Analysis of Volatile Aldehydes in Smokeless Tobacco with a Rapid, One-Step Extraction and Derivatization with UHPLC-MS/MS Quantification

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AGENDA

1. Standardized aldehyde method for STP´s needed
2. Aims for a new STP Aldehyde method
3. Method description and validation
4. Stability of sample extracts
5. Result comparison: UHPLC-MS/MS and GC-MS
6. Pros and cons of the UHPLC-MS/MS compared to the GC-MS method
7. Economical considerations

FDA demands Aldehyde analysis in Smokefree Tobacco Products (STP´s)

FDA Harmful and Potentially Harmful Components (HPHC) for STP´s. (First STP-list 2012)

Acetaldehyde	Arsenic	NNN
Formaldehyde	Cadmium	NNK
Crotonaldehyde	Benzo[a]pyrene	Nicotine

CORESTA recommended method No 74 is only for smoke

NO recommended method for STP´s yet.

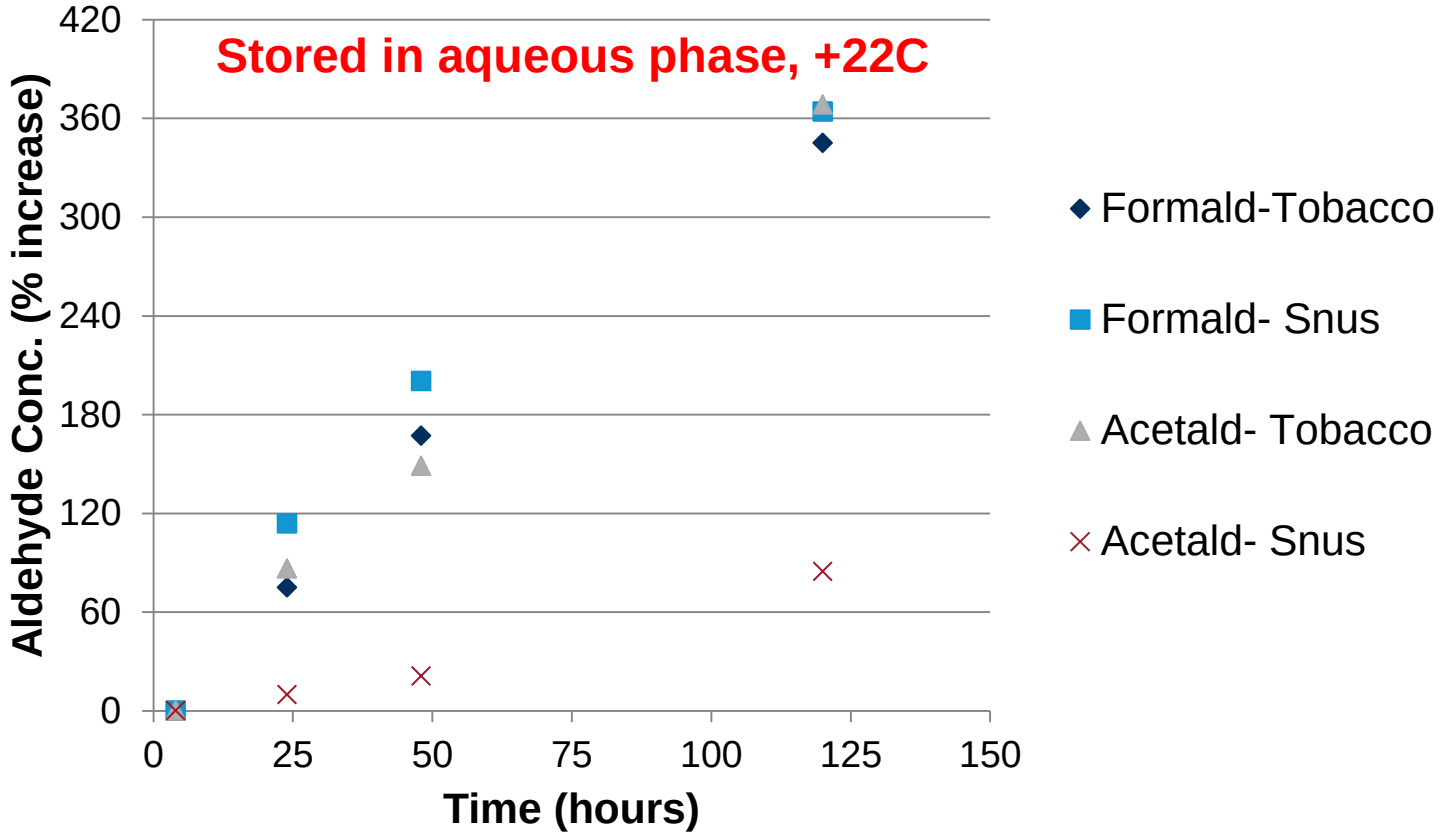
Initial challenges

1. Swedish Match sent tobacco samples for analysis to 4 international labs: Up to 1000% difference in concentration for formaldehyde and acetaldehyde

