

Detection of Extracts from Pineapple  
Peels and Cores in Juice  
*Pure Juice Project WP 8*

Polyphenolic Screening & Isotopic Testing

by  
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Authenticity Services

# What problems are we looking to stop?

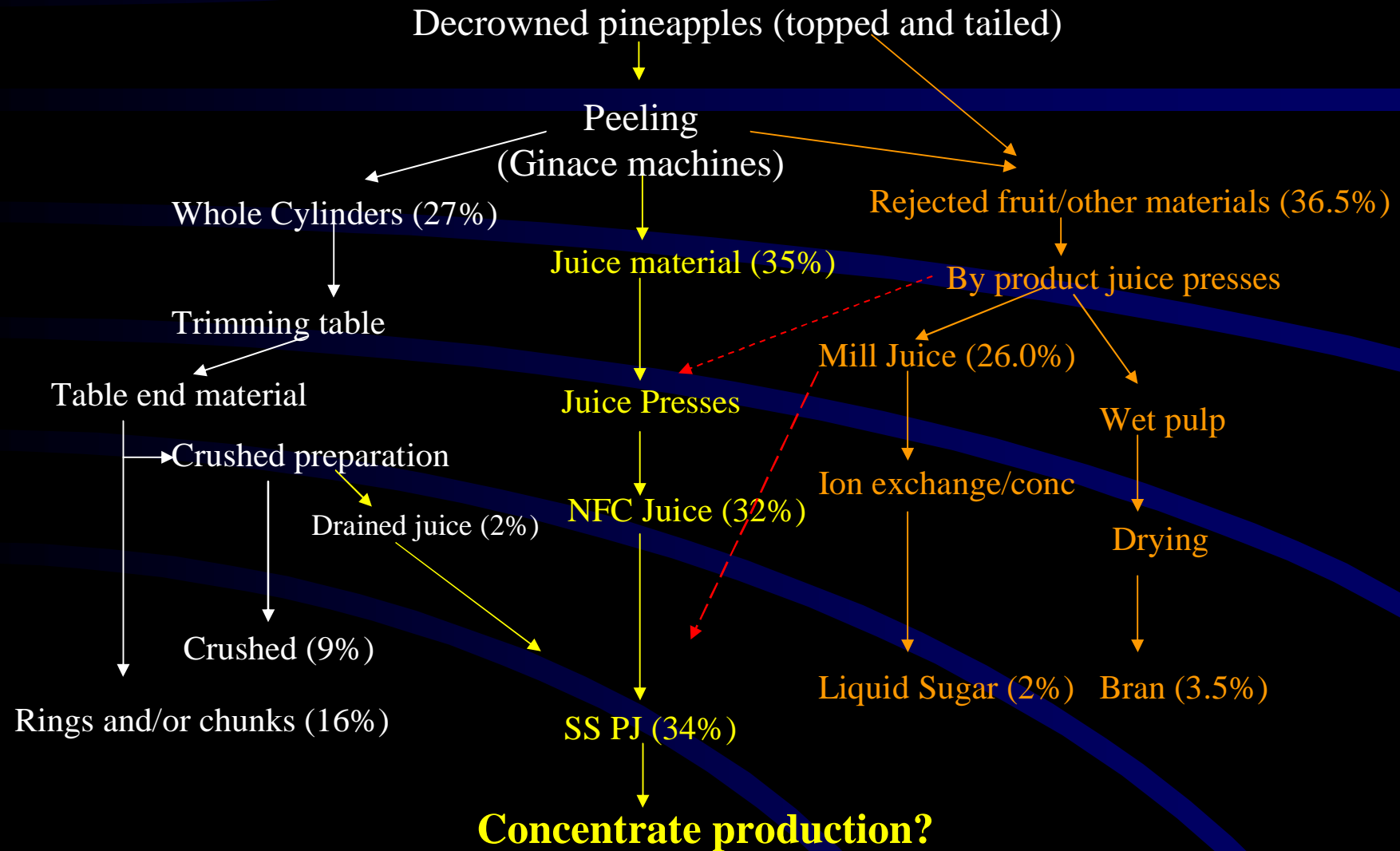
## Problem I

- Pineapple juice is often produced as a by product in factories that manufacture canned products (i.e. rings, chucks, crushed).
- In these factories there is an excess of skins and cores. These can be processed on the “juice” line extending the product with sub-standard material.

## Problem II

- “Cloud” stability in pineapple juice is sometimes bad, due to poor fruit quality &/or processing conditions. Under these conditions pectin or another hydrocolloid may be added to enhance the product’s stability and not declared.

# Typical Processing of Pineapples



# Existing Methods

## Problem I

- Distortion of the glucose to fructose ratio ( $> 1.2$ )
- **Inclusion of excessive levels of skins and cores**
- Elevation of some of the minerals (potassium, calcium and phosphate)
- **Inclusion of excessive amounts of skin**
- Elevated levels of nitrate ions
- **Inclusion of excessive amounts of core**
- S. Wallrauch. *Flussiges Obst* (59) 92/1 20 - 26

## Problem II

- Detection of elevated pectin levels in juice using IFU 26 (ethanolic ppt and carbazole method)
- **Addition of pectin (apple or citrus) as a stabiliser**

# Project Objectives

- *RSSL*
  - To determine the usefulness of a polyphenol profiling method to detect the presence of added peel or core extracts in pineapple juice.
  - Assess the usefulness of carbon isotope ratio analysis to detect added pectin as a stabiliser in pineapple juices.
- *CheLab*
  - Assess conventional analytical methods to detect the addition of peel and core extracts to pineapple juice.
  - Assess the usefulness of polysaccharides as a means to detect peel and core extracts to pineapple juices.

# HPLC methods

- A number of isocratic and gradient HPLC conditions were examined to optimise the separation of the polyphenols
- Finally modified the one referenced at the bottom of the page by using acetic acid as solvent on a C<sub>18</sub> column and detection at 280 nm or DAD
- Replaced phosphate buffer (0.05M pH 3.0) with acetic acid as it is easier on piston seals. However, can not work below *ca* 240 nm with DAD and if required have to use phosphate buffer. In our hands both methods gave the same results.

