A Comparison of Methods for Feed Fat Analysis

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Overview

- Why this discussion?
  - Lipid has nutritional significance in livestock feed
- Methods vary
  - Ether extract
  - Acid ether extract
  - Fatty acid analysis

Definitions

- Lipids – plant compounds insoluble in water but soluble in organic solvents
- Crude fat content is estimated by extracting a ground feed sample with diethyl ether
  - Weende Experiment Station (1860)

CRUDE FAT OR ETHER EXTRACT-- AOAC

- Sample
  - 2 g, dry
  - Pre-extract with water if large amounts of water-soluble materials are present
- Extract in Soxhlet with dry diethyl ether
  - 4 hr @ condensation rate of 5-6 drops/sec, or16 hr @ 2-3 drops/sec
- Evaporate ether, cool, weigh

AOAC 920.39

What compounds are extracted ?

- Lipids
  - Nonglycerol-based
    - waxes, alkanes
  - Glycerol-based
    - Simple
    - Compound
- Non-lipid
  - Water
  - Fat-soluble vitamins
  - Pigments

Fatty Acids in Glycerol Lipids

Lipid	Source	FA, %
Triglyceride	Cereal seeds Oilseeds	95
Phospholipid	Plant membrane	72
Galactolipid	Forages	56

CONTENT AND COMPOSITION OF ETHER EXTRACT FROM FORAGE LEAVES

	% of DM	% of EE
Ether Extract	5.3	100
Fatty Acids	2.3	43
Non-Fatty Acid		
Galactose	0.41	8
Glycerol	0.46	9
Chlorophyll	0.23	4
Waxes	0.9	17
Other	1.0	19

Palmquist and Jenkins, 2003

LIPID COMPOSITION (%) OF CRUDE SOYBEAN OIL

Triacylglycerol	95 – 97
Phosphatides	1.5 – 2.5
Other matter	1.6
Sterols	0.33
Tocopherols	0.15 – 0.21
Hydrocarbons	0.014
Free fatty acids	0.3 – 0.7

Pryde, 1980

Acidified ether extract-AOAC

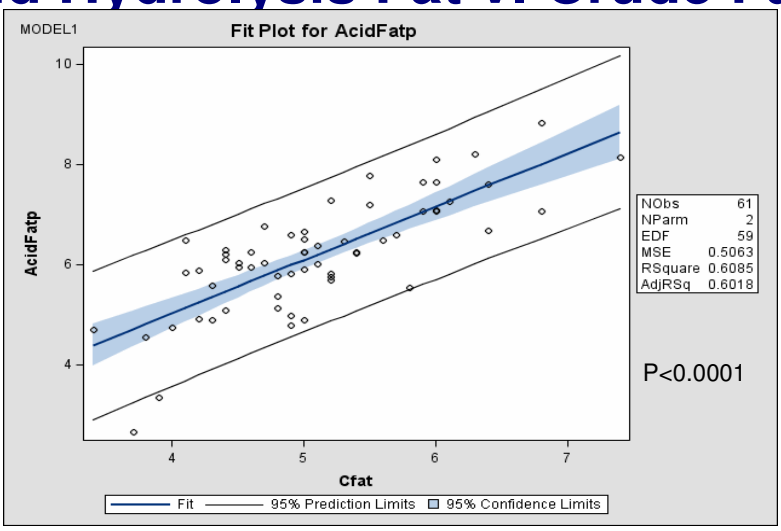
- Required for extruded feeds and some high calcium feeds
- Sample
  - 2 g, dried
  - 2 ml EtOH
  - 10 ml 8 N HCl
  - 30 - 40 min @ 70 - 80°C, with shaking
  - Wash with ether, filter
  - Evaporate ether, weigh residue

AOAC 954.02

EE vs Acid EE

	EE			
	Lab 1	Lab 2	Lab 3	Acid EE
Corn	3.1	2.7	4.0	5.8
Alfalfa	3.6	3.7	3.8	6.2
TMR	4.5	4.1	4.5	6.0
Ca Salt	1.2	2.4		85.1