2. Sample extracts not stable

Aldehyde stability problems in aqueous phase

Aldehydes water-soluble, but concentration increase during aqueous sample storage



Selective and sensitive alternative methods for aldehyde-analysis

- **UHPLC-MS/MS**

This method presented here today

- **GC-MS**

Presented at CORESTA in October 2013

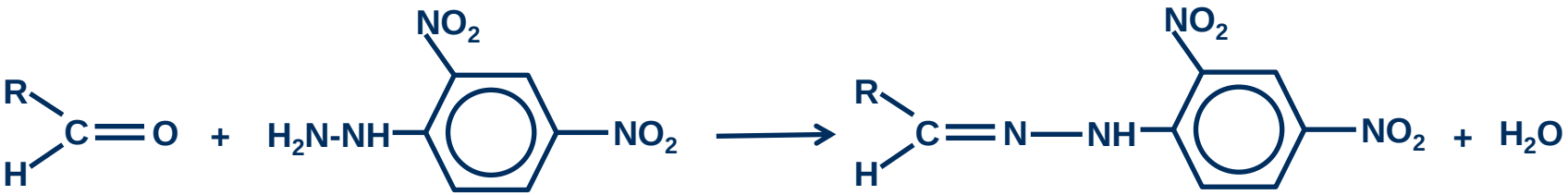
Aims for a new Aldehyde method for STP´s

1. **A sample preparation method resulting in stable sample extracts**
2. **Simple, quick and convenient sample preparation method**
3. **Fast separation with selective and sensitive detection**
4. **Accurate and precise method with good between-lab reproducibility**

High sample capacity with low cost per sample

Sample preparation: DNPH-derivatization of aldehydes

2,4-Dinitrophenylhydrazine (DNPH)



Aldehyde **DNPH** **Aldehyde-derivative**

Reasons for DNPH-derivatization

1. **Capture the aldehyde in solution- less volatile**
2. **Increase the mass of the molecule- promotes MS-detection**

Sample preparation- 1-step extraction/derivatization

1. 1g of tobacco, add 40 ml ammonium formate (pH 3.0)
2. Spike the 6 calibrators with analytes
3. 100 µl IS (formald-d2, acetald-d4) to all calibrators and samples
4. 1 ml DNPH solution (3 mg DNPH /ml Acetonitrile)
5. 10 ml Isohexane
6. Extraction and derivatization by orbital shaking <u>60 min</u>
7. Remove isohexane layer for analysis (within 30 min)

<u>GC-MS</u>
3-step extraction/filter/derivatization

- **Extraction 1 hour**
- **Filter samples**
- **Derivatization 2 hours**

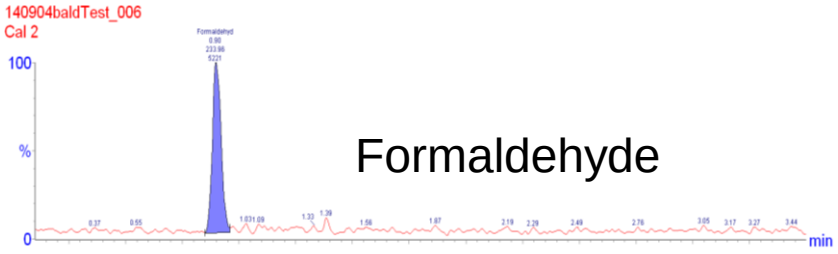
UHPLC-MS/MS method

UHPLC	
Mobile Phase A	10 mM Ammonium acetate, pH 4.7 (± 0.1)
Mobile Phase B	Acetonitrile
Flow Rate	0.45 mL/min
Gradient	See below
Injection Volume	10 µL
Run Time	4.1 min
Column	
Analytical Column	**UHPLC:** Waters Acquity UPLC BEH C18 column, 2.1mm x 100mm, 1.7µm particle size
Column Temp.	60 °C
MS/MS Detector	
Ionization:	Electrospray
Polarity:	Negative mode
UHPLC gradient	

