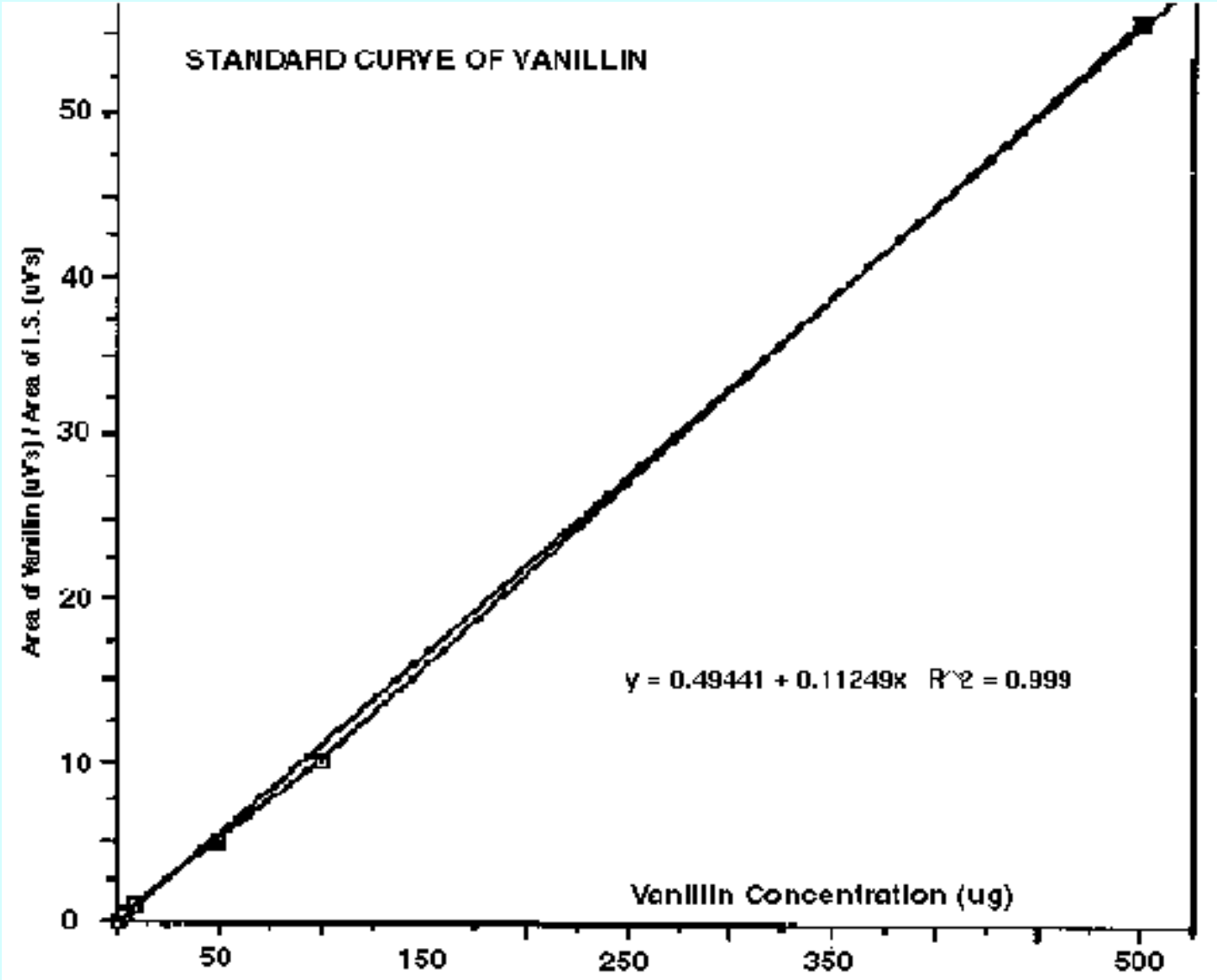


Vanilla β-Diketones & γ-Pyrones

- Pentacosane-2,4-dione
- 16-pentacosene-2,4-dione
- Heptacosane-2,4-dione
- 18-heptacosen-2,4-dione
- Nonacosane-2,4-dione
- 20-nonacosen-2,4-dione
- Hentriacontane-2,4-dione
- 22-hentriacontene-2,4-dione
- 2-(10-nonadecenyl)-2,3-dihydro-6-methyl-4H-pyran-4-one
- 2-(14-tricosenyl)-2,3-dihydro-6-methyl-4H-pyran-4-one
- 2-(12-heneicosenyl)-2,3-dihydro-6-methyl-4H-pyran-4-one

(Common in Plant Waxes – Tulloch, 1976)
(Occurrence in Vanilla Beans – Ramoroson, et al. 2000)

Calibration Curve of Vanillin Concentration



Direct Thermal Desorption-GC-MS Analysis of Vanilla Beans

Over 200 Volatile & Semi-Volatile Compounds Identified in Vanilla Beans to Date

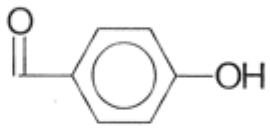
A Typical DTD-GC-MS Analysis of a Single Sample Yields About 80-100 Compounds

Vanillin Concentration & Vanillin/p-Hydroxybenzaldehyde Ratios in Vanilla Bean Samples

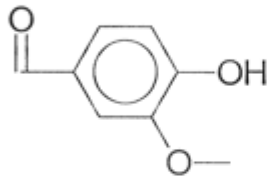
Vanillin/p-Hydroxybenzaldehyde Ratio as Adulteration Indicator

- Hoffman, Patrick, Harmon, A., Ford, P. and Zapf, M., Weber, A., King, S, Grypa, R., Philander, E, Gonzalez, L., Lentz, K., Noakes, J. and Culp, R., Vanilla 1st International Congress: Analytical Approaches to Vanilla Quality and Authentication (2005)
- Jurgebs, U., Lebensmittelchem. Grichtl. Chem., 35:97, (1981)
- Johns, T.V., Vanilla 1st International Congress: Indian Vanilla, Cultivation, Curing, Quality & Authenticity (2005)

Typical values for Van/p-HB ratio are 8-25. However, higher values reported for Indian vanilla beans.