Acid Hydrolysis Fat v. Crude Fat



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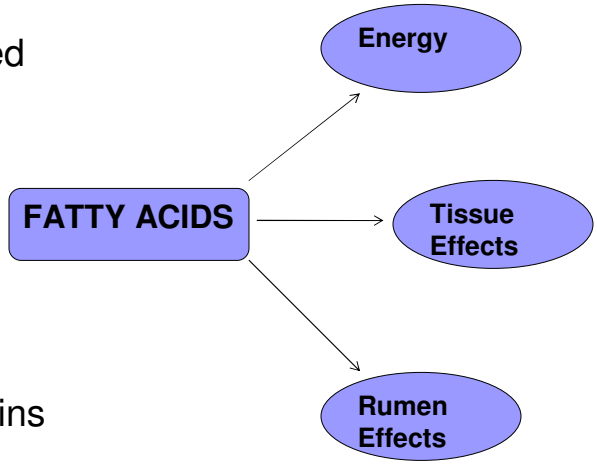


GLC as an alternative to ether extract

- One-step methods are available
- High precision
- Quantity and quality (fatty acid profile) in one analysis
  - Oxidized fatty acids (unavailable) are not analyzed

Ether Extract vs Fatty Acids

Forage	Ether Extract (%)	Fatty Acid (% of EE)
Alfalfa	3.50	2.28
Corn grain	4.23	4.03
Corn Silage	3.19	2.21