Kirksey *et al.* In: Methods to detect adulteration of fruit juice beverages, Vol. 1, page 145 – 166. Published by AG Science Inc, Auburndale Florida.

# HPLC method

Centrifuged juice at 40k g for 10mins to clarify

Detection UV: 280 nm or DAD

Column: : ODS 2, 5 um, 250 mm x 4 mm

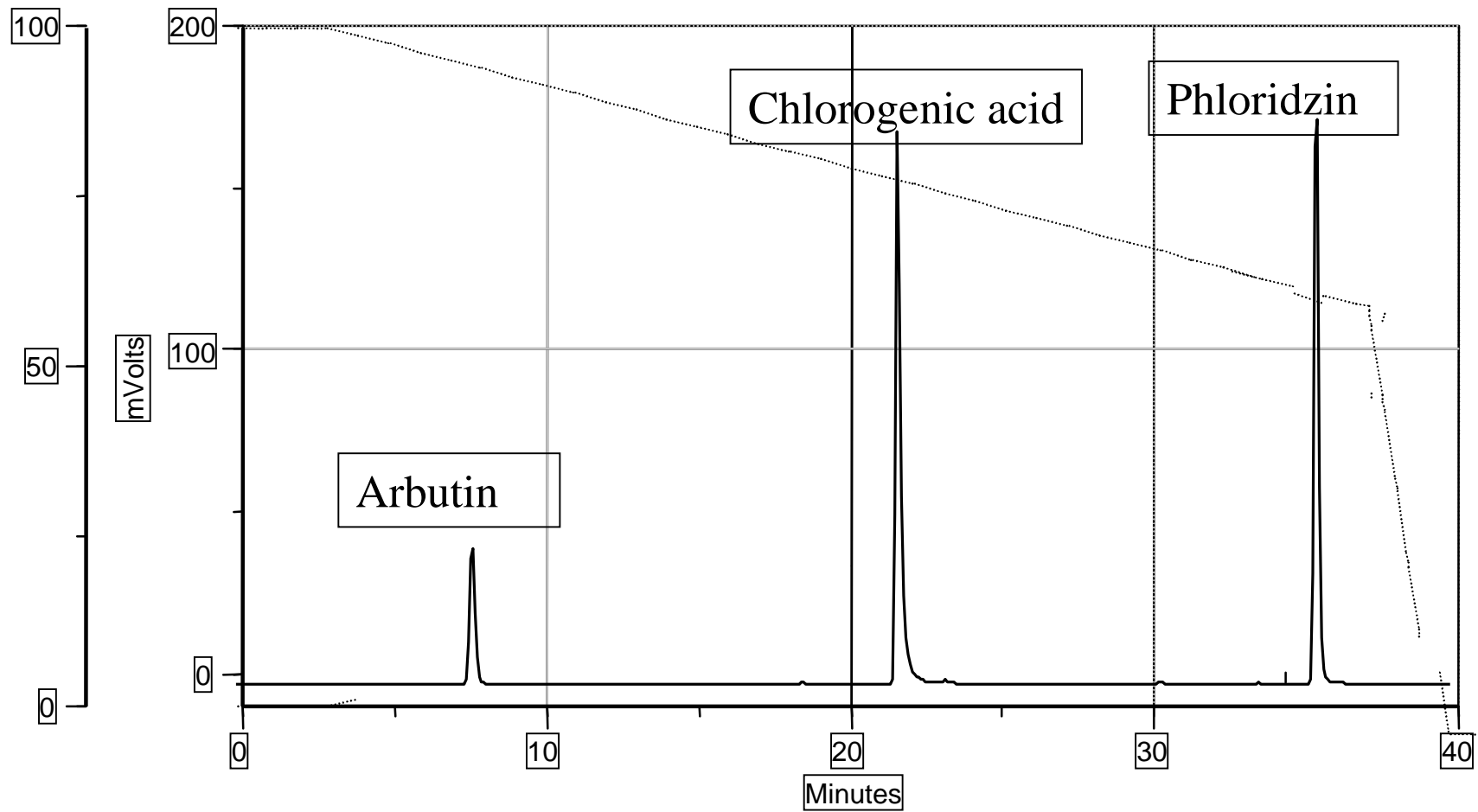
