TITLE : RESIDUAL ANALYSIS OF CARBONYLS IN TOBACCO AND TOBACCO PRODUCTS BY HPLC

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Abstract

A Simple method developed for the determination of Carbonyls residues in tobacco and tobacco products using HPLC with Photo-diode Array (PDA) detector.

This method involves derivitization and ultra sonication for the rapid and complete extraction of carbonyl compounds from tobacco leaves. Tobacco leaves were minced and ultra sonicated in acidified 2,4-dinitrophenyl hydrazine (DNPH acidified with phosphoric acid) in acetonitrile for 2hrs and then holding for 30 min to allow for the complete reaction of aldehydes and ketones with DNPH, and subsequent chromatographic separation on LicChrospher 100 RP- 18e 250mm x 5 micron (Merck make) under gradient condition at 365nm. Method has been optimized for various parameters I,e like concentration and derivitization of carbonyl with DNPH, sample extraction technique and extraction time.

The method has been validated by standard validation protocols i.e.Limit of detection, Limit of Quantification, Recovery, Repeatability and Reproducibility. Recoveries of (73 – 103.4%) were obtained with a linear regression coefficient of 0.9998 for the range of 0.022- 4.52 mg/Kg of selected 8 carbonyls. Tobacco samples of various grades were analyzed.

Introduction

Carbonyl compounds have been drawing more and more attention as they are believed some carbonyls have been proven to be carcinogenic or risk for human health. Tobacco leaves are important source of carbonyls. It is hypothesized that these carbonyls are formed from lipid and wax constituents in tobacco leaves.

	Name of carbonyl	Molecular formula	Structural formula
1.	Formaldehyde	HCHO	CH_2O
2.	Acetaldehyde	C_2H_4O	CH_3CHO
3.	Acetone	C_3H_6O	CH_3CH_2CHO
4.	Acrolein	C_3H_4O	$CH_2=CHCHO$
5.	Propionaldehyde	C_3H_6O	CH_3CH_2CHO
6.	Crotonaldehyde	C_4H_6O	$CH_3CH=CHCHO$
7.	Methyl Ethyl Ketone	C_4H_8O	$C_2H_5COCH_3$
8.	Butyraldehyde	C_4H_8O	$CH_3CH_2CH_2CHO$