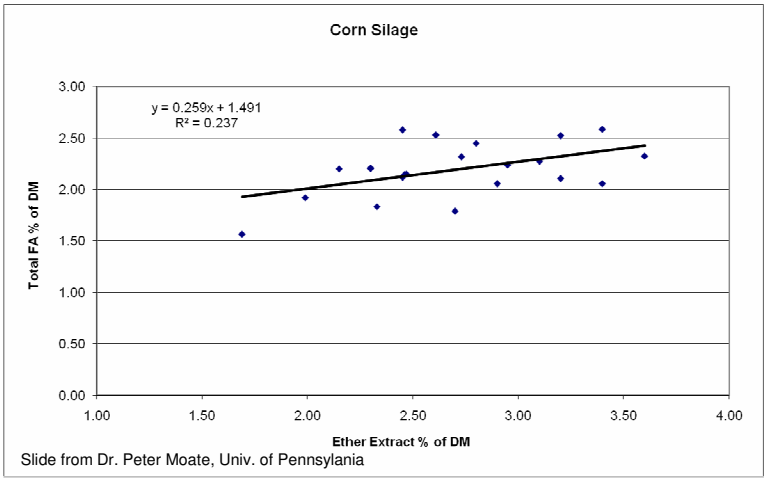
From CPM for Dairy

RELATION BETWEEN FATTY ACIDS AND ETHER EXTRACT IN MIXED DIETS

- FA = - 0.98 + (1.03 x EE)
  - r^2 = 0.87
  - P < 0.0001
  - RMSE = 0.71
  - N = 18

Allen, 2000

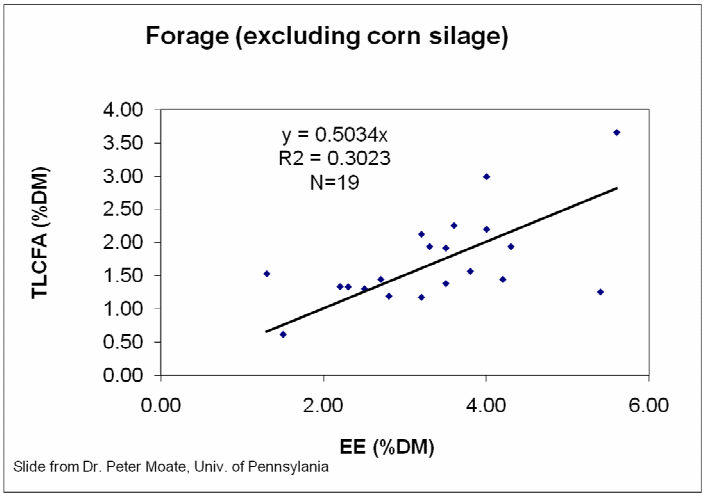
TFA vs EE



Slide from Dr. Peter Moate, Univ. of Pennsylania

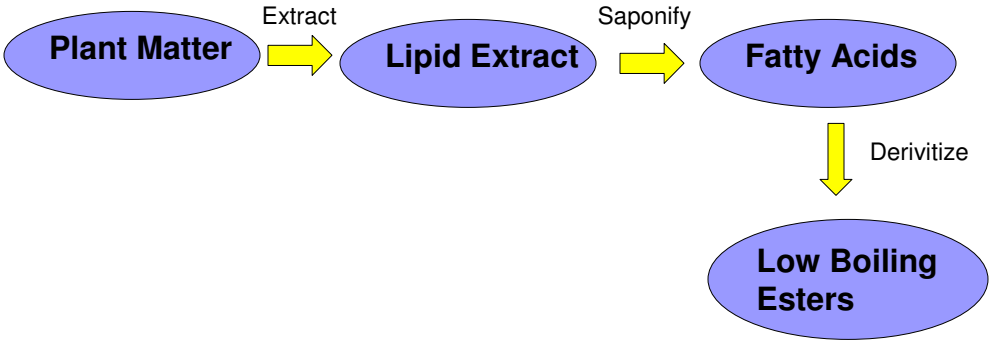
From CPM for Dairy

TFA vs EE

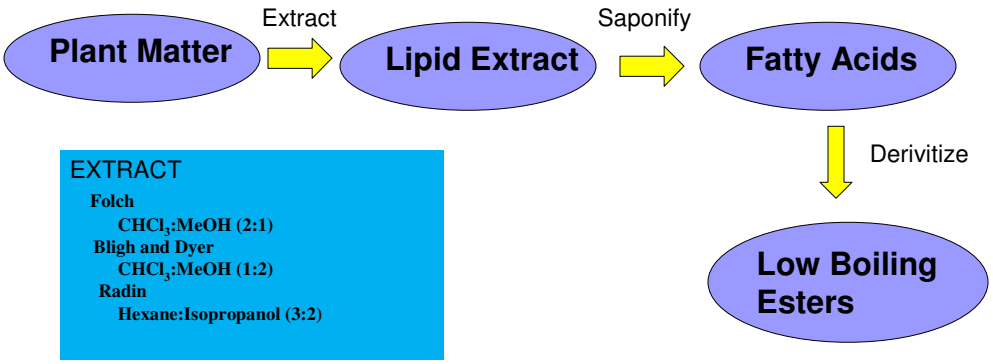


From CPM for Dairy

Fatty Acid Analysis



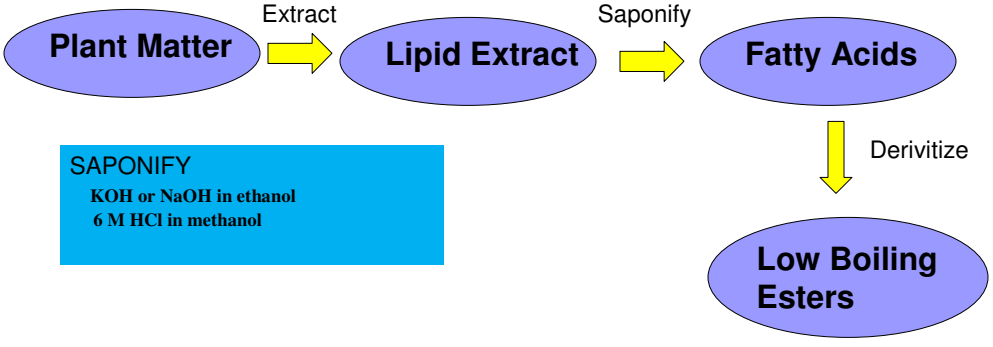
Fatty Acid Analysis



EXTRACT

Folch
$CHCl_3$:MeOH (2:1)
Bligh and Dyer
$CHCl_3$:MeOH (1:2)
Radin
Hexane:Isopropanol (3:2)