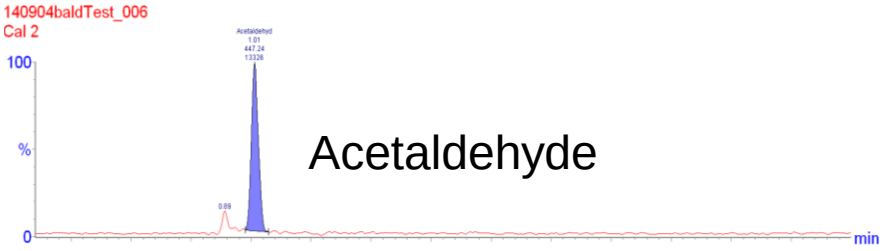
Time (min)	Flow-rate (mL/min)	10 mM NH4Ac, pH 4.7 (%)	Acetonitrile (%)
Initial	0.45	45	55
0.2	0.45	45	55
1.0	0.45	0	100
1.5	0.45	0	100
1.8	0.45	45	55
4.1	0.45	45	55

LC-MS/MS Chromatograms Waters QP- Lowest Standard

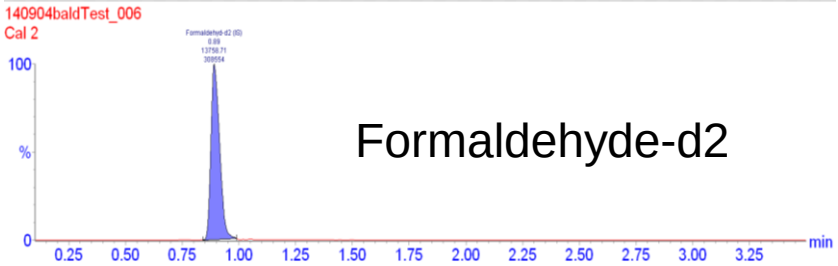
Calibration Standard – 0.1µg/g (Croton 0.05 µg/g)