Literature

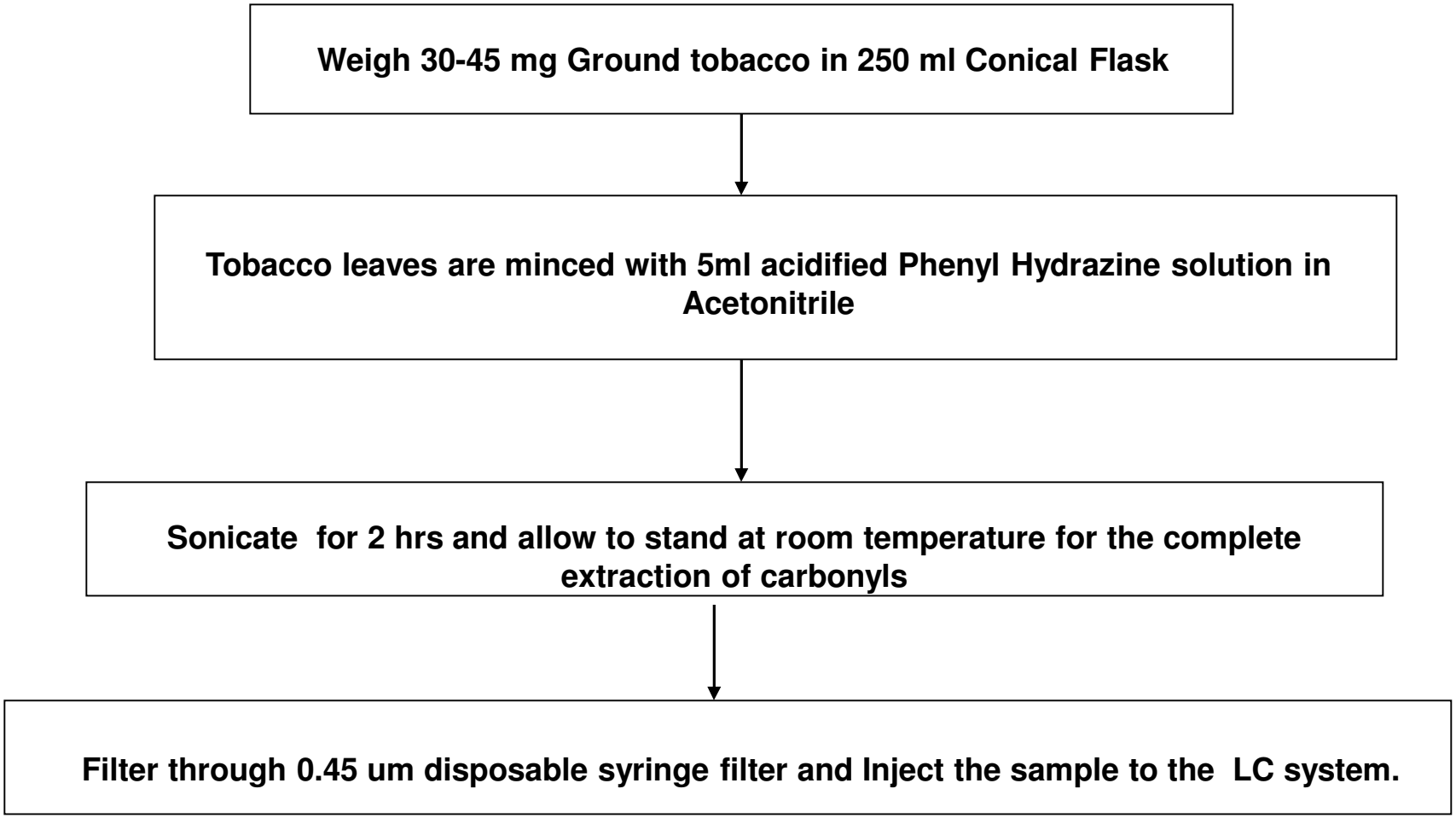
A Few analytical methods available for the quantification of residual Carbonyl in various sample matrix (Tobacco smoke, Tree leaves etc) however no methods available for tobacco leaves.

a) Juan Huang. Yanli Feng. Jiamo Fu. Guoying Sheng, A method of detecting carbonyls compounds.

b) Health Canada test method T-104. Determination of selected Carbonyls in mainstream Tobacco smoke.

c) Journal of Chromatography A volume 693, issue 2, 24 February 1995,

SAMPLE PREPARATION



LC Conditions

Column temperature: 30°C

Injection volume : 20ul

Gradient elution:

Mobile phase –A : Acetonitrile : THF : IPA in % (30 :10 :1) make up to 1 liter with distilled water

Mobile phase – B : Acetonitrile : THF : IPA in % (65: 1: 1) make up to 1 liter with distilled water

Mobile phase – C Pure Acetonitrile

Flow rate : 1.5 ml /min

Run time : 35 min

Post run time : 10 min

Gradient programming :

Time in Min	Pump A Flow %	Pump B Flow %	Pump C Flow %
0.0	100	0.0	0.0
8.0	70	30	0.0
20.0	47	53	0.0
27.0	0.0	100	0.0
30.0	0.0	0.0	100
32.0	0.0	0.0	100
34.0	95	5.0	0.0
Method end Action :	100	0.0	0.0