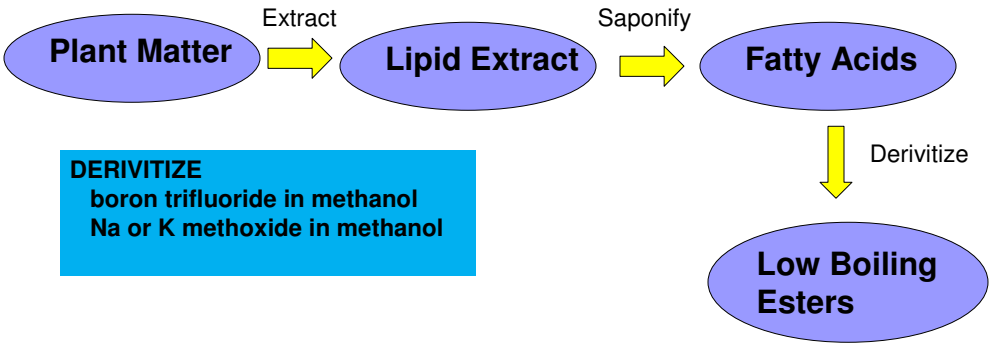
Fatty Acid Analysis



SAPONIFY

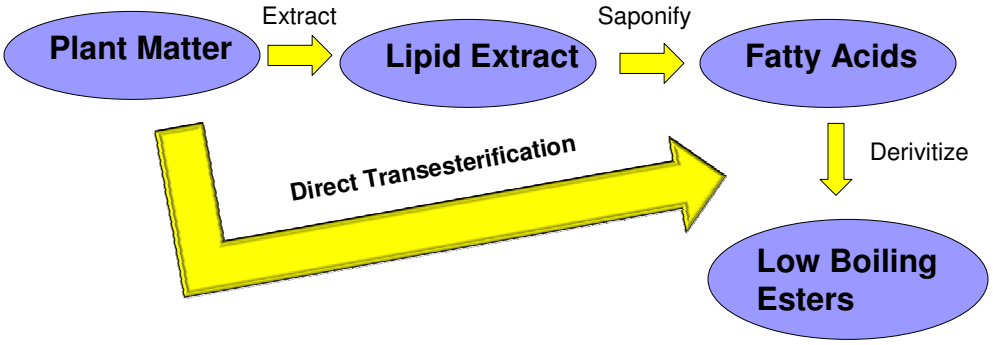
KOH or NaOH in ethanol
6 M HCl in methanol

Fatty Acid Analysis



DERIVITIZE
- boron trifluoride in methanol
- Na or K methoxide in methanol

Fatty Acid Analysis



FATTY ACID CONTENT AND COMPOSITION OF SOME FEEDSTUFFS

Feedstuff	FA, % DM	16:0	18:0	18:1	18:2	18:3
Barley	1.6	27.6	1.5	20.5	43.3	4.3
Maize	3.2	16.3	2.6	30.9	47.8	2.3
Dehy Alfalfa	1.4	28.5	3.8	6.5	18.4	39.0
Ryegrass	--	11.9	1.0	2.2	14.6	68.2
Cottonseed	18.6	25.3	2.8	17.1	53.2	0.1

Palmquist,1988

Possible Errors in Fatty Acid Analysis

- Non-fatty acid peaks

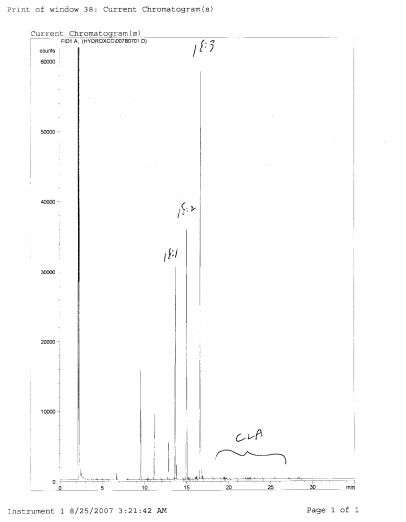
Non-Fatty Acid Peaks

- **Methyl levulinate**
  - **Levulinic acid is produced during acid hydrolysis of samples containing sugar**
  - **Elutes on most GLC chromatograms near methyl 13:0**
- **BHT**
  - **This commonly-used antioxidant elutes with methyl 14:0 on most GLC chromatograms**

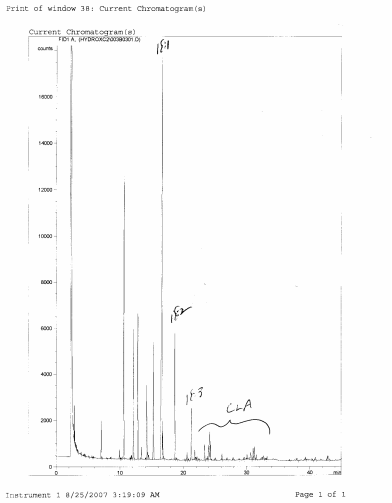
Possible Errors in Fatty Acid Analysis

- Non-fatty acid peaks
- Method of drying

TMR + linseed oil GOOD



TMR + linseed oil BAD



Effect of Drying Method on In Vitro Samples

Drying Method	FA, mg/g
n	8
Oven-dried @ 55C	17.9 ± 2.4
Freeze-Dried	19.6 ± 2.6

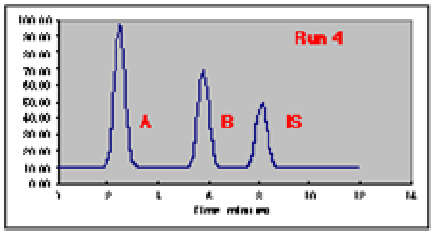
Possible Errors in Fatty Acid Analysis

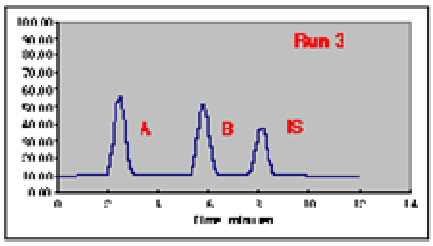
- Non-fatty acid peaks
- Method of drying
- Choice of Internal Standard