p-Hydroxybenzaldehyde



Vanillin

Vanillin Concentration and Vanillin/p-Hydroxybenzaldehyde Ratio

Vanilla Bean Sample	Species	Vanillin Conc. % w/w	Van/p-HB
Madagascar A	*Vanilla planifolia*	2.24	15.86
Madagascar B	*Vanilla planifolia*	1.67	11.95
Madagascar A55	Unidentified	2.67	14.12
Madagascar	*Vanilla Pompona*	2.33	6.58
Comoros	*Vanilla planifolia*	2.92	18.66
Mexican	*Vanilla planifolia*	2.28	14.69
Tongan	*Vanilla planifolia*	1.04	22.31
Indonesian	*Vanilla planifolia*	0.15	15.67
Hawaiian	*Vanilla planifolia*	2.22	24.74
Ugandan	*Vanilla planifolia*	1.19	18.06
Tahitian	*Vanilla tahitensis*	0.58	7.13
Peruvian	Wild ? Species	0.44	12.08

Vanillin Concentration and Vanillin/p-Hydroxybenzaldehyde Ratio

Vanilla Bean Sample	Species	Vanillin Conc. % w/w	Van/p-HB
PNG - A	*Vanilla tahitensis*	1.66	13.45
PNG - B	*Vanilla tahitensis*	0.90	19.62
PNG - C	*Vanilla tahitensis*	1.05	8.33
Indian - A (Kerala)	*Vanilla planifolia*	1.22	15.57
Indian - B (SBOVP)	*Vanilla planifolia*	2.43	21.33
Indian - C (SBNOVKP)	*Vanilla planifolia*	1.92	**32.37**
Indian - D (SBNOVKH)	*Vanilla planifolia*	1.97	**33.18**
Indian - E (Chipmanglur)	*Vanilla planifolia*	2.86	**25.93**
Indian - F (Aldur)	*Vanilla planifolia*	2.82	**74.09**

Vanillin Concentration and Vanillin/p-Hydroxybenzaldehyde Ratio

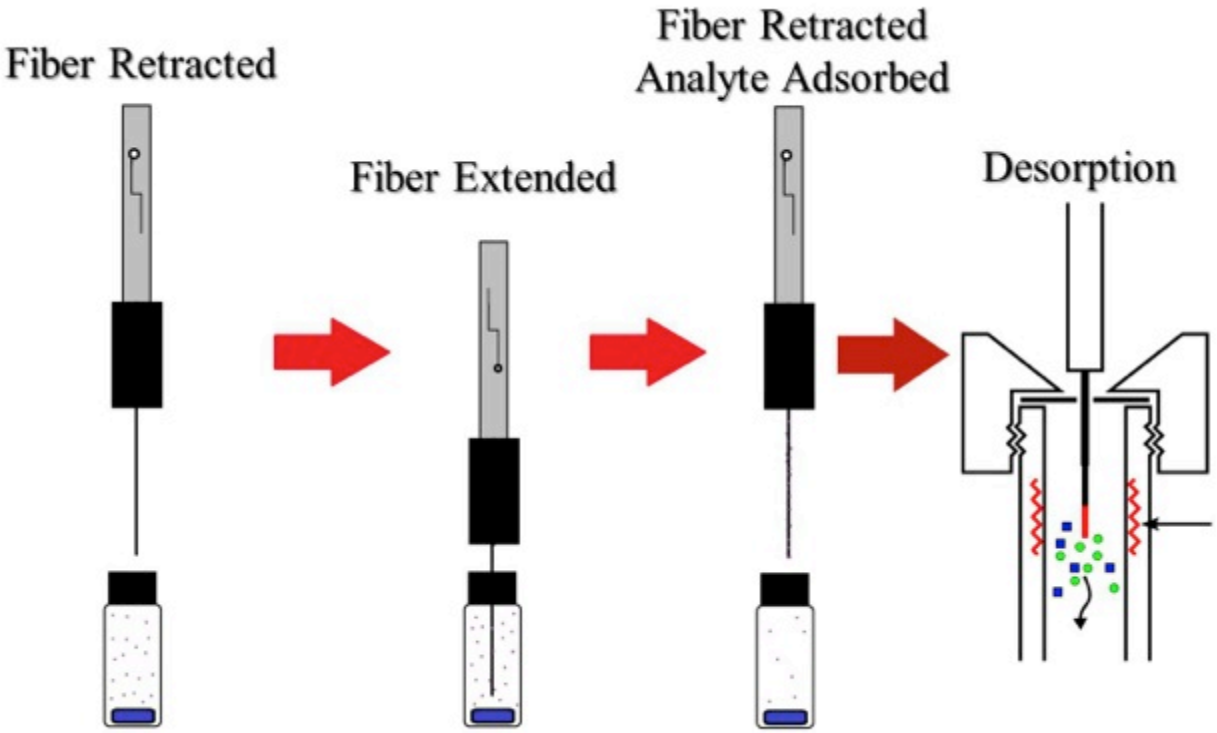
Statistical Analysis

Statistical Data	Vanillin Conc. % w/w	Van/p-HB
Mean, n=15 (all regions except Indian)	1.56	14.88
SD	± 0.86	± 5.29
Min. (Indonesian)	0.15	6.58
Max. (Comoros)	2.92	24.74
Mean, n=6 (Indian Beans)	2.20	33.75
SD	± 0.63	± 20.86
Min. (Kerala Region)	1.22	15.57
Max. (Chipmanglur region)	2.86	74.09

Solid Phase Microextraction GC-MS (SPME-GC-MS)

- SPME technique developed and patented by Dr. Janusz Pawliszyn, University of Waterloo, Canada in early 1990's
- Commercialized and marketed by Varian, Supelco, Leap Technologies & Gerstel
- Automated (AS 8200, CTC CombiPal)
- Coating on thin fused silica or SS fibre is used to adsorb volatile/ semi-volatile compounds from headspace of sample
- Fibre coatiings available in polydimethylsiloxane (PDMS), polyacrylate (PA), polyethylene glycol (Carbowax) and other functionalities
- Fibre is thermally desorbed by insertion into GC injection port to deliver adsorbed compounds for analysis
- Rapid and simple analysis technique

