Precision and accuracy of individual alkaloid measurements

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Abstract:

In the last several years there have been changes made in alkaloid accumulation in our commercial lines with more changes to come very soon as the industry moves beyond the LC lines to mutant lines. This especially concerns the lowering of nornicotine accumulation and the formation of NNN (N'-nitrosonornicotine). Additionally, myosmine is another minor alkaloid found in tobacco and is the first degradation product of nornicotine. Myosmine may also be nitrosated to NNN (Zwickenpflug, 2000). In burley tobacco we are concerned about the carcinogen NNN of the TSNAs (tobacco-specific nitrosamines) found in tobacco as burley conversion of nicotine to nornicotine is not stable in present lines and leads to NNN accumulation. There have been many improvements in measuring tobacco alkaloids over the last 50 years. Many are questioning the accuracy of the measurement of nornicotine and myosmine in tobacco by the routine method generally accepted by tobacco researchers. The objective of this proposal is for the lab to become part of a multi-lab study to develop a new protocol to measure nornicotine and myosmine precisely and accurately. This will allow KTRDC to better describe the alkaloid composition of burley tobacco variety trials and of new breeding lines being developed within the KTRDC programs.

Summary of Progress:

Methods and results

Based on the CORESTA collaborative study method, "determination of nicotine and minor alkaloids in tobacco and tobacco product by GC-MS method", a tentative modified GC/MS/MS method (MeOH extract method) was established in our lab.

MeOH extract method: The weighed tobacco sample is wetted with 5N sodium hydroxide (NaOH) for 30mins and extracted with methanol (MeOH) by shaking for 30mins. The extraction solution is filtered with PTFE membrane filter into a sample vial for the analysis with the gas chromatography-mass spectrometer which is equipped with CAM GC column (30m X 0.25mm, 0.25μm). GC program and MS parameters are shown in Table 1 and Table 2.

Table1: GC Program

rate	temperature	hold time	total time
	110 C	1 min	1 min
10 C/min	190 C	0	9 mins
45 C/min	280 C	0	11mins

Table2: Quantification and Qualification Transitions for Alkaloids

Name	Quantification Transition	Qualification Transition
	(m/z)	(m/z)
nicotine	162 > 184	162 > 119
quinoline	129 > 102	
7-methylquinoline	143 >115	
nornicotine	148 >119	148 > 106
myosmine	146 > 118	146 > 91
anabasine	162 > 84	162 > 133
anatabine	160 > 131	160 > 82

Alkaloids in a total of 11 samples were measured. They are CORESTA Reference Smokeless Products (CRP) 1-4, Reference cigarette-3R4F, Fine cut MST, Burley, Virginia, Oriental, Fire cured and Dark air cured tobacco. Preliminary results are shown in Table 3.