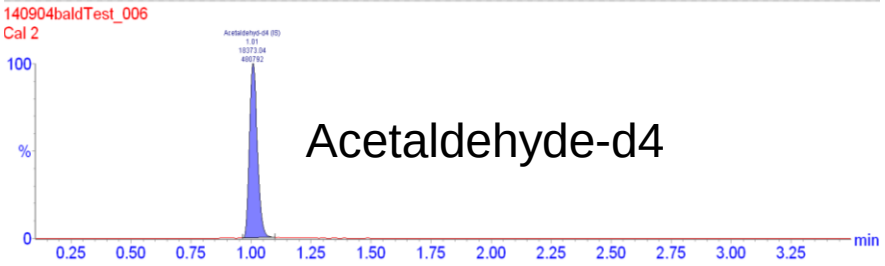
Formaldehyde



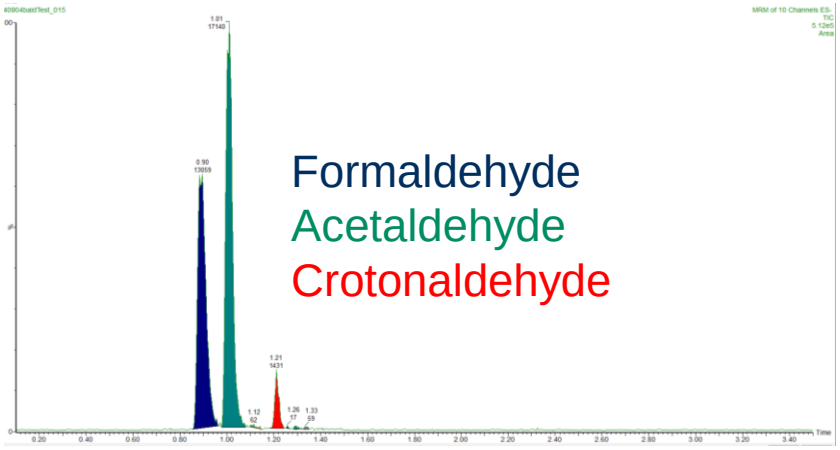
Acetaldehyde



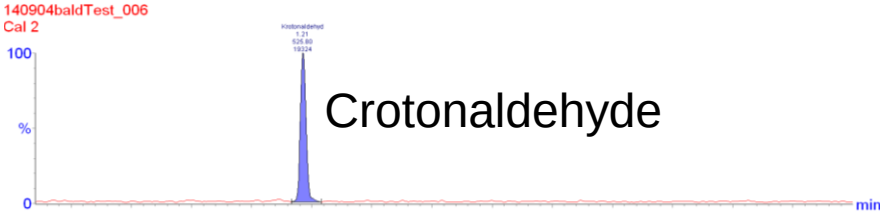
Formaldehyde-d2



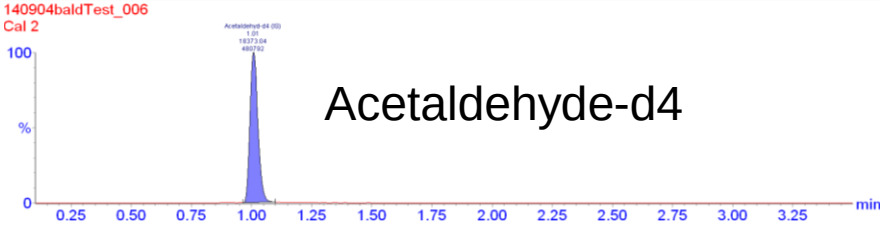
Acetaldehyde-d4



Formaldehyde
Acetaldehyde
Crotonaldehyde



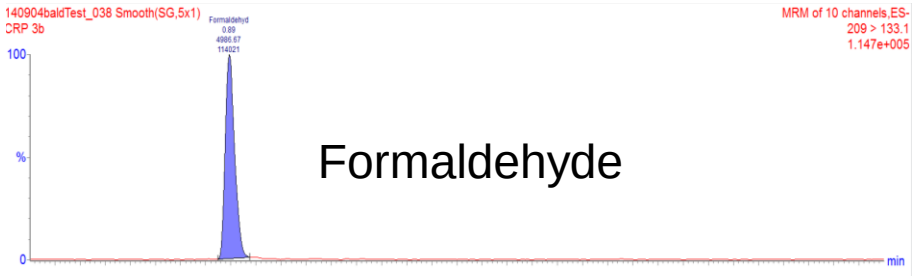
Crotonaldehyde



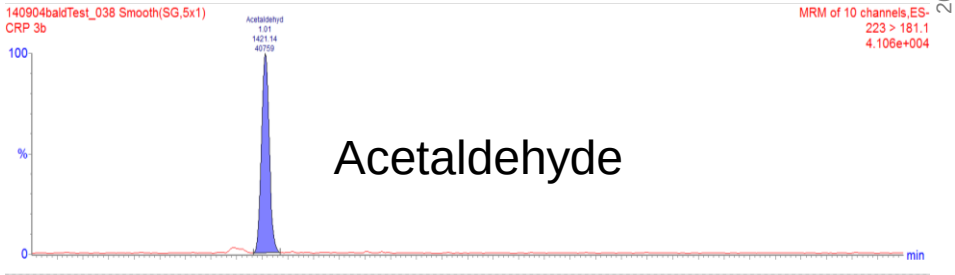
Acetaldehyde-d4

LC-MS/MS Chromatograms Waters QP- Tobacco sample

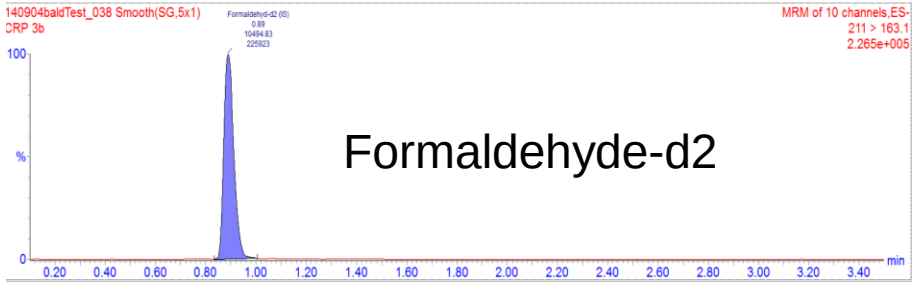
Tobacco (CRP3) Form ca 6 µg/g, Acet 2 µg/g and Croton 0.05 µg/g