Solvent A: 0.5 % acetic acid

Solvent B: 70 % acetonitrile

Flow rate: 1.2 ml/min

Time	%A	%B
0	100	0
3	100	0
38	56	44
40	0	100
50	0	100
50.1	100	0
70	100	0

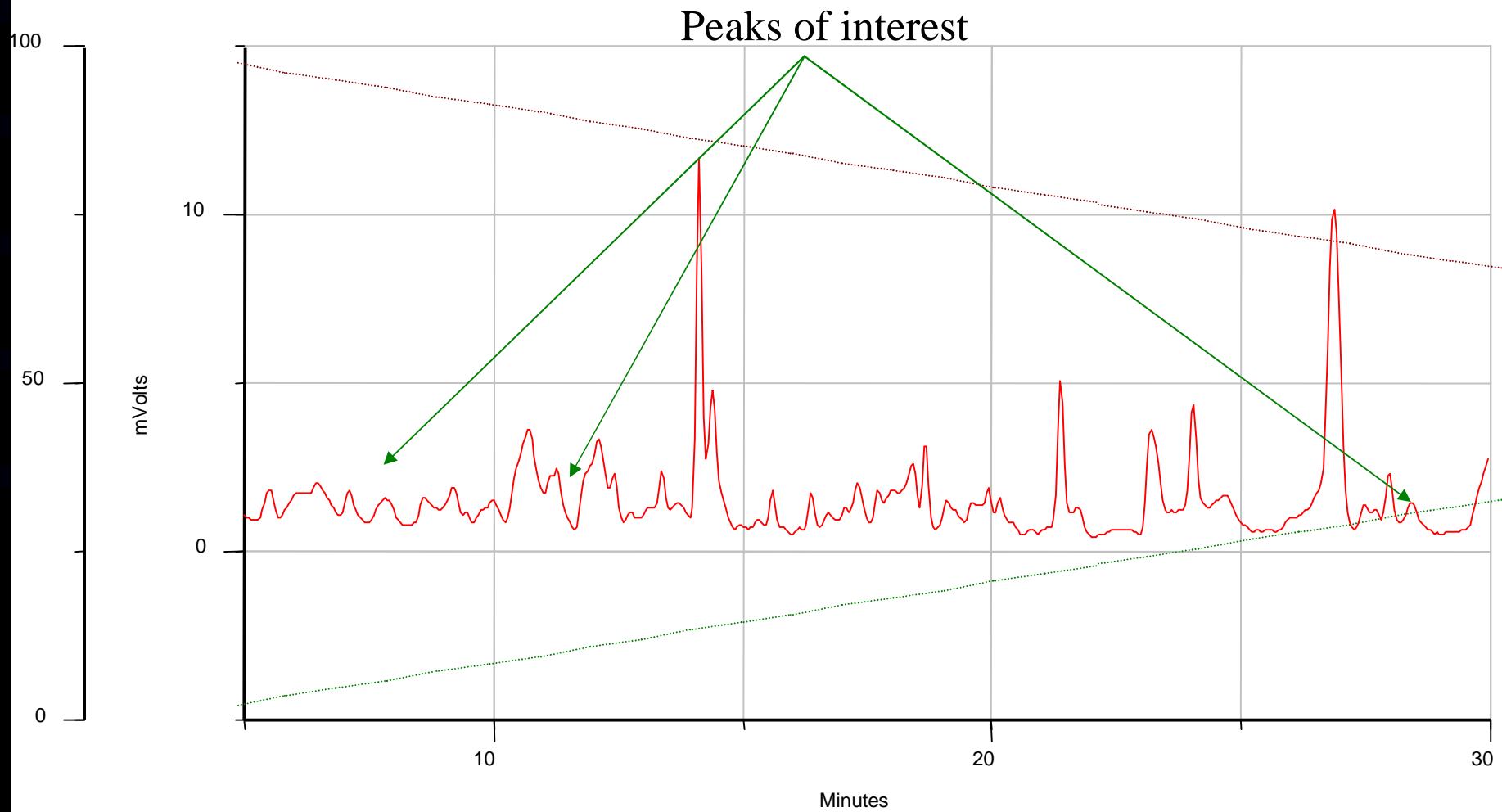
# Batch peak R.T. variability fixed with three standards



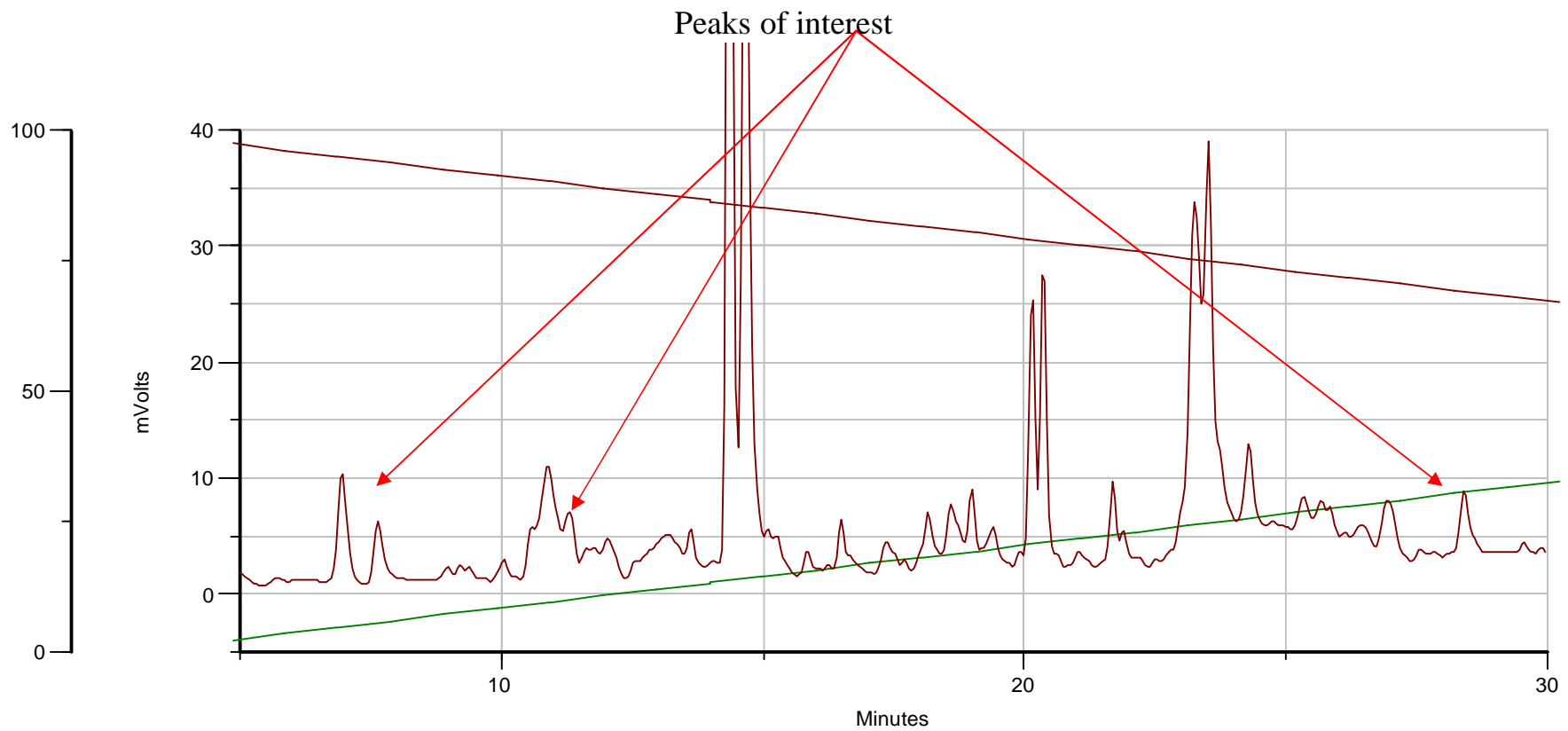
## Why use standards?