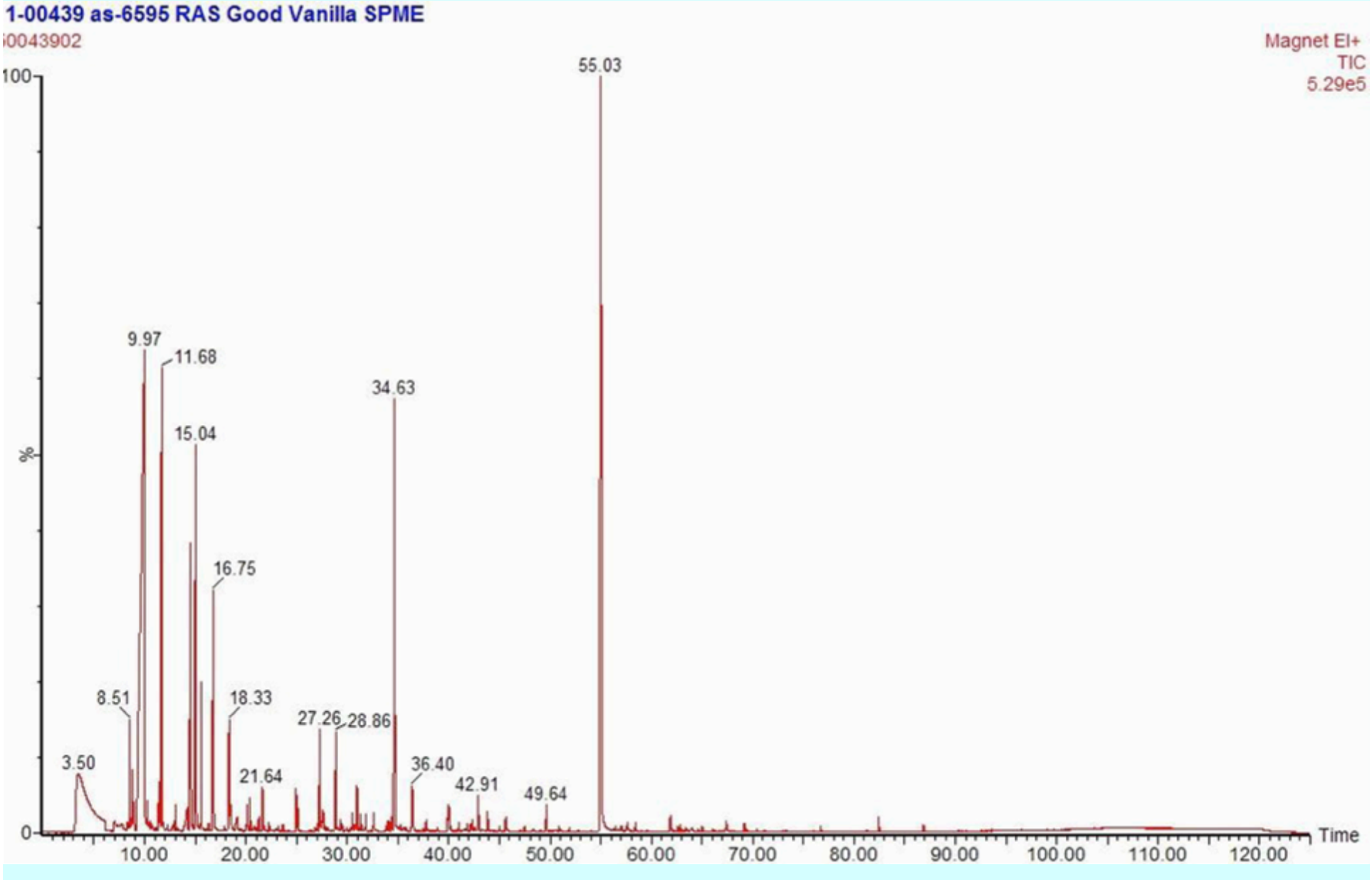
SPME


Fiber Retracted
Fiber Extended
Fiber Retracted
Analyte Adsorbed
Desorption

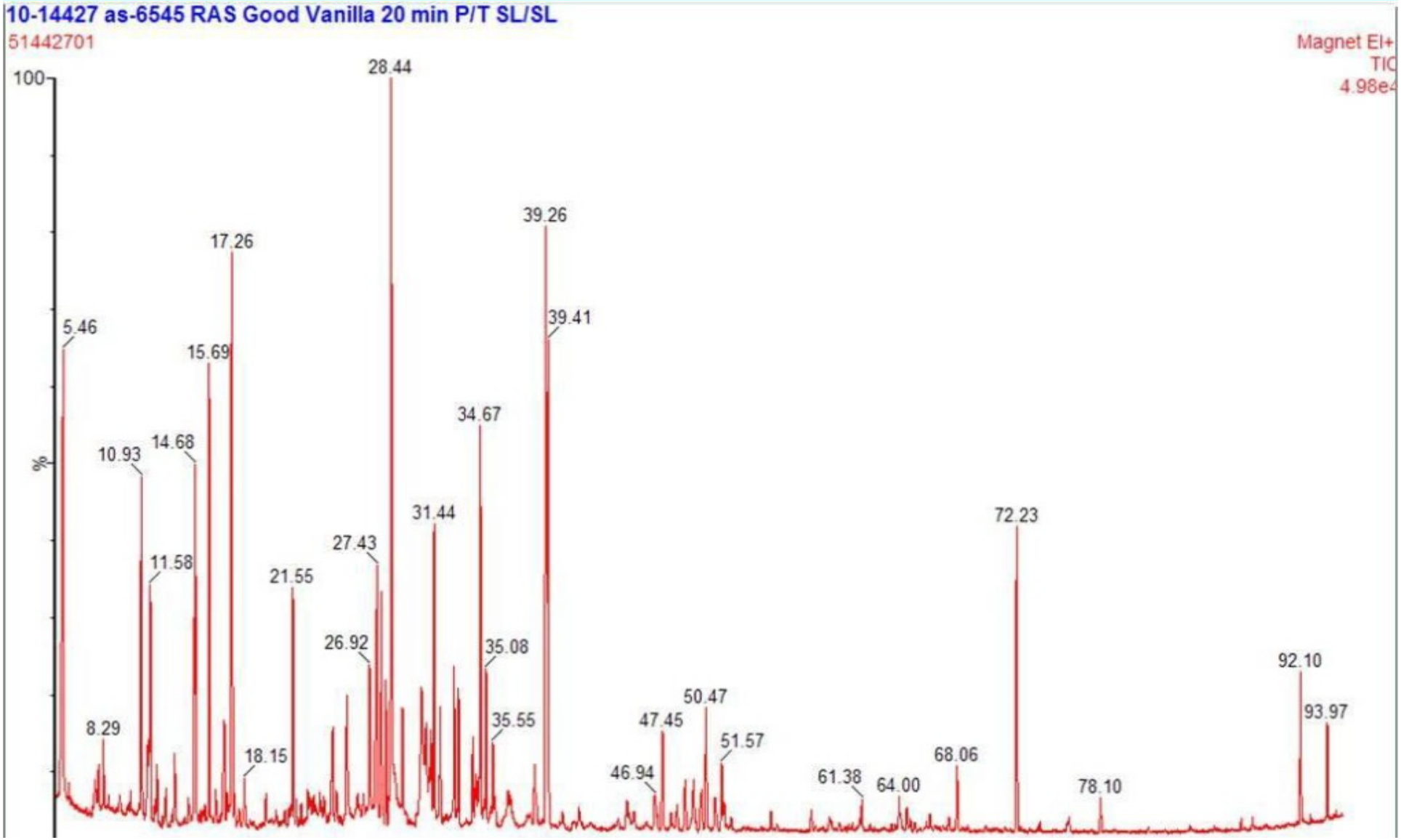
SPME Limitations

- Can introduce bias in measurement based on compounds affinity to adsorb onto coated fibre (based on vapor-phase equilibrium and affinity to adsorb to coating). Not used as sample introduction for GC-C-IRMS due to potential for isotope fractionation.
- Tends to favor non-polar analytes and discriminate against very polar compounds. Mixed fibre coatings can help.
- Less efficient in collecting high boiling species (will not detect compounds such as waxy long chain β-diketones and γ-pyranones at high boiling end of vanilla bean chromatograms)
- Does not readily lend itself to quantification
- Fibre is small and has limited surface area available for adsorption.
- Less loading capacity and sensitivity than techniques such as Purge & Trap-Thermal Desorption-GC-MS or DTD-GC-MS.
- Despite limitations technique is very popular and widely utilized due to ease of use and simplicity

Vanilla Chromatogram by SPME-GC-MS



Vanilla Chromatogram by Purge & Trap-Thermal Desorption-GC-MS



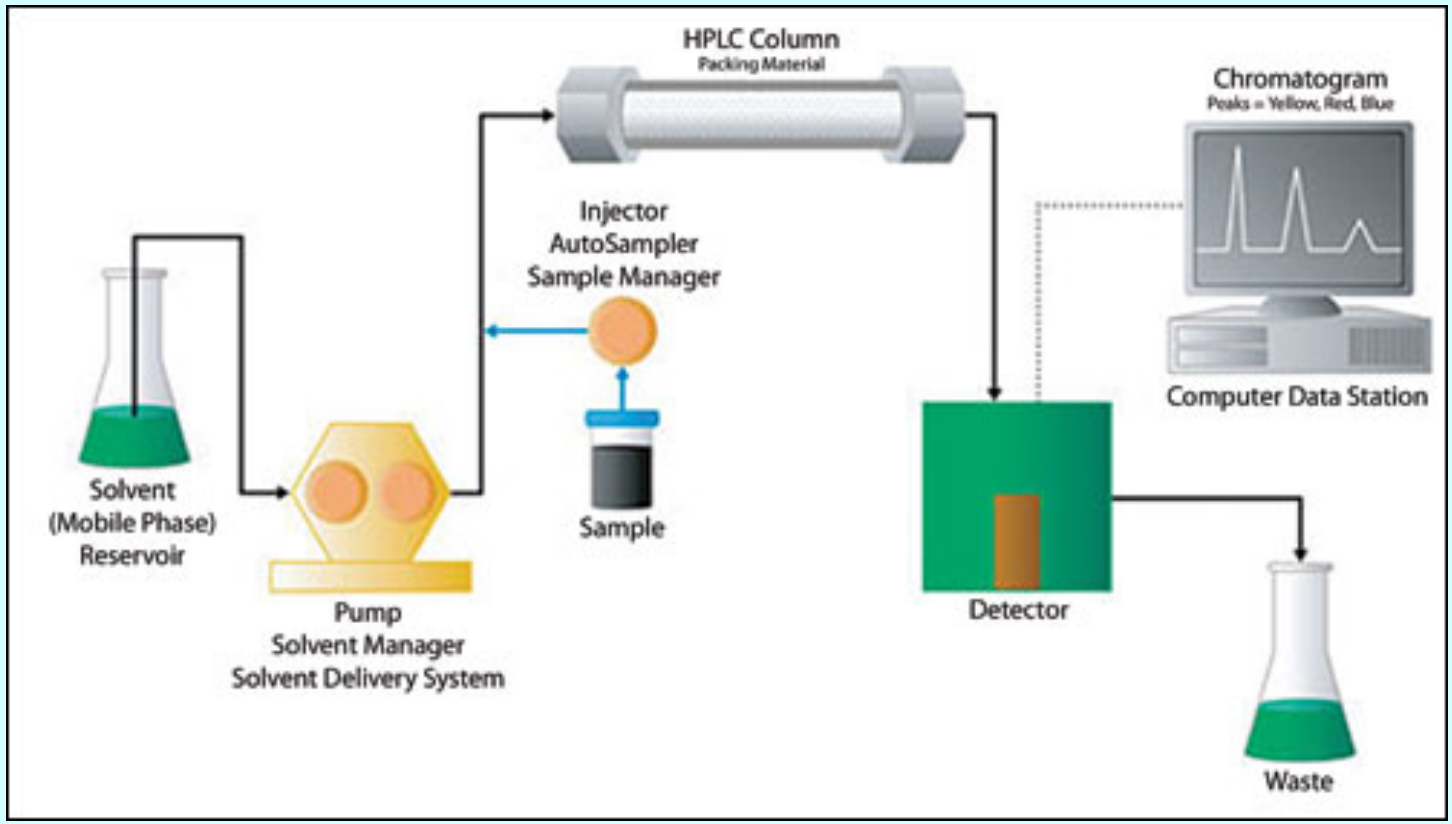
HPLC or HPLC-MS Analysis

- Can be used to analyze non-volatile components of vanilla not amenable to GC-MS (i.e., glucovanillin)
- Trace level non-volatile components of vanilla less investigated, represents an unexplored frontier in vanilla chemistry