Chromatograms of standard and Sample

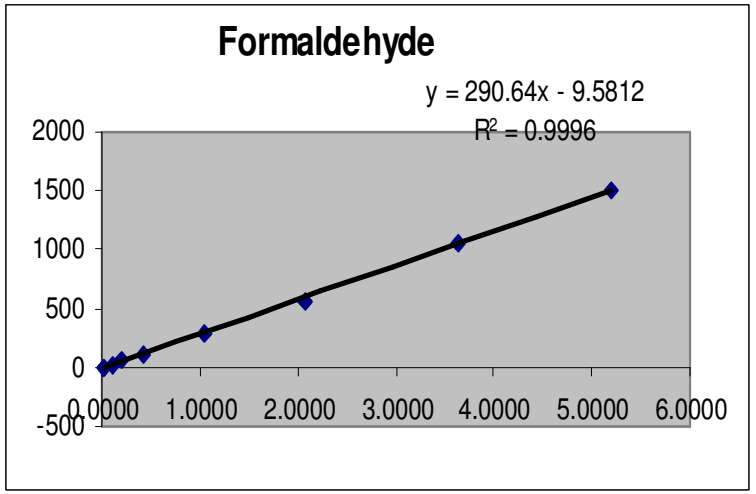
Limit of detection and limit of quantification

Lowest carbonyl standard solution of Carbonyls were used

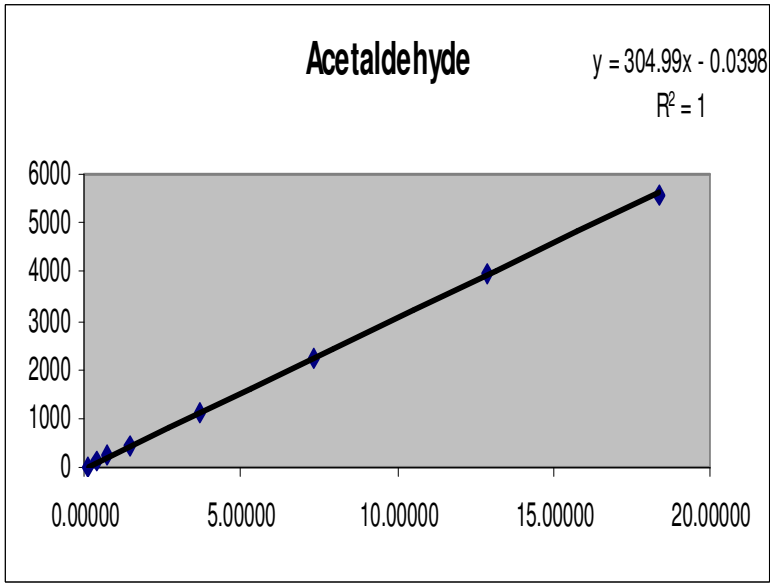
Name of Carbonyl	LOD in ppm	LOQ in ppm
Formaldehyde	0.40	1.20
Acetaldehyde	0.54	1.65
Acetone	0.44	1.32
Acrolien	0.31	0.95
Propionaldehyde	0.33	1.01
Crotonaldehyde	0.29	0.89
MEK	0.20	0.67
Butyraldehyde	0.31	0.94

VALIDATION: LINEARITY

Sl NO	Formaldehyde Conc	Peak Area
1	0.026	6.7
2	0.104	28.5
3	0.207	55.4
4	0.415	111.2
5	1.037	281.2
6	2.075	569.7
7	3.631	1051.5
8	5.187	1505.1
	Corr Coefficient :	**0.9996**