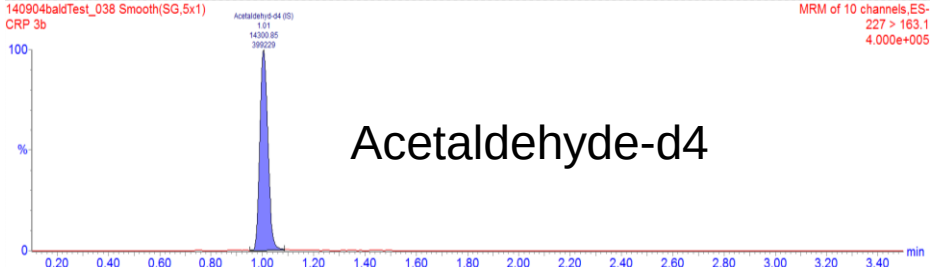
Formaldehyde



Acetaldehyde

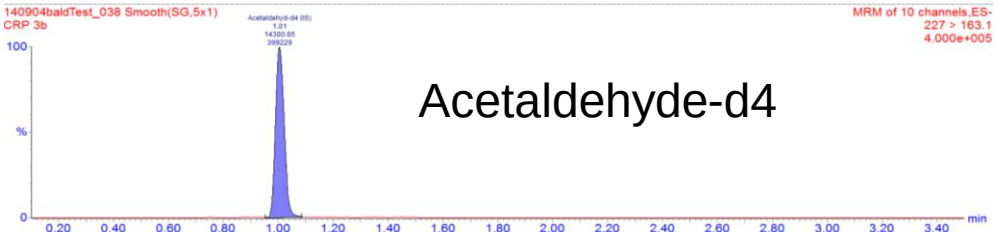


Formaldehyde-d2



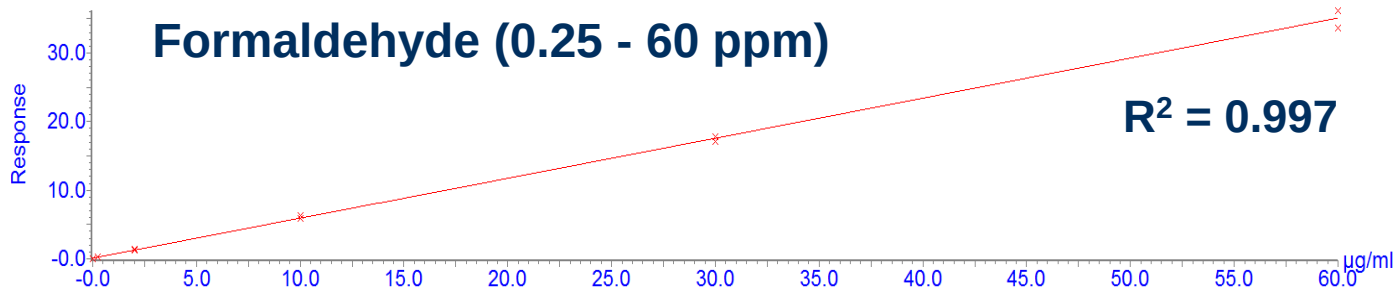
Acetaldehyde-d4

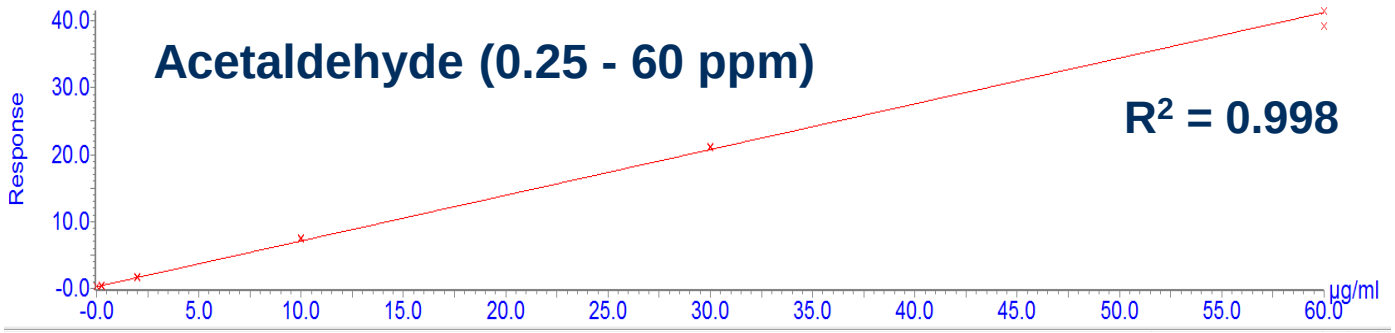
Crotonaldehyde

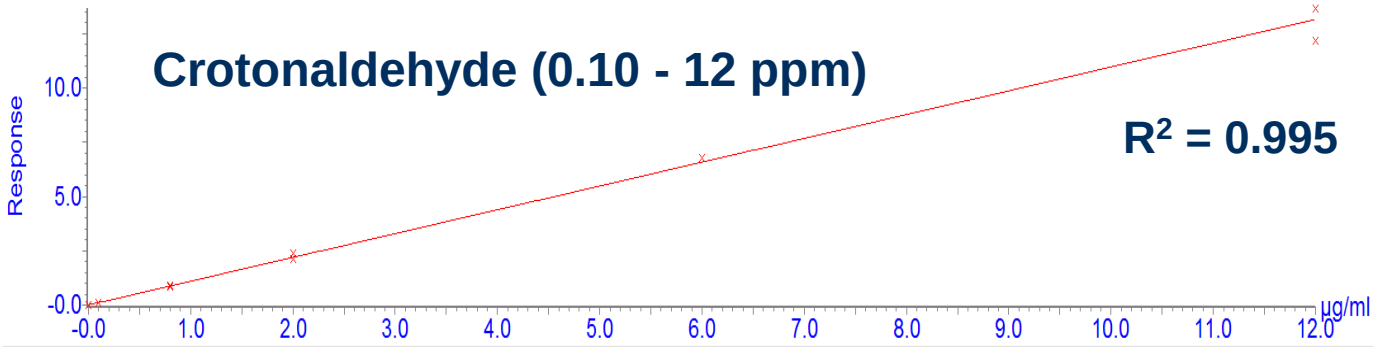