- Most commonly used for direct analysis of vanilla extract
- Very good for measuring organic acids, major phenolic species in vanilla and especially compounds with good UV-Vis absorbance characteristics
- Good for ratio measurements. No discrimination based on volatility.
- Excellent for quantification. Very stable and reproducible when properly calibrated.
- Recent developments in methodology such as Ultra High Pressure HPLC (UHPLC) or Fast LC offer rapid analysis times, lower solvent consumption, reduced waste

HPLC or HPLC-MS Analysis Limitations

- Compounds must have chromophore or UV-absorbing groups in the structures to be measurable by UV-Vis detectors
- Detector response factors (RF) differ greatly as a consequence
- Many compounds in vanilla are invisible to UV-Vis detectors
- Universal HPLC detectors such as Refractive Index (RI) lack high sensitivity
- For HPLC-MS analysis compounds must be ionizable by techniques such as Atmospheric Pressure Chemical Ionization (APCI) or Electrospray (ESI). Many compounds in vanilla do not readily ionize
- HPLC has much lower chromatographic resolution than GC

HPLC Method Overview



Source: www.comsol.com

Fast LC Method

- 2008 – New instrumentation unveiled that can take advantage of smaller particles size columns
- Offers faster analysis, higher resolution
- UPLC – Ultra-high pressure Liquid Chromatograph

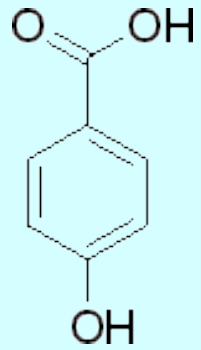
Why faster HPLC?