- The peaks of interest are unknowns so proper standards can not be used
- Chromatograms are very busy so little or no room for internal standard/s (best option)
- External standards help fix retention times within and between runs
- Allows detector response to be monitored with time

# HPLC Profile for Pineapple Concentrate



# HPLC Profile of Commercial Skin Extract

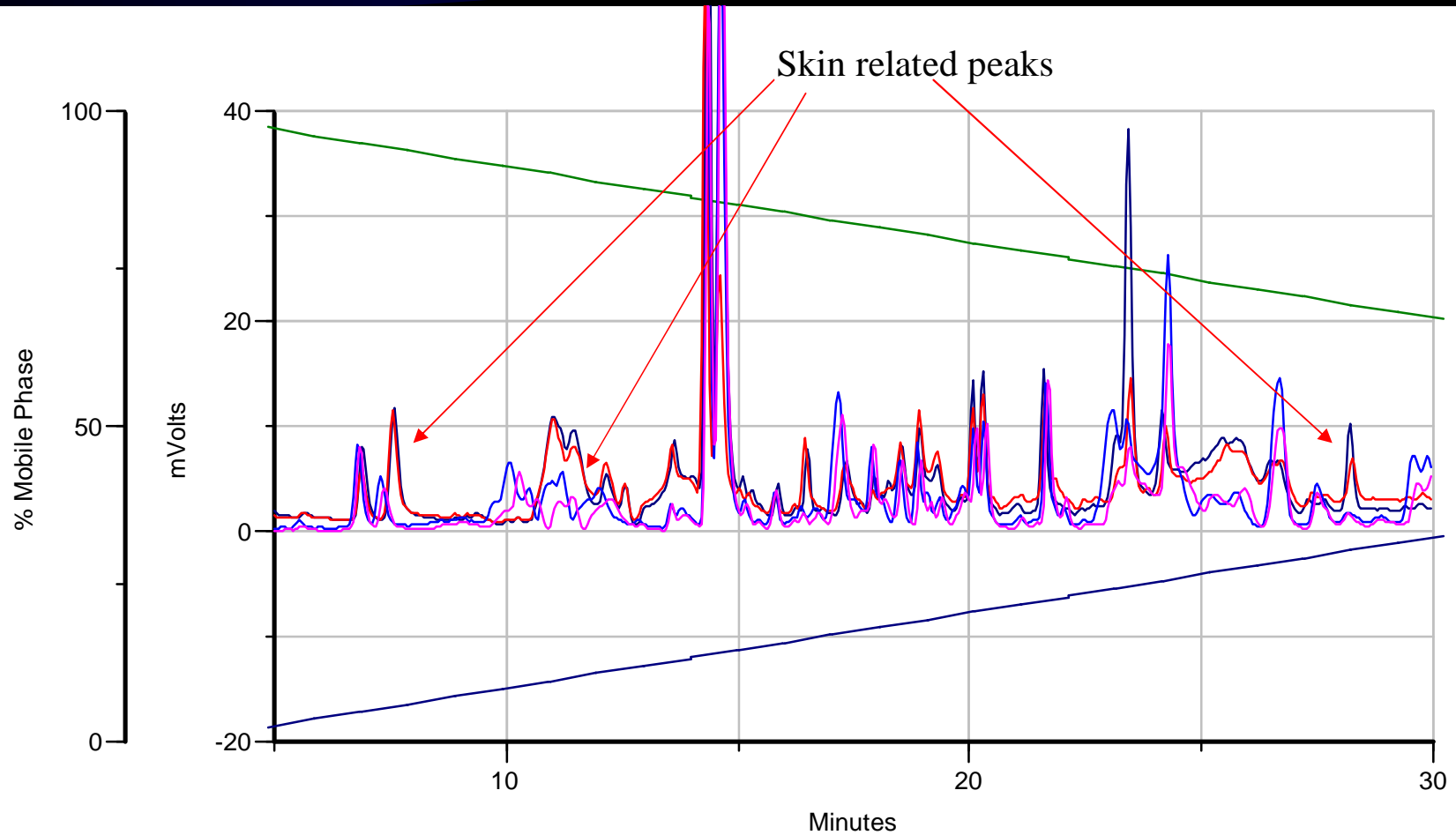


c:\gilson\rssl\system\_1\polyphen.006\polyphen.gdt : UV channel 1 : skin summerpride box1: Inj. Number: 7