Acetaldehyde-d4

Aldehyde standardcurves







Validation results

	r (%)	Rw (%)	Accuracy (%)
Formaldehyde	5.7	5.7	102-105
Acetaldehyde	7.3	11.4	102-109
Crotonaldehyde	8.2	9.3	71-88

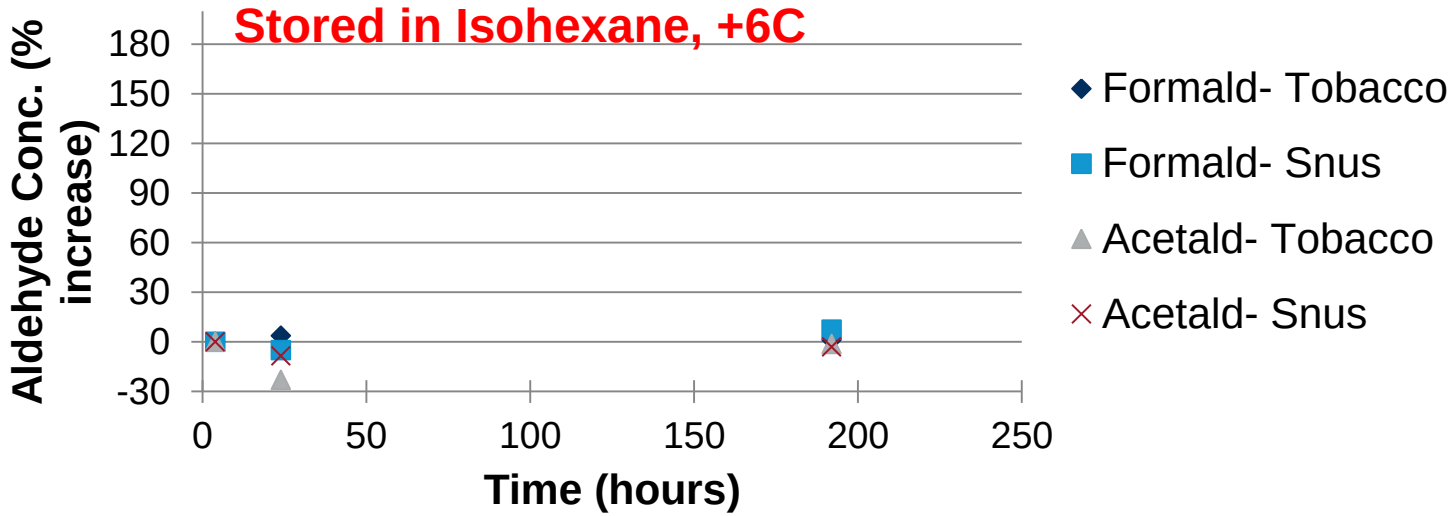
r = Repeatability: Pooled r (%); 7 sample types, 6 replicates