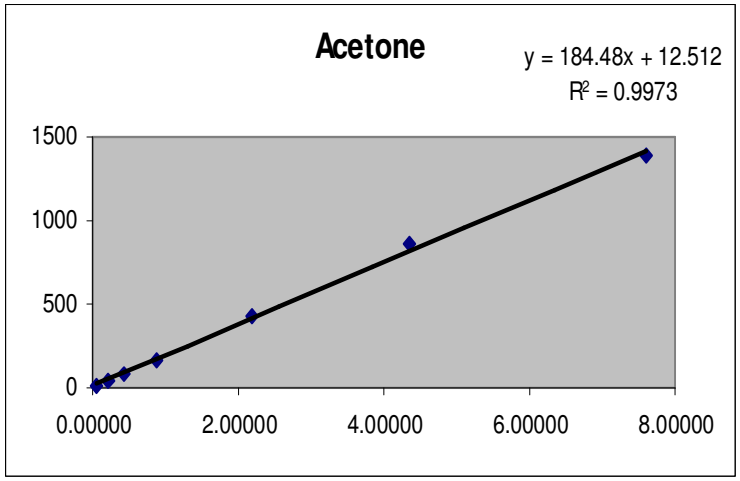


Sl NO	Acetaldehyde conc	Peak Area
1	0.092	27.4
2	0.368	114.5
3	0.735	222.2
4	1.470	441.2
5	3.674	1115.1
6	7.350	2246.0
7	12.863	3951.3
8	18.376	5584.9
	Corr Coefficient :	**1.000**

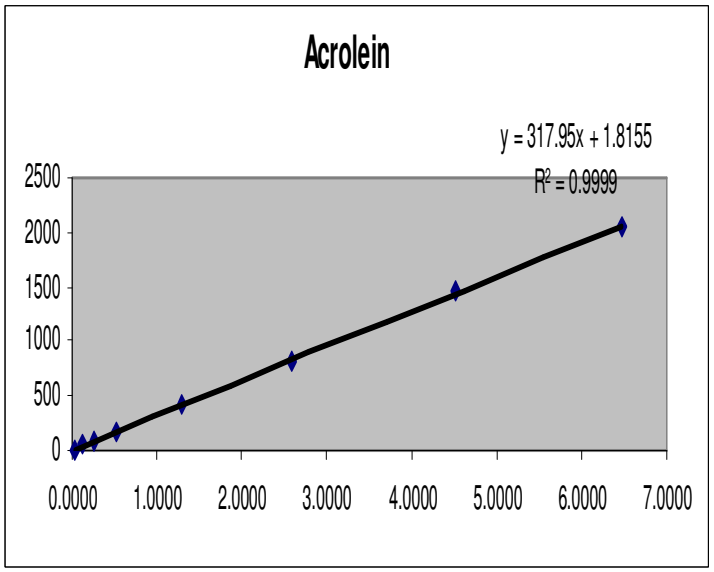


LINEARITY

Sl NO	Acetone conc	Peak Area
1	0.054	9.8
2	0.217	43.1
3	0.435	83.7
4	0.870	167.3
5	2.174	432.6
6	4.348	865.6
7	7.609	1383.2
8	10.870	1813.6
	Corr Coefficient :	**0.9973**

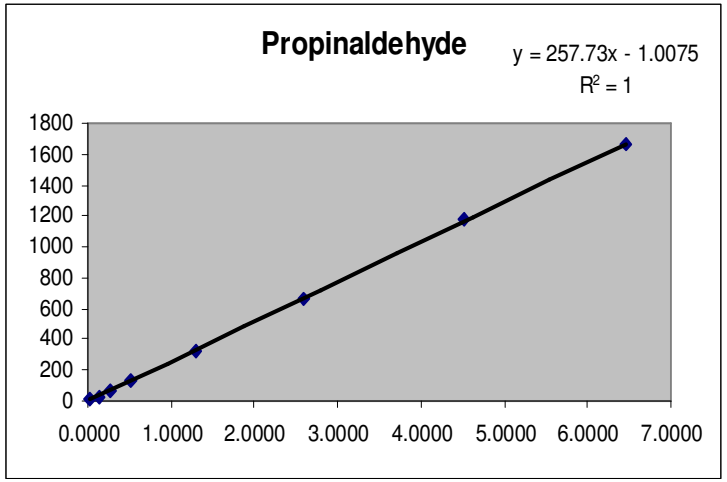


Sl NO	Acrolien conc	Peak Area
1	0.032	10.2
2	0.129	42.6
3	0.259	82.8
4	0.517	164.5
5	1.293	410.1
6	2.587	827.0
7	4.522	1458.8
8	6.467	2044.3
	Corr Coefficient :	**0.9999**