Table 3: Alkaloid measured with modified CORESTA collaborative study method

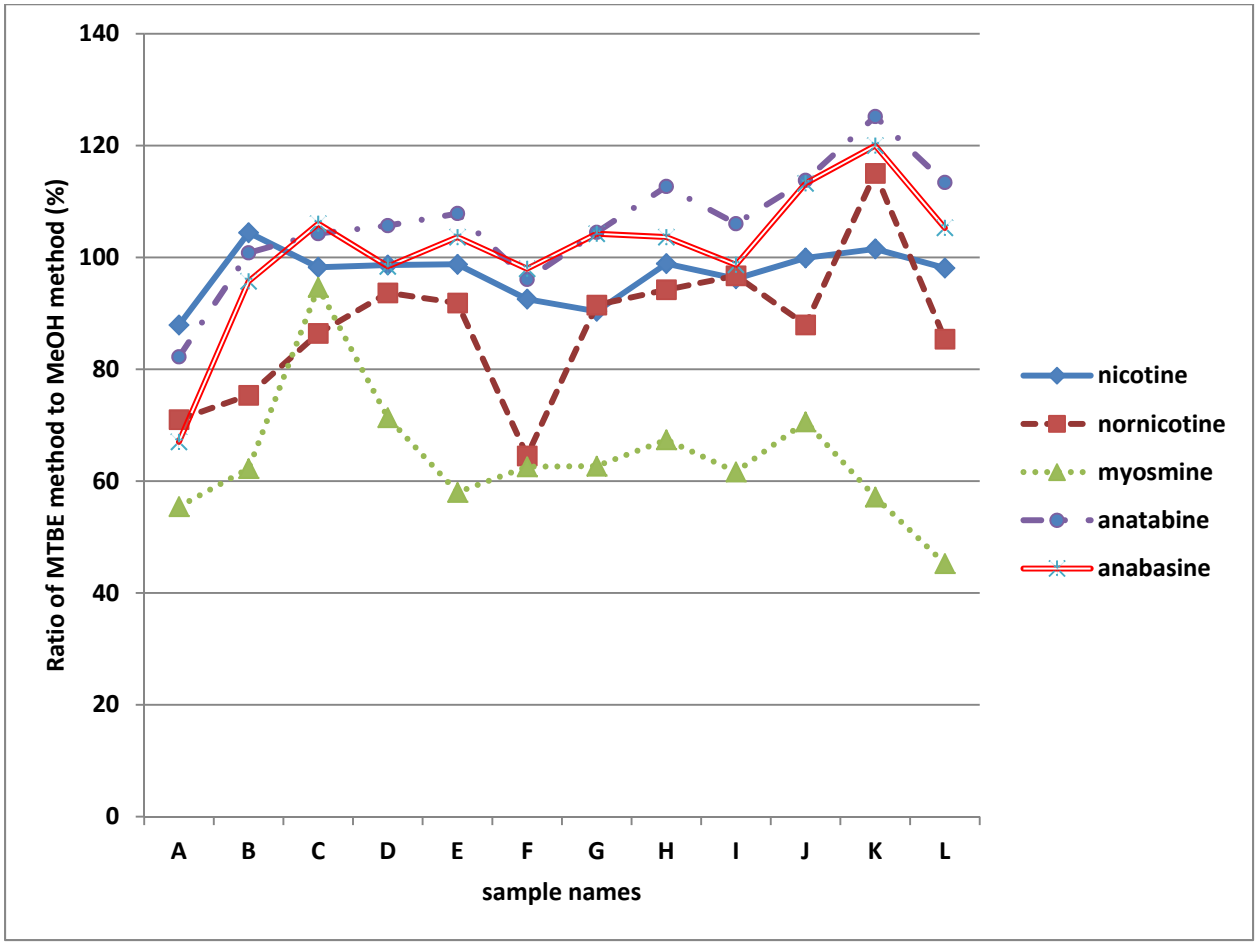
sample	Replicate	Nicotine	Nornicotine	Anabasine	Myosmine	Anatabine
		µg/g	µg/g	µg/g	µg/g	µg/g
CRP1	1	11848	280.7	50.7	41.9	147.1
	2	11422	267.4	42.6	36.1	142.2
	3	11636	275.1	54.1	33.2	144.5
CRP2	1	10968	244.6	42.2	21.7	169.8
	2	11182	254.1	42.8	22.2	175.2
	3	10760	180.8	39.0	13.9	160.4
CRP3	1	19778	538.3	79.9	59.6	412.2
	2	20707	590.4	80.7	64.5	444.0
	3	20475	586.9	80.6	63.6	438.3
CRP4	1	11257	532.4	41.9	23.5	412.0
	2	11151	534.2	41.3	22.5	398.9
	3	10695	488.6	39.3	22.8	367.1
3R4F	1	16576	642.0	79.5	33.1	586.4
	2	16734	563.5	72.6	25.4	582.6
	3	16924	631.0	73.4	30.6	578.0
Fine cut MST	1	9330	129.8	33.3	13.0	99.0
	2	9725	148.1	32.5	16.3	104.9
	3	9506	191.8	37.2	24.9	113.7
Burley	1	13006	1583.7	78.4	95.3	491.4
	2	12982	1550.8	75.4	93.4	467.2
	3	12520	1724.6	72.8	97.3	473.6
Virginia	1	16957	467.9	62.1	25.5	814.0
	2	16856	544.4	66.2	27.9	841.3
	3	16960	512.7	64.9	26.1	809.1
Oriental	1	6495	399.0	20.7	17.7	102.4
	2	6556	301.3	20.2	15.3	99.9
	3	6894	373.4	22.0	16.8	109.5
Fire cured	1	22350	390.2	62.7	24.6	374.9
	2	22381	389.6	65.3	24.1	391.6
	3	21935	388.6	61.1	23.6	384.4
Dark air cured	1	13662	575.9	64.8	29.4	553.8
	2	13602	657.5	69.4	24.2	606.4
	3	14052	741.7	68.8	20.6	597.8

Comparison of routine MTBE extract method to Methanol extract method

MTBE extract method: The weighed tobacco sample is wetted with 2N sodium hydroxide (NaOH) for 15mins and extracted with Methyl tert-butyl ether (MTBE) by shaking for 2.5 hours. The extraction solution is filtered through a PTFE membrane into a sample vial and analyzed by a gas chromatography-mass spectrometer (GC-MS) with the same program and parameters as those used in the methanol method.

Alkaloids in a total of 12 samples were measured by both methods. Three replications of each sample were run. Results from MTBE method and MeOH method were compared. The ratio of MTBE method to MeOH method was calculated and shown in Figure 1.

Figure 1: Ratio of MTBE method to MeOH method



Conclusion

Based on the average ratios of the MTBE method to the MeOH method for the 12 samples, the results for nicotine, anatabine and anabasine are similar between these two methods. However, for nornicotine and myosmine, results of MTBE method are about 12% and 35%, respectively, lower than that of MeOH method. So, the methanol extraction method is more efficient than the MTBE method for minor alkaloid analysis. However, due to the high concentration of NaOH used in the methanol extraction method, the syringe needle and injection port liner and septum in the GC need to be changed often.