- Increase in throughput and productivity
- Decrease in turnaround time
- Customer
  - Decrease time required per investigative experiment allows more variations to be evaluated in the same time frame
- Analytical Lab
  - Rapid, thorough evaluations of separation parameters leads to better methods being developed quicker

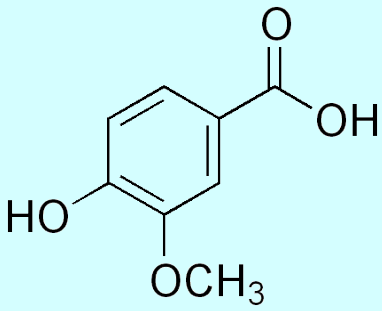
Recent HPLC Vanilla methods

Author	Year	Column
Thompson	1988	300mm x 3.9mm x 10um
Lamprecht	1994	250mm x 100mm x 5um
Voisine	1995	250mm x 100mm x 5um
Negishi	1996	150mm x 4.6mm x 5um
Waliszewski	2006	150mm x 4.6mm x 5um
Sinha	2007	250mm x 4.6mm x 5um
Cicchetti	2009	250mm x 4.6mm x 5um
Toth	2012	50mm x 2.1mm x 1.7um 50mm x 4.6mm x 1.8um

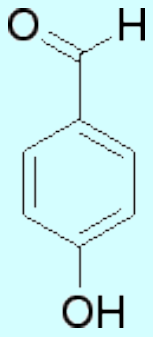
Compounds of interest



4-hydroxybenzoic acid (1)

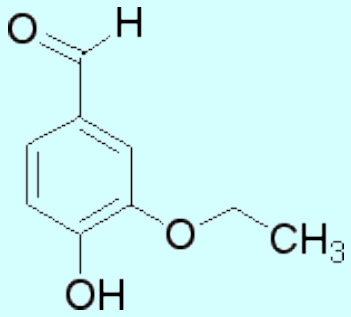


vanillic acid (2)



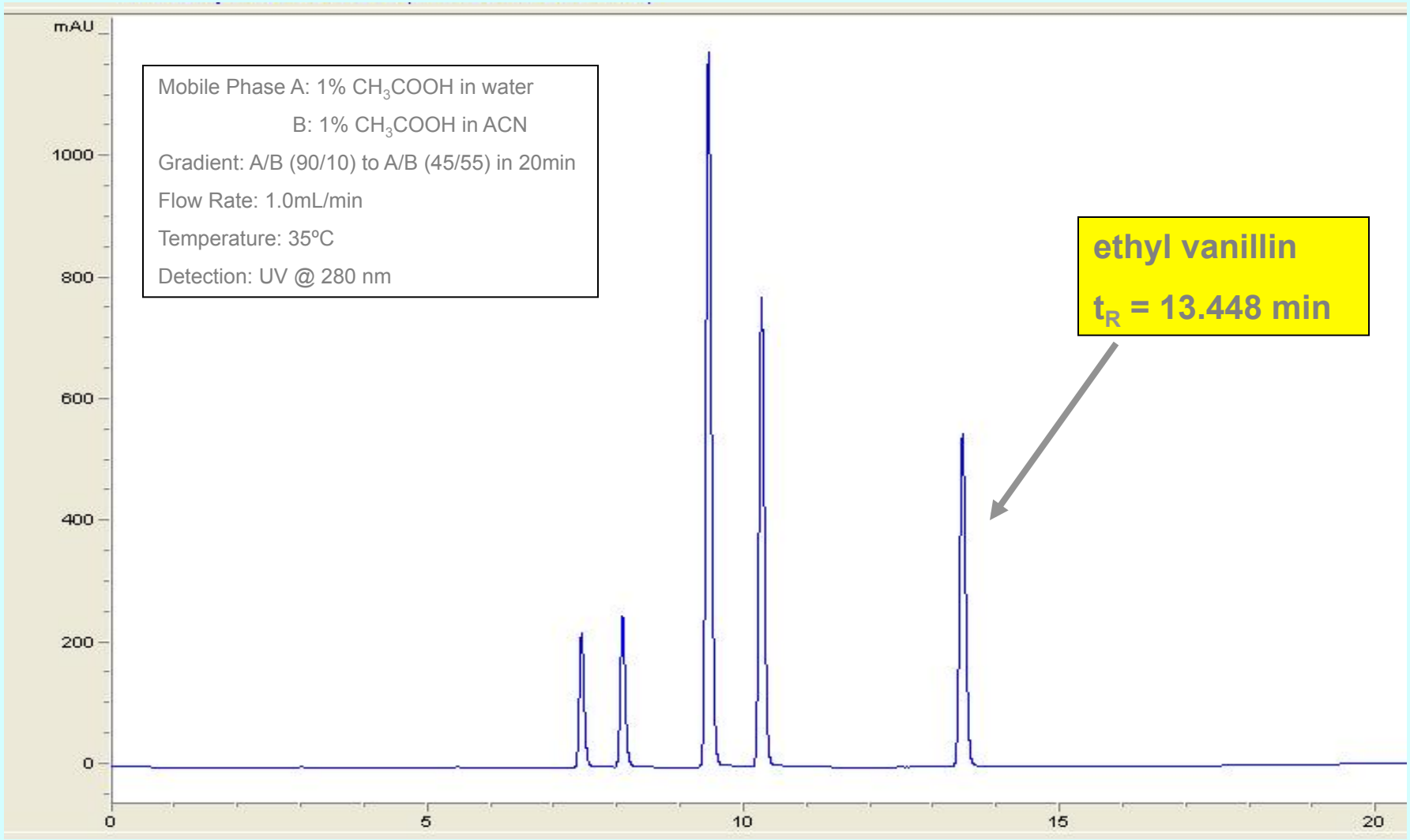
4-hydroxybenzaldehyde (3)

vanillin (4)