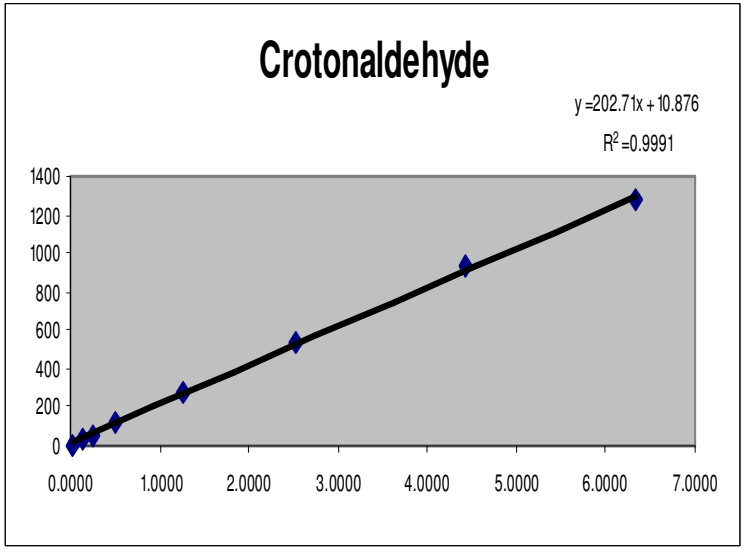


LINEARITY

Sl NO	Propinaldehyde conc	Peak Area
1	0.032	7.6
2	0.129	33.4
3	0.259	65.1
4	0.518	129.7
5	1.294	330.4
6	2.588	667.3
7	4.528	1174.2
8	6.469	1660.6
	Corr Coefficient :	**1.000**

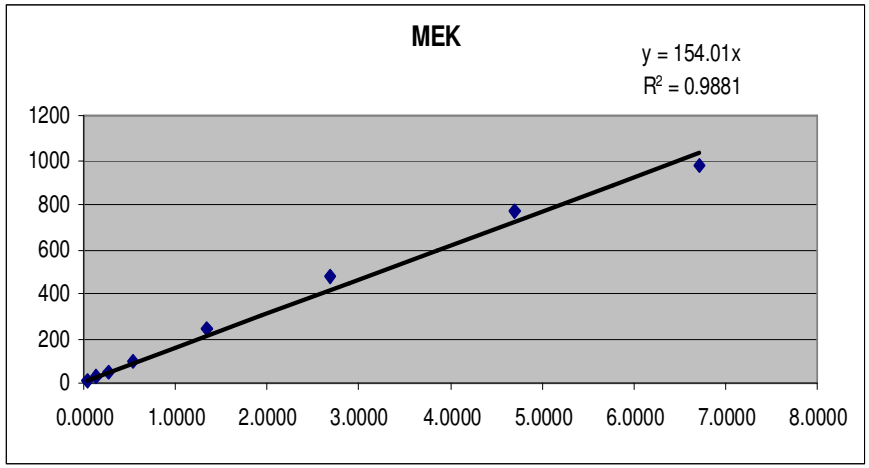


Sl NO	Crotonaldehyde conc	Peak Area
1	0.032	7.9
2	0.127	29.3
3	0.254	56.9
4	0.507	112.7
5	1.268	277.3
6	2.536	540.0
7	4.438	930.9
8	6.340	1274.4
	Corr Coefficient :	**1.000**