Internal Standards

- Known amount of unique fatty acid added to sample
- Amount of unknown fatty acid determined from its ratio to the internal standard
- Errors affect sample and internal sample the same so final results are not affected
- Corrects for
  - Errors in extraction
  - Errors in derivitization

Example of Incomplete Extraction





Amt_{IS} = 1 mg
$Area_{IS}$ = 100
$Area_{Unk}$ = 200

$$Amt_{Unk} = 1\ mg * \frac{Area_{Unk}}{Area_{IS}}$$

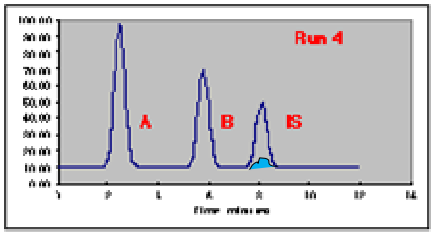
Amt_{Unk} = 2 mg

Amt_{IS} = 1 mg
$Area_{IS}$ = 75
$Area_{Unk}$ = 150

$$Amt_{Unk} = 1\ mg * \frac{Area_{Unk}}{Area_{IS}}$$

Amt_{Unk} = 2 mg

Example of Background IS



Amt_{IS} = 1 mg
$Area_{IS}$ = 100
$Area_{Unk}$ = 200

$$Amt_{Unk} = 1\ mg * \frac{Area_{Unk}}{Area_{IS}}$$

Amt_{Unk} = 2 mg

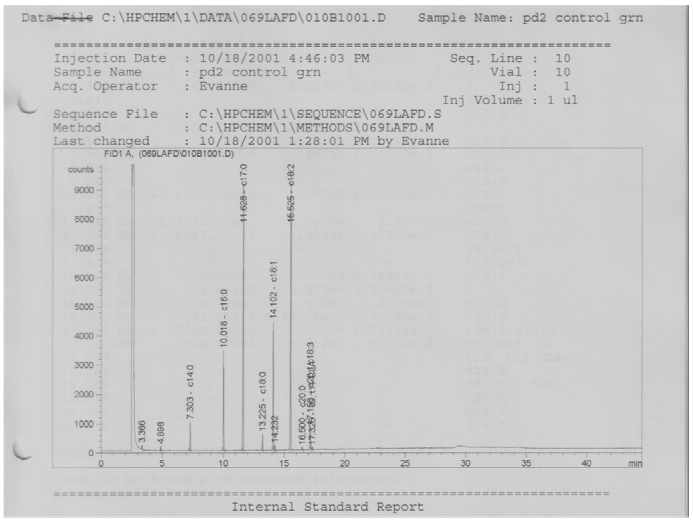
Amt_{IS} = 1 mg
$Area_{IS}$ = 110
$Area_{Unk}$ = 200