Aqueous

Organic

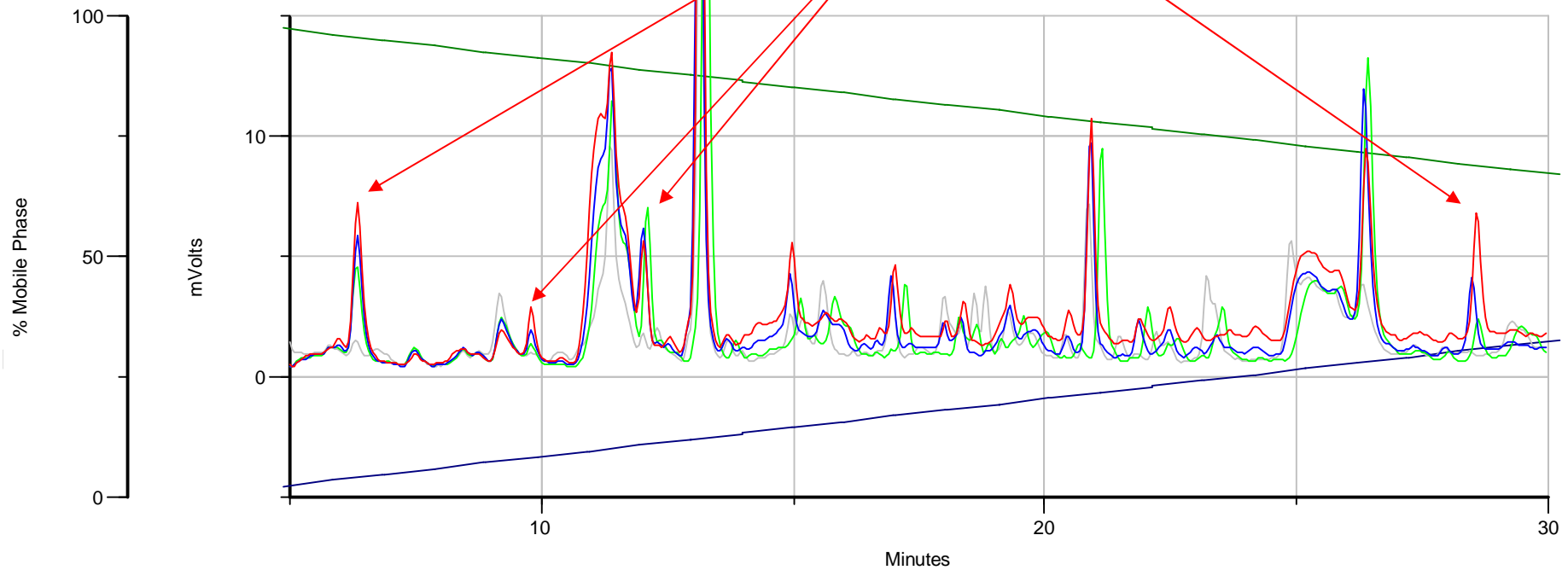
# HPLC Traces for juice and skin extracts



- c:\gilson\rssl\system\_1\polyphen.007\polyphen.gdt : UV channel 1 : skin philippine F200plant: Inj. Number: 21
- c:\gilson\rssl\system\_1\polyphen.007\polyphen.gdt : UV channel 1 : skin philippine f200first: Inj. Number: 23
- c:\gilson\rssl\system\_1\polyphen.004\polyphen.gdt : UV channel 1 : Philippine f200 first: Inj. Number: 6
- c:\gilson\rssl\system\_1\polyphen.004\polyphen.gdt : UV channel 1 : philippine f200 plant: Inj. Number: 8

# Serial addition of skin extract

Skin related peaks



- c:\gilson\rssl\system\_1\polyphen.017\polyphen.gdt : UV channel 1 : 10535 : Inj. Number: 7
- c:\gilson\rssl\system\_1\polyphen.017\polyphen.gdt : UV channel 1 : 10535 5%mexico: Inj. Number: 8
- c:\gilson\rssl\system\_1\polyphen.017\polyphen.gdt : UV channel 1 : 10535 10%mexico: Inj. Number: 9
- c:\gilson\rssl\system\_1\polyphen.017\polyphen.gdt : UV channel 1 : 10535 20%mexico: Inj. Number: 10

# Samples examined

	2003	2004	2005	Total	Total of totals
<b>SGF</b>					
<b>NFC</b>	8	16	4	28	63
<b>Concs</b>	10	7	7	24	80
<b>Grade B</b>			10	10	12
<b>Mill Juice</b>		3	1	4	5
<b>Fruit</b>	13			13	45
<b>RSSL</b>					
<b>NFC</b>		15	20	35	
<b>Concs</b>	14	20	22	56	
<b>Grade B</b>	2			2	
<b>Mill Juice</b>	1			1	
<b>Fruit</b>	2	20	10	32	
<b>Totals</b>	<b>50</b>	<b>81</b>	<b>74</b>	<b>205</b>	

# Typical Peak Areas for Marker Peaks in Juice, Core & Skin Extracts

<b>Approx retention time</b>	<b>7 min</b>	<b>12 min</b>	<b>28 min</b>	<b>30 min</b>
<b>Juice</b> N = 188	13500 CV = 15%	15400 CV = 65%	21800 CV = 55%	12150 CV = 52%
<b>Core Ext</b> N = 45	8100 CV = 15%	36150 CV = 25%	23250 CV = 17%	131200 CV = 17%
<b>Skin Ext</b> N = 62	158500 CV = 45%	551000 CV = 30%	99500 CV = 55%	95000 CV = 76%

# Validation exercise

- Provided a copy of the SOP to a person who had not used the method before
- Provided two training samples to familiarise them with the procedure
  - a) pure juice
  - b) a 50/50 mix of juice plus skin extract
- Examined 10 samples as blind duplicates and asked to assess the samples on the basis of pure juice and 50% spike sample provided (samples a and b were not within the assessment set)