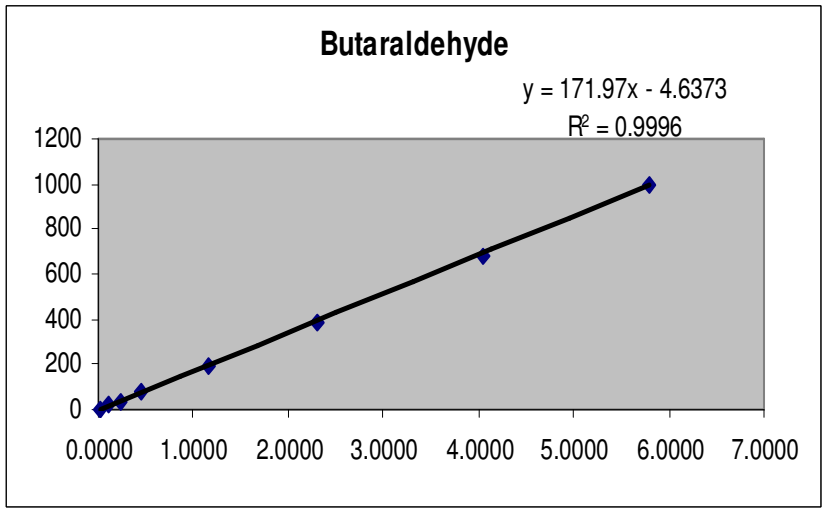


LINEARITY

Sl NO	MEK Conc	Peak Area
1	0.034	6.1
2	0.134	25.3
3	0.269	49.1
4	0.538	97.0
5	1.344	240.2
6	2.688	479.1
7	4.705	766.5
8	6.721	971.5
	Corr Coefficient :	**0.9907**



Sl NO	Butaraldehyde conc	Peak Area
1	0.029	4.4
2	0.116	19.7
3	0.231	39.1
4	0.463	76.4
5	1.157	189.9
6	2.315	380.9
7	4.051	684.6
8	5.787	1001.4
	Corr Coefficient :	**1.000**



RECOVERY

Studies conducted by adding a known amount of Carbonyls to the tobacco sample

		Carbonyl in ppm		
	Carbonyl name	Spike area	Recovered area	% Recovery
1	Formaldehyde	123.9	97.2	78.5
2	Acetaldehyde	219.2	228.8	96.1
3	Acetone	80.0	75.5	94.3
4	Acrolien	94.5	82.5	87.3
5	Propionaldehyde	63.0	63.5	100.7
6	Crotonaldehyde	86.3	64.3	74.5
7	MEK	43.9	45.4	103.4
8	Butyraldehyde	43.0	36.0	83.6

PRECISION

Tobacco samples of straight grade were analyzed over a period of time and results were reproducible. Rsd was well within the limit.

Precision

Trial	Formaldehyde (ppm)	Acetaldehyde(ppm)	Acetone (ppm)	Acrolien (ppm)
1	10.66	10.33	18.18	4.90
2	9.48	10.41	20.96	4.88
3	9.36	9.25	16.62	6.01
Avg	9.83	8.87	18.59	5.26
Sd	0.59	0.65	2.20	0.65
RSd	5.97	7.30	11.83	12.29
Trial	**Propionaldehyde (ppm)**	**Crotonaldehyde(ppm)**	**MEK (ppm)**	**Butyraldehyde (ppm)**
1	7.92	1.76	0.87	1.31
2	6.37		1.27	1.75
3	7.15	1.48	1.10	1.70
Avg	7.15	1.62	1.08	2.04
Sd	0.633	0.198	0.164	0.300
RSd	8.85	12.22	15.18	14.71

ACCURACY

Certified Reference materials carbonyls were analyzed by this method and results are as follows.

Sl No	Name of Carbonyl	Purity % (CRM)	Purity % by Method	Error %
1	Formaldehyde - 2,4- DNPH	99.90	99.78	0.12
2	Acetaldehyde - 2,4- DNPH	99.90	99.18	0.72
3	Acetone - 2,4- DNPH	99.90	98.95	0.95
4	Acrolein - 2,4- DNPH	99.90	97.24	2.66
5	Propionaldehyde - 2,4- DNPH	99.90	99.50	0.40
6	Crotonaldehyde - 2,4- DNPH	99.90	99.92	-0.02
7	Methyl - Ethyl Ketone- 2,4- DNPH	99.90	99.73	0.17
8	Butyraldehyde - 2,4- DNPH	99.90	99.90	0.00

Conclusion

1 Easy sample preparation and fast analysis

2 Good resolution of Carbonyl peak from tobacco impurities

3 No matrix interference as evidenced from the recovery studies.