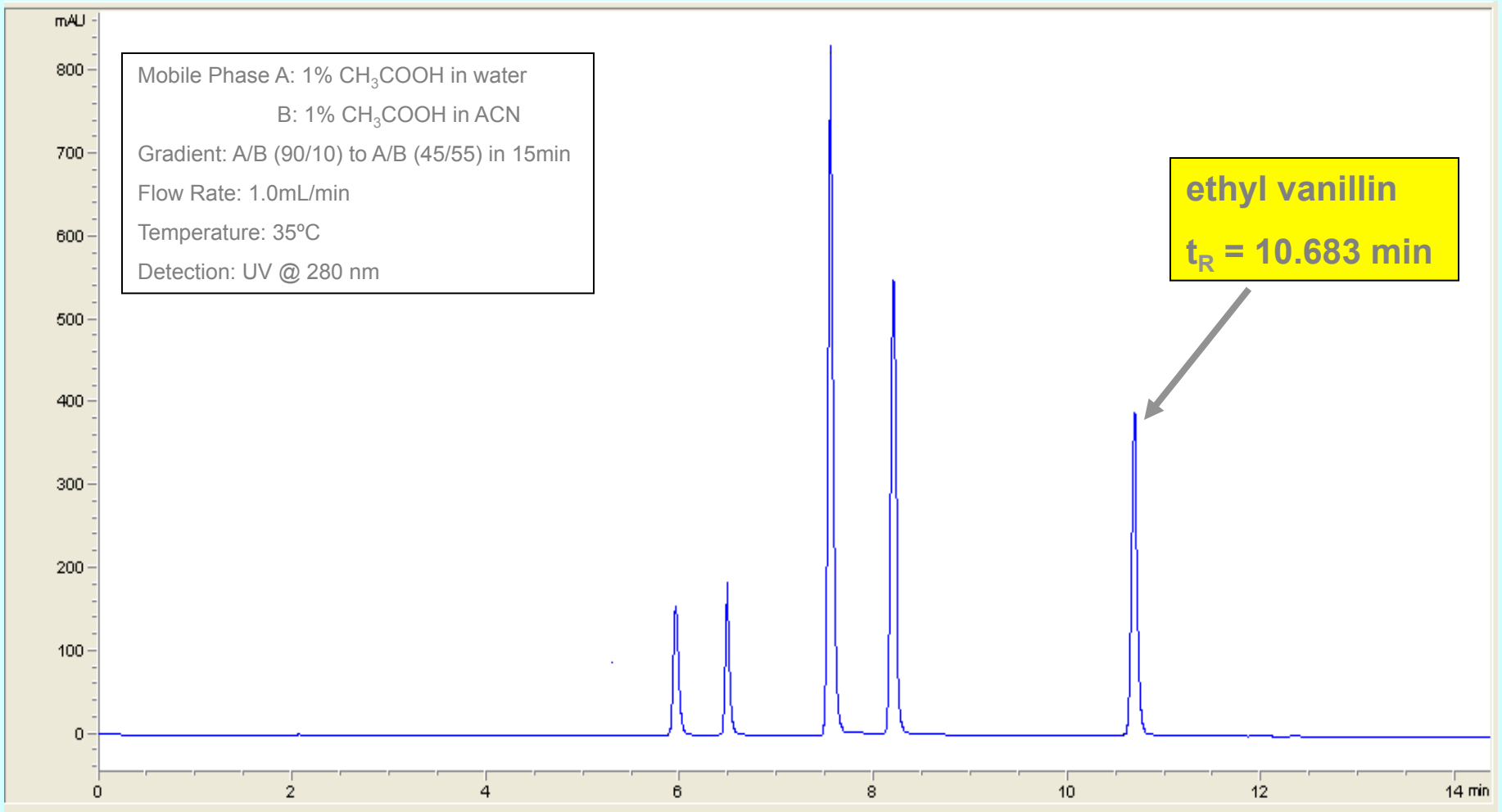


ethyl vanillin (5)