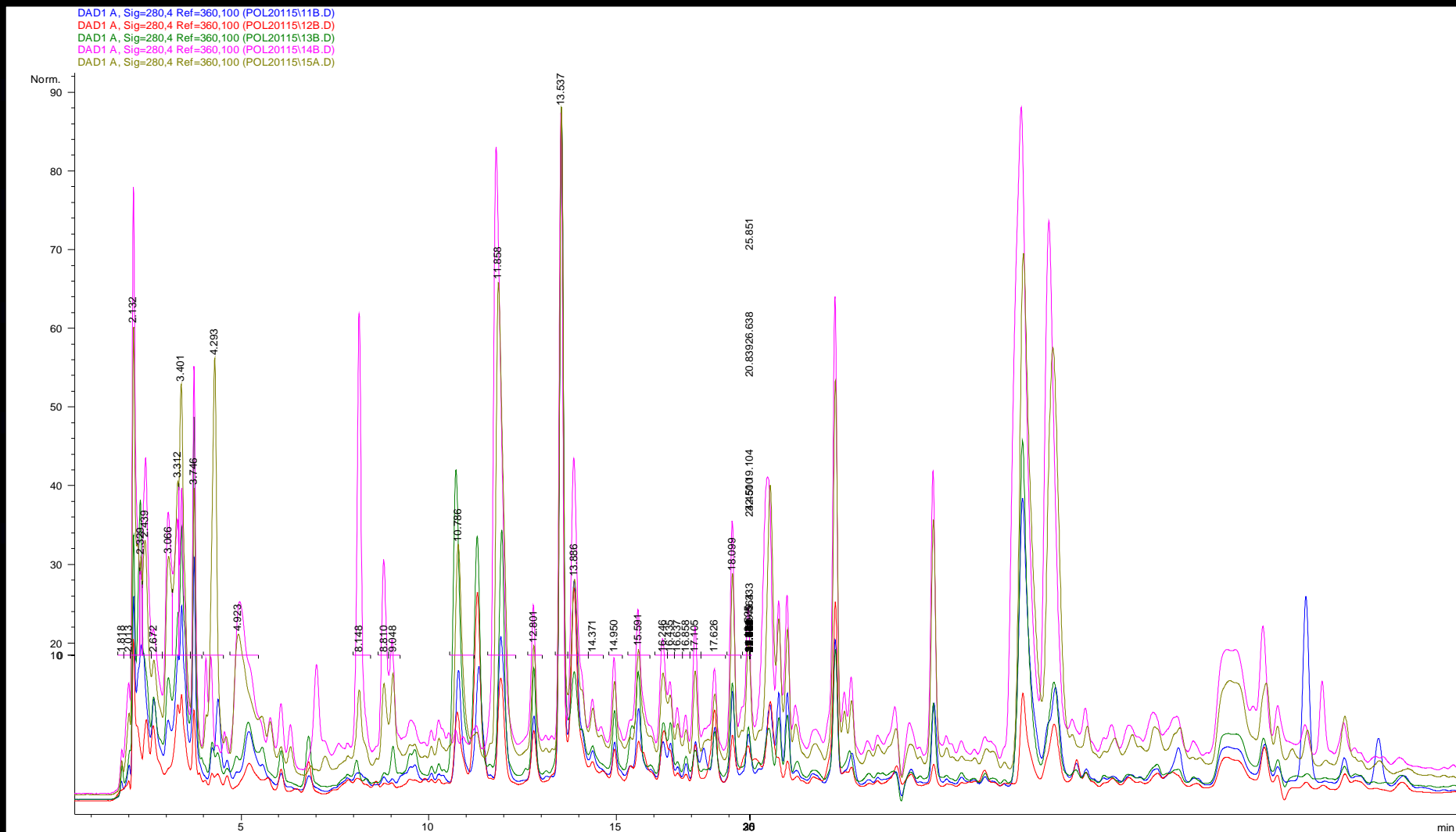
# Validation Exercise Results

	Sample		Blind duplicate 1	Blind duplicate 2
			Found	Found
1	Pure Juice (A)		pure	pure
2	20% skin added		22% skin	24% skin
3	20% skin added		18% skin	22% skin
4	30% skin added		33% skin	35% skin
5	Pure Juice (B)		pure	pure
6	30% skin added		35% skin	33% skin
7	10% skin added		pure	pure
8	10% skin added		pure	pure
9	Pure Juice (C)		pure	pure
10	Pure Juice (D)		pure	pure