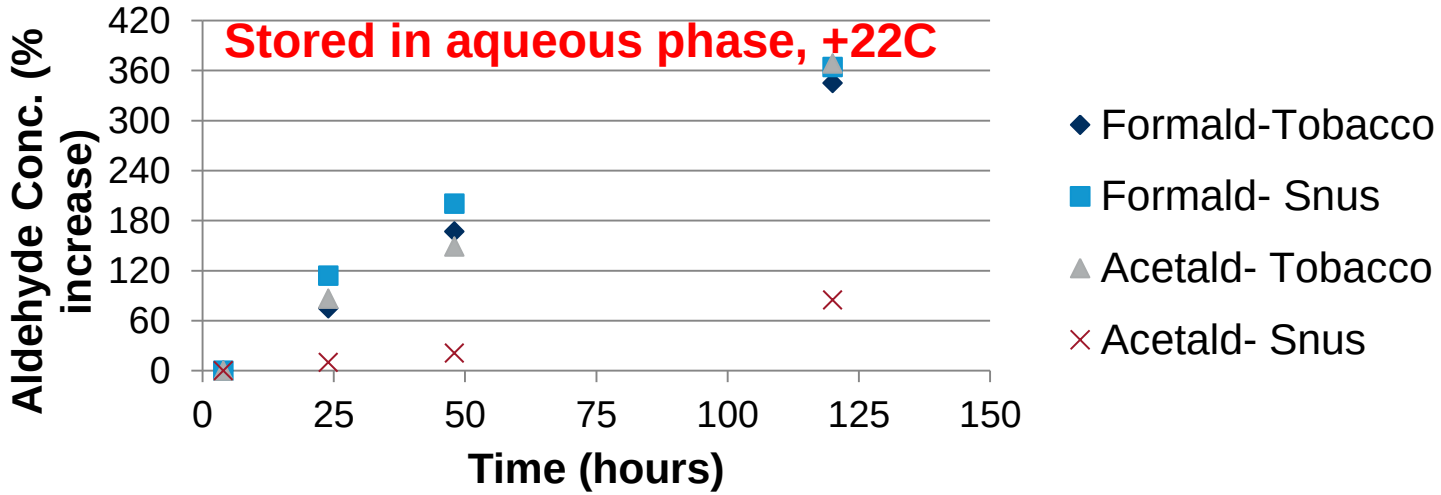
Rw = Intermediate precision: Pooled Rw (%); 3 sample types, 6 days, 3 replicates

Accuracy: Lowest-Highest; 3 sample types, 3 concentration levels

7 sample types
Snus- loose
Snus- pouch
Snus- dry pouch
Chewing tobacco
Moist snuff
Raw tobacco
Fiber based matrix

Stability of DNPH derivatives in Isohexane in +6ºC





Result comparision- different methods and labs

Form-aldehyde (ppm)	UHPLC-MS/MS (Swedish Match)	UHPLC-MS/MS (EuroFins, Sweden)	GC-MS (Swedish Match)
CRP1	1.0	1.0	1.3
CRP2	1.5	0.9	1.5
CRP3	7.0	-	6.0
CRP4	0.3	-	0.4

Acet-aldehyde (ppm)	UHPLC-MS/MS (Swedish Match)	UHPLC-MS/MS (EuroFins,Sweden)	GC-MS (Swedish Match)
CRP1	6.8	8.8	10.2
CRP2	2.6	2.9	3.3
CRP3	1.8	-	2.3
CRP4	0.4	-	0.6

Pros and cons UHPLC-MS/MS vs. GC-MS

	UHPLC-MS/MS	GC-MS
Sample Preparation Time	2 hours	4 hours
Instrument Cycle Time	6 minutes	18 minutes
Chromatography	Completely resolved peaks	Completely resolved peaks
Sensitivity (LOQ)	0.05 - 0.25 µg/g	0.1 - 0.145 µg/g
Capacity	120 samples per day and person (2 x 60)	40 samples per day and person
Cost per sample	$18	$54
Instrument Cost	$275.000	$125.000

Economical consideration: UHPLC-MS/MS vs GC-MS

	UHPLC-MS/MS	GC-MS
Instrument cost	$275.000	$125.000
Analysis capacity	120 / day	40 / day
Cost per analysis	$18	$54
Swedish match 2013: 3077 analyses	$55.000	$165.000

For Swedish Match:

The higher price for UHPLC-MS/MS,

ca $150.000, becomes saving in just 17 months.

And this is just regarding one method…

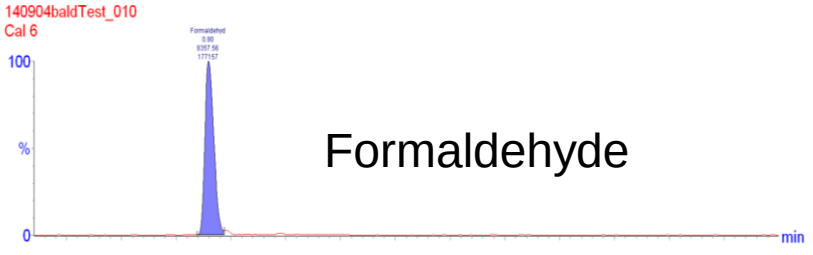
Summary 1-step extraction/derivatization UHPLC-MS/MS method

1. **Sample extracts stable for 8 days**
2. **Fast and convenient sample preparation-**
   60 samples in 2 hours
3. **Quick separation (6 min) with selective and sensitive detection (LOQ 0.05-0.25 ppm)**
4. **Accurate and precise method in accordance with other labs and alternative GC-MS method**