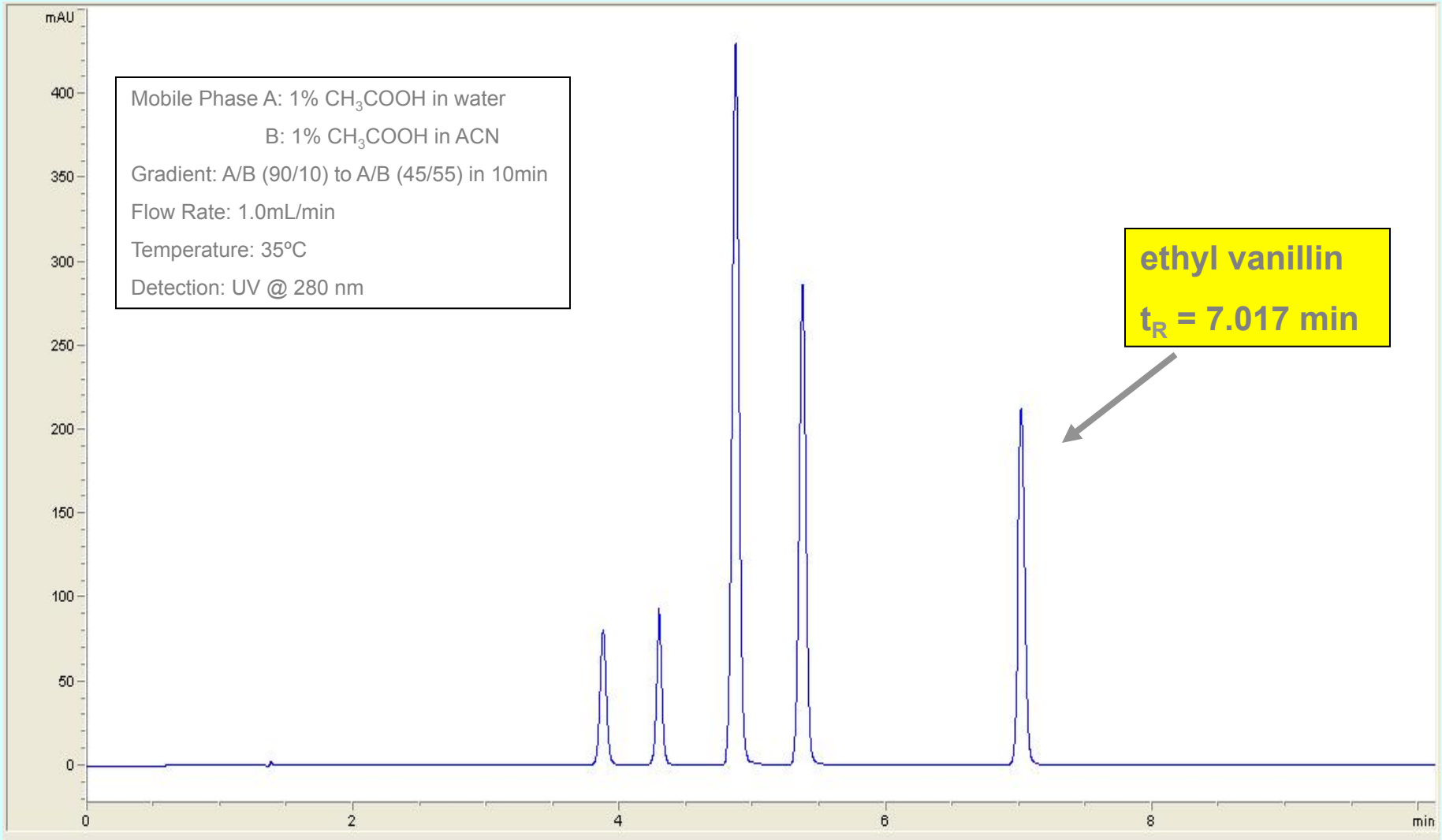
Restek Ultra C-18
250mm x 4.6mm x 5um



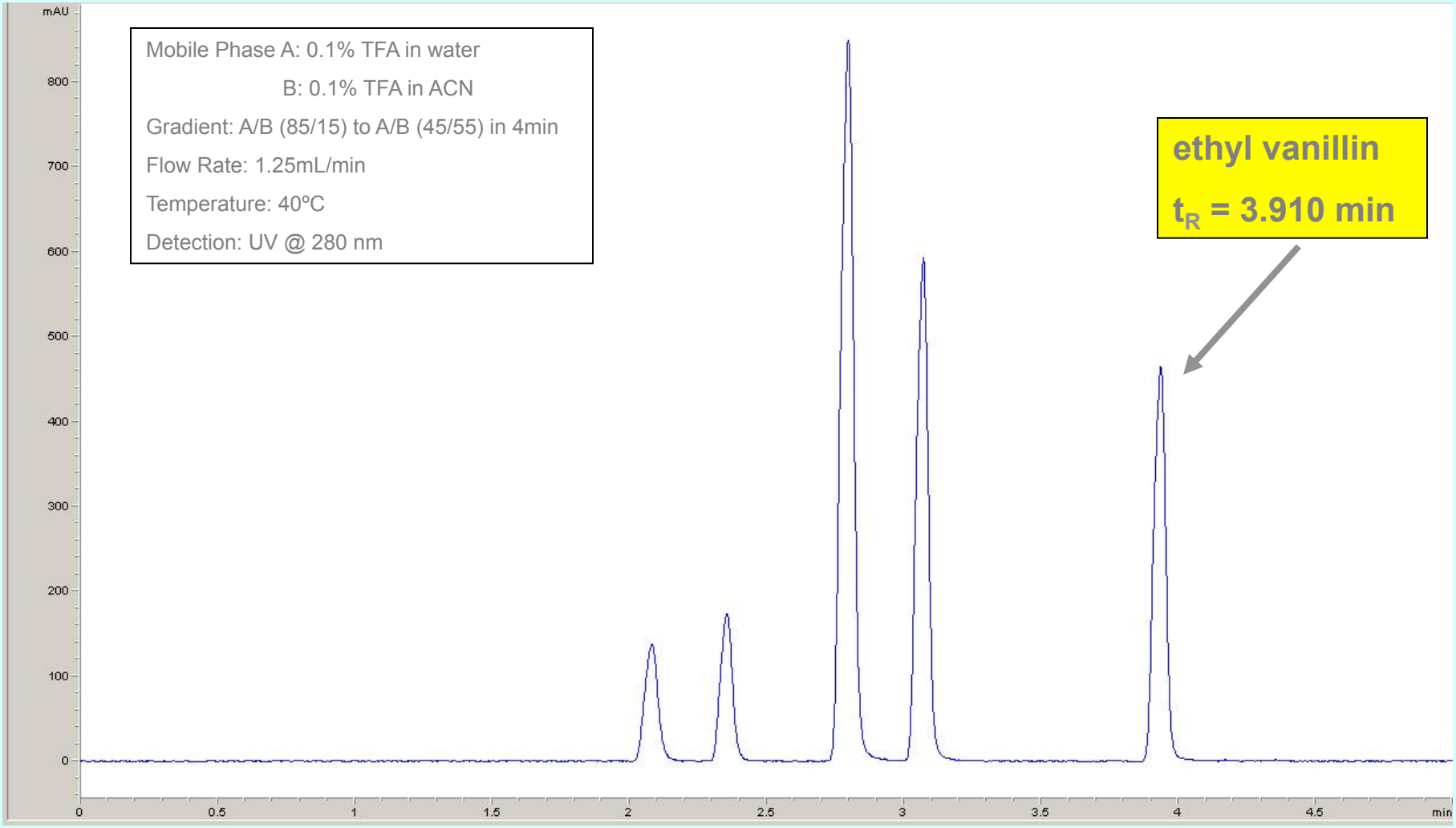
Phenomonex Luna C18(2) 150mm x 4.6mm x 3um