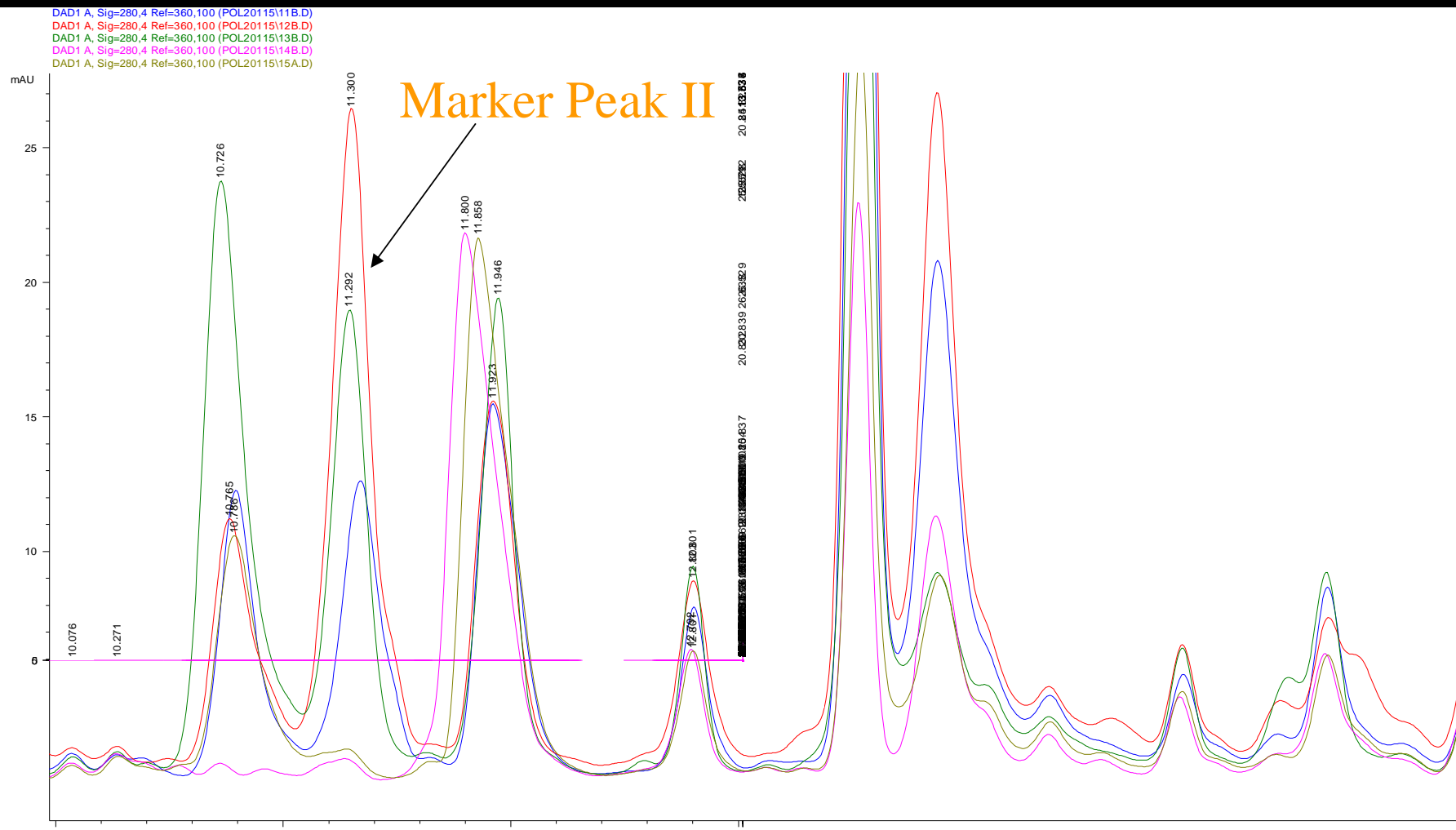
# Final validation samples

- Two sets of samples received for assessment
- Set one AN 11 – 15
- Showed variable profiles typical of juice samples we have examined
- Set two AN 6 – 10
- All showed very similar profiles with little or no variation in components present (unusual)

# HPLC traces for AN 11 - 15

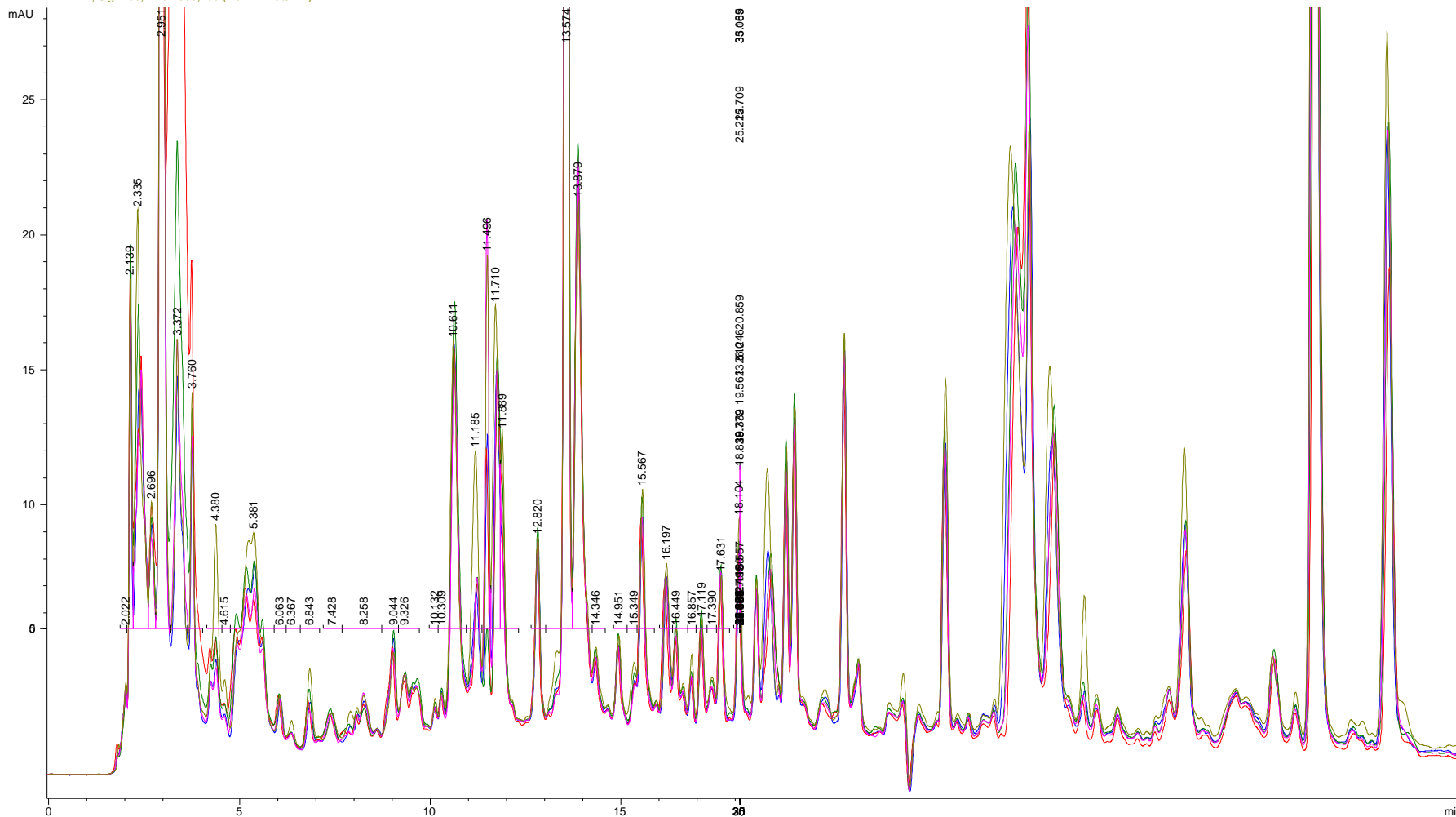


# AN 11 – 15 Marker peak II



# South African samples AN 6 -10

DAD1 A, Sig=280,4 Ref=360,100 (POL22115/10A.D)  
DAD1 A, Sig=280,4 Ref=360,100 (POL22115/6A.D)  
DAD1 A, Sig=280,4 Ref=360,100 (POL22115/7A.D)  
DAD1 A, Sig=280,4 Ref=360,100 (POL22115/8A.D)  
DAD1 A, Sig=280,4 Ref=360,100 (POL22115/9A.D)