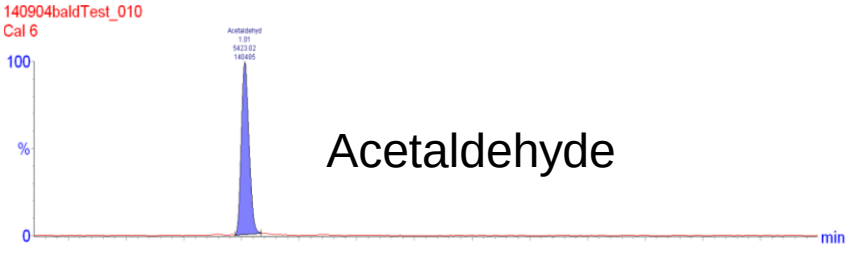
High sample capacity with low cost per sample

LC-MS/MS Chromatograms Waters QP

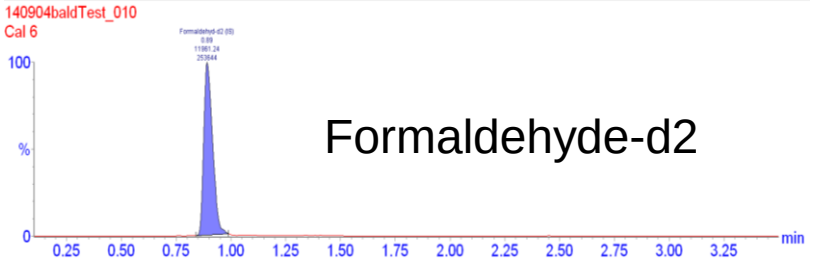
Calibration Standard – 1 µg/mL (Croton 0.1 µg/mL)



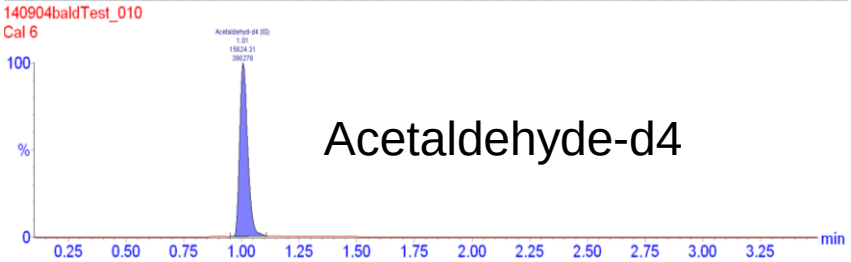
Formaldehyde



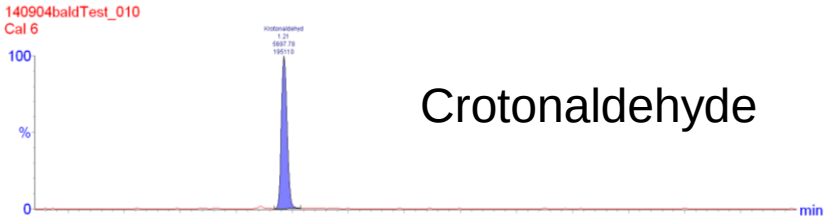
Acetaldehyde



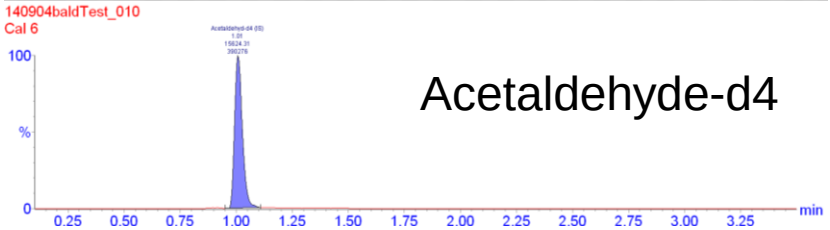
Formaldehyde-d2



Acetaldehyde-d4



Crotonaldehyde



Acetaldehyde-d4

Stability of PFBHA-Acetaldehyde in water in room temp

PFBHA-Acetaldehyde in room temp after adding H_2SO_4 (step 8): +0 vs +18 hours

Area/ IS area
PFBHA-water- Acc. to meth. (+0h) vs. +18h in room temp

Sample	+0h	+18 h	Change %
CRP 1 isohex	23,6	34,3	45
CRP 2 isohex	3,5	8,6	144
CRP 3 isohex	4,6	13,6	196
CRP 4 isohex	1,2	2,6	125
R2-Tob isohex	1,6	5,3	221

DNPH-Acetaldehyde increased 53% in r.t. 4h to 24h