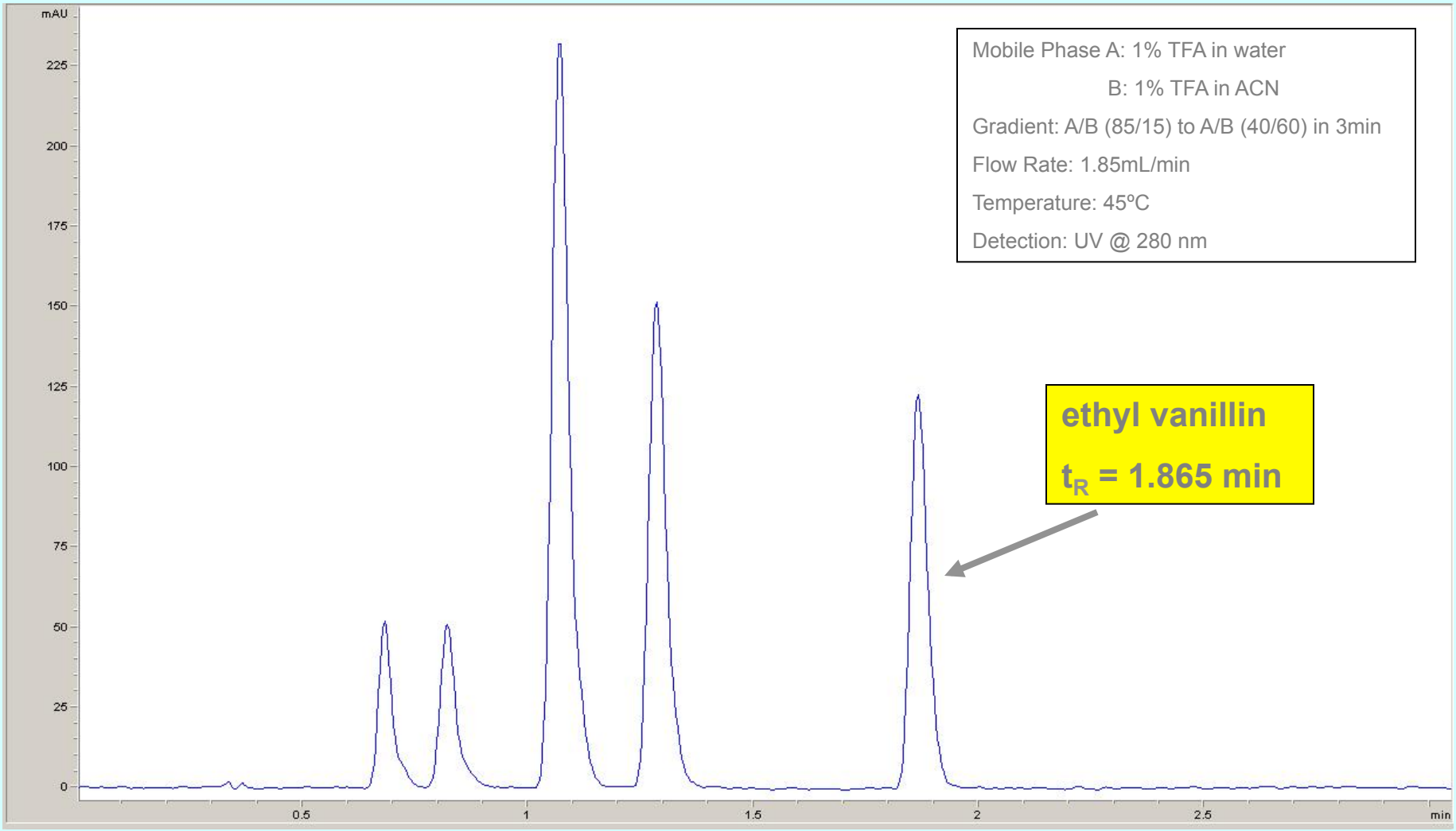
Phenomonex Luna C18(2) 100mm x 4.6mm x 3um



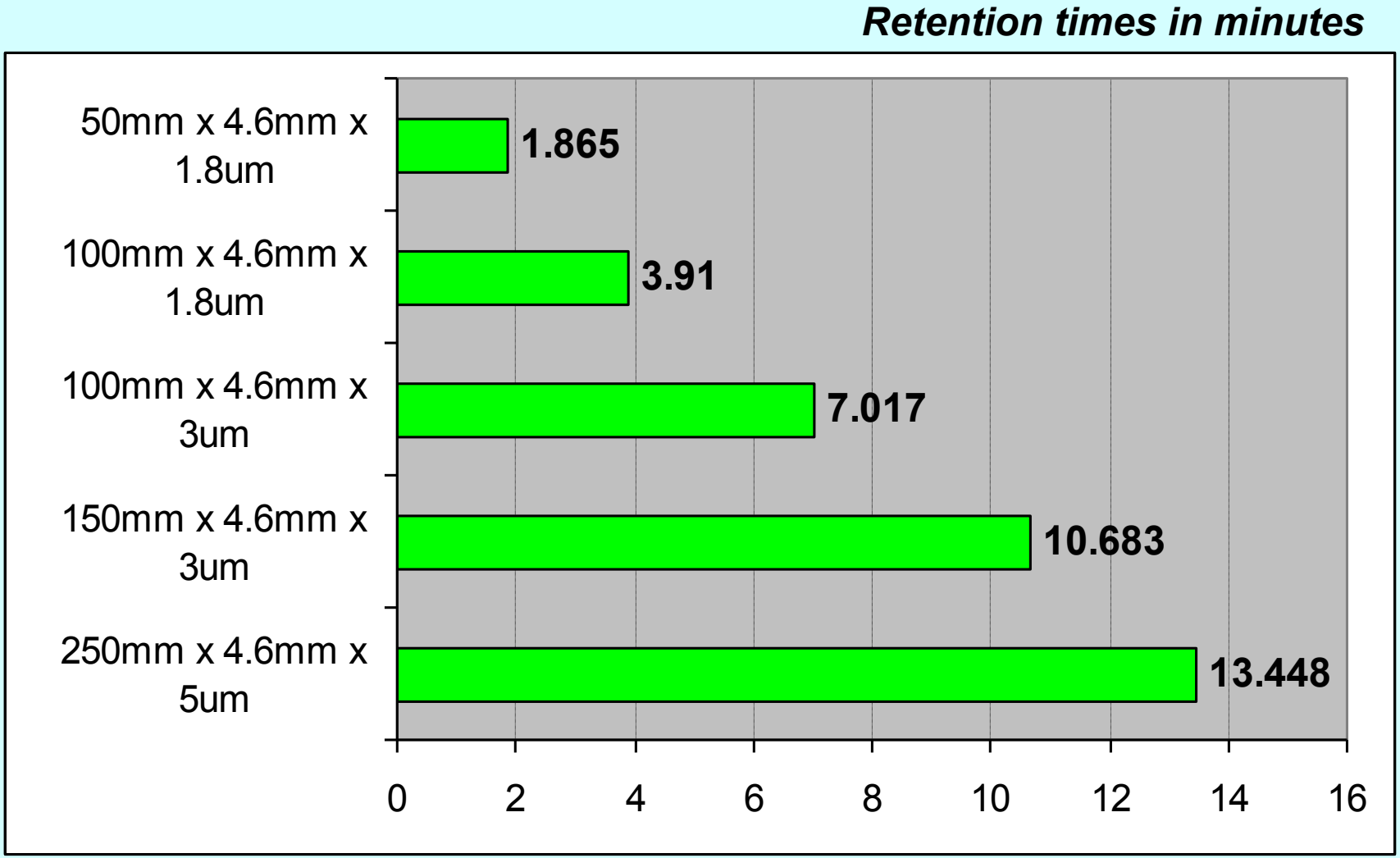
Agilent Zorbax Eclipse Plus 100mm x 4.6mm x 1.8um



ES Industries Epic C18 SD 50mm x 4.6mm x 1.8um



Fast LC - Time savings



Acknowledgements

Graduate Students Conducting Vanilla Research

- Judy Chen, 1992
- Jide Adedeji, 1993
- Keun Joong Lee, 2006
- Stephen Toth, 2012
- Maria Galeas, 2014