$$Amt_{Unk} = 1\ mg * \frac{Area_{Unk}}{Area_{IS}}$$

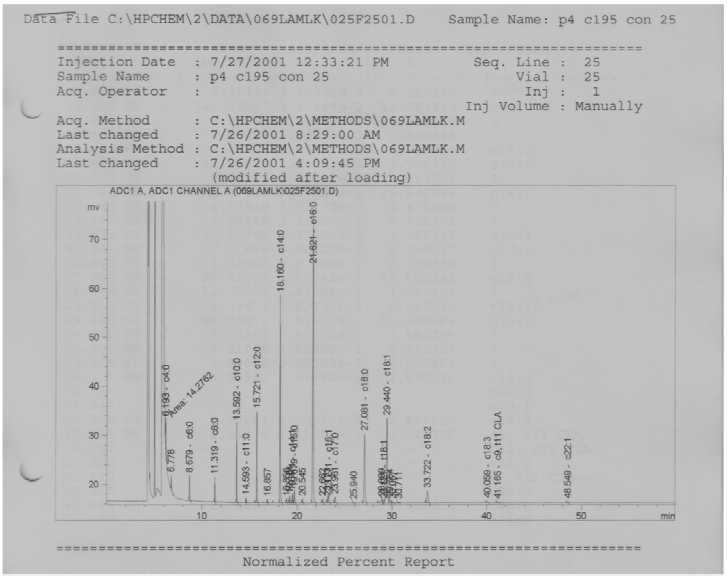
Amt_{Unk} = 1.82 mg

TMR GC

C17:0 and C19:0 often used for feed

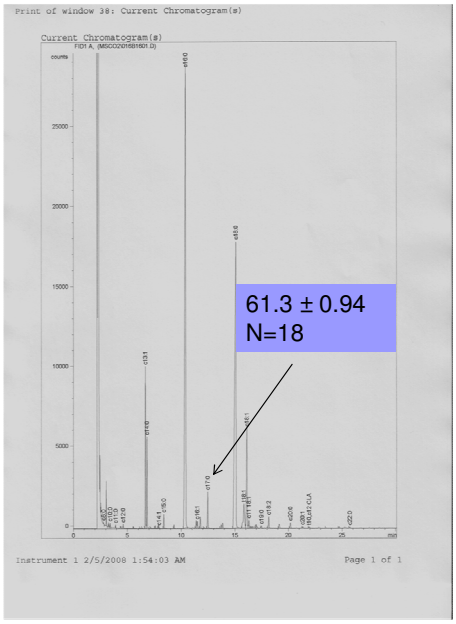


Milk GC



Energy Booster (MSC)

EE = 98.2 ± 0.6
n=18



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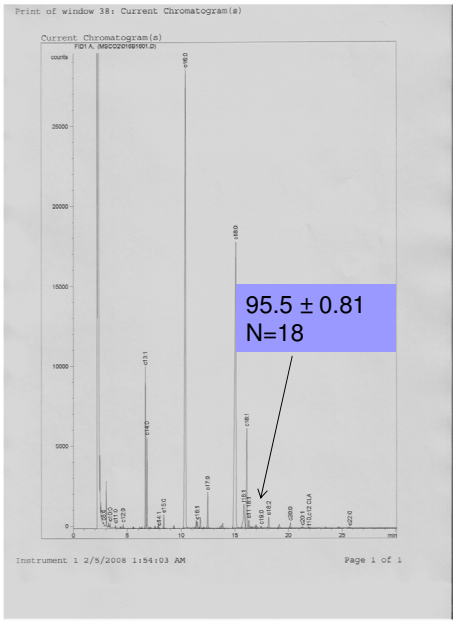

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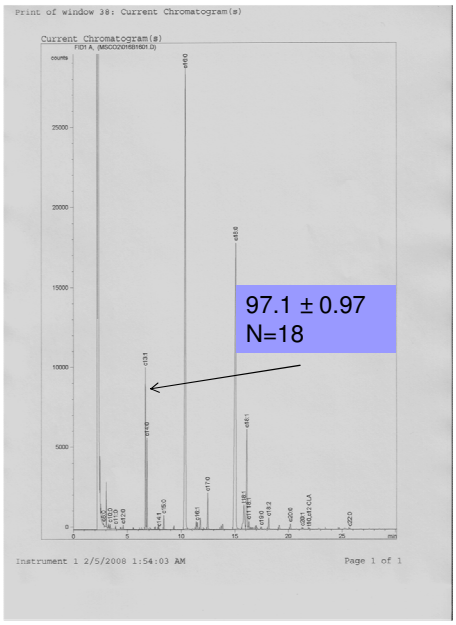

Possible Errors in Fatty Acid Analysis

- Non-fatty acid peaks
- Method of drying
- Choice of Internal Standard
- CLA isomerization

GC analysis of CLA mix (% of total FA)

Forage	Actual	Direct Methylation
c9,t11	16.35	6.12
t10,c12	9.20	3.72
t9,t11	7.28	20.91
Total	30.8	32.8

SUMMARY:EE vs Fatty acids

- EE
  - Low cost
  - AOAC approved
  - Not nutritionally uniform
  - High variance
- Fatty acids
  - High cost
  - Not AOAC approved
  - Nutritionally uniform
  - Precise (Direct Transesterification works great)

SUMMARY: Errors in GC analysis

- Proper collection and storage to prevent lipid destruction
- Pay attention to drying procedure – freeze-drying is always preferable
- Watch choice of internal standard
  - Initial background run to select IS
- Samples with CLA require special derivitization steps

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