# Marker peak I AN 6 - 10



# RSSL's conclusions for final assessment samples AN 6 –15

<b>Code</b>	<b>Peak I 7 min</b>	<b>Peak II 12 min</b>	<b>Peak III 20 min</b>	<b>Peak IV 28 min</b>	<b>RSSL's Assessment</b>	<b>Level</b>
<b>AN6</b>	<b>++</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>Skin added</b>	<b>5%</b>
<b>AN7</b>	<b>+++</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>Skin added</b>	<b>10%</b>
<b>AN8</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>Juice</b>	<b>0%</b>
<b>AN9</b>	<b>++++</b>	<b>+++</b>	<b>++</b>	<b>+++</b>	<b>Skin added</b>	<b>15%</b>
<b>AN10</b>	<b>++</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>Skin added</b>	<b>10%</b>
<b>AN11</b>	<b>+</b>	<b>+</b>	<b>absent</b>	<b>absent</b>	<b>Skin added</b>	<b>10%</b>
<b>AN12</b>	<b>+++</b>	<b>+++</b>	<b>absent</b>	<b>absent</b>	<b>Skin added</b>	<b>20%</b>
<b>AN13</b>	<b>+++</b>	<b>+++</b>	<b>absent</b>	<b>absent</b>	<b>Skin added</b>	<b>20%</b>
<b>AN14</b>	<b>absent</b>	<b>absent</b>	<b>absent</b>	<b>absent</b>	<b>Juice</b>	<b>0%</b>
<b>AN15</b>	<b>absent</b>	<b>absent</b>	<b>absent</b>	<b>absent</b>	<b>Juice</b>	<b>0%</b>

# Final Assessment samples

<b>Code</b>	<b>Origin</b>	<b>Grade</b>	<b>RSSL's Assessment</b>	<b>Level</b>
<b>AN6</b>	<b>South Africa</b>	<b>50 % B</b>	<b>Skin added</b>	<b>5%</b>
<b>AN7</b>	<b>South Africa</b>	<b>100 % B</b>	<b>Skin added</b>	<b>10%</b>
<b>AN8</b>	<b>South Africa</b>	<b>100 % A</b>	<b>Juice</b>	<b>0%</b>
<b>AN9</b>	<b>South Africa</b>	<b>20 % B</b>	<b>Skin added</b>	<b>15%</b>
<b>AN10</b>	<b>South Africa</b>	<b>50 % B</b>	<b>Skin added</b>	<b>10%</b>
<b>AN11</b>	<b>South Africa/Thailand</b>	<b>100 % A</b>	<b>Skin added*</b>	<b>10%</b>
<b>AN12</b>	<b>Philippines</b>	<b>100 % A</b>	<b>Skin added*</b>	<b>20%</b>
<b>AN13</b>	<b>South Africa</b>	<b>100 % B</b>	<b>Skin added</b>	<b>20%</b>
<b>AN14</b>	<b>Indonesia/Kenya</b>	<b>100 % A</b>	<b>Juice</b>	<b>0%</b>
<b>AN15</b>	<b>Indonesia/Kenya</b>	<b>100 % A</b>	<b>Juice</b>	<b>0%</b>

\* Two false positive samples

# Conclusions

- Levels of marker peaks in juices is variable, and some skin extracts had quite low values in third year samples
- Detection limit for detection of skin extracts can be in the region of 15 to 20% but very dependant on skin extract
- Core extracts can not be reliably seen in juice by polyphenol profiling

# Masking Skin Extract Addition

- Cloud stability of pineapple juices is generally poor and stabilisers are sometimes added to limit cloud loss
- Addition of skin extracts, which sometimes have little cloud, can add to these problems
- Both stability problems can be overcome by the addition of a thickening agent, such as pectin
- Most commercial pectins are either derived from citrus fruits, typically lemon, or apple as they are by-products of juice processing

# Carbon isotope ratios in whole juice isolated pectins and stabilisers

	Whole Juice	Isolated Pectin	Citrus pectin	Apple pectin
Number	50	50	10	5
Mean (permil)	-12.55	-12.8	-25.8	-25.7
Std Div	-0.97	-1.15	-1.56	-1.48
CV (%)	7.7	9.0	6.0	5.8

# Addition of C<sub>3</sub> Derived Pectin

Whole Juice	Un-spiked Pectin	Spiked Pectin	Pectin level	Spike level
-11.80	-12		210	0
-11.75		-12.7		5
-11.78		-13.4		10
-11.90		-14.8		20
-12.30	-12.3		295	0
-12.40		-13.0		5
-12.38		-13.7		10
-12.35		-15.0		20

# Pectin Addition in Commercial Juices?

Origin	Commerical Juice type	Whole juice (permil)	Pectin ratio (permil)		Difference (permil)	Conclusions
Thialand	conc	-11.8	-12.3		-0.5	OK
Indonesia	conc	-12.3	-12.9		-0.6	OK
Thialand	conc	-11.9	-11.8		0.1	OK
Malayasia	conc	-11.2	-11.5		-0.3	OK
Kenya	conc	-12.4	-12.4		0	OK
UK	conc	-11.2	-11.6		-0.4	OK
Costa rica	conc	-11.6	-12		-0.4	OK
Spain	Finished prod	-11.5	-11.4		0.1	OK
Spain	Finished prod	-12.3	-12.8		-0.5	OK
Azores	NFC	-13.5	-13.6		-0.1	OK
Costa rica	NFC	-11.5	-11.8		-0.3	OK
Costa rica	NFC	-12.7	-12.4		0.3	OK
Costa rica	NFC	-12.9	-13.5		-0.6	OK
Spain	Finished prod	-11.6	-13		-1.4	Added pect.
Spain	Finished prod	-11.4	-13.3		-1.9	Added pect.
Poland	Finished prod	-15.6	-11.8		3.8	Added C3 sug

# Conclusions

- Carbon isotope ratio shows addition of C<sub>3</sub> stabilisers to pineapple juices at 10 to 20% level
- Can detect sugar addition if added from C<sub>3</sub> source by shift in whole juice vs. pectin values
- *Note previous work by RSSL as part of FSA funded project found a weaker correlation between sugars and pectin than between individual sugars and citric acid.*

# Thanks

## **My co-workers**

Joaquim Magalhaes

Jonathan Nodjiadjim

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