Determination of water in tobacco by near infra-red spectroscopy

Application Notes

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Introduction

As part of a program of work into the study of the tobacco/water system, the development of a method for the accurate and reproducible measurement of water content has been carried out. Of the various methods available for determining water, oven drying and the Karl Fischer titration have had greatest use in R & DE.

Recently, another method, involving the use of near infra-red (NIR) spectroscopy and which is specific for water, has been developed. This method has been evaluated and used as a reference to compare the validity of oven drying and the Karl Fischer titration.

Experimental

Apparatus

- A Cary 5 double beam spectrophotometer covering the 175-3300 nm range
- A solvent dispenser. A suitable container would be a 5 liter aspirator with a silica gel moisture trap in the neck, and the bottom outlet connected to a double-action 25 mL automatic pipette or an electrically operated dispenser. Because of the drying effect of the silica gel on the surface of the methanol it is recommended that the solvent is stirred continuously, with a magnetic stirrer, for example, during use.
- Hot-air oven at II0 °C
- 50 mL conical flasks with B24 stoppers
- 5 mL syringe with wide bore needle
- Normal laboratory glassware

Reagents

Methanol (specially dried), CH, OH, water content <0.05 %.

Procedure

Note: The determination is normally carried out on samples already prepared for other analyses. If the sample is in leaf form special arrangements for the extraction will be necessary depending on sample size and form.

1. 0.49-0.51 g of sample was weighed to the nearest mg into a dry 50 mL conical flask, to which 25 mL of dry methanol was added, and the flask stoppered.
2. Two standard water solutions were also prepared by weighing 90-110 mg water, to the nearest 0.1 mg, into a dry 50 mL conical flask, to which 50 mL of dry methanol was added, and the flask stoppered.

Method

3. Two flask blanks were prepared by dispensing 25 mL of dry methanol into each of two dry 50 mL flasks and stoppered immediately.
4. The samples were allowed to extract overnight at room temperature.

Note: For urgent analyses, the extraction may be speeded up by 30 minutes shaking on a laboratory shaker, followed by a 1 hour settling period. This may produce slightly low results on some tobacco types.

5. The spectrophotometer was used according to the manufacturer's instructions, to scan the spectral region from 1800-1950 nm in the absorbance mode, with a range of 0-0.5 absorbance units.
6. Two dry 10 mm stoppered glass cell were filled with dry methanol and placed in the spectrophotometer.

Note: For large numbers of samples it is convenient to use three cells, one reference and two for samples. This allows the operator to prepare the next sample during the two minutes waiting period.

7. A waiting time of two minutes was allowed to equilibrate the solution to instrument conditions, before scanning from 1800-1950 nm. This was used as a check for cleanliness of cells and operating conditions. The result should be a baseline, flat to within 0.02 absorbance units.
8. The sample cell was filled from the flask blank and placed in the spectrophotometer. The two minute waiting time was allowed before scanning the same range of 1800-1950 nm.
9. The sample extracts were run in the same manner, using a dry syringe to transfer aliquots of the supernatant solution to the sample cell. This reduced spillages on the optical face of the cell.

Note: Particles of tobacco in suspension will result in a high background absorbance; therefore care is required in opening the flask and removing the aliquot of sample.

10. The absorbance of the peak was measured at 1943 nm and the background absorbance at 1845 nm.

Method

Calculation

1. Subtract the relevant background absorbance from each peak value.
2. Calculate the calibration factors from the formula

   $F= W_s \ / V_s \times A_s$

   Where:
   W_s= weight (mg) of standard

   V_s = volume (mL) of standard (normally 50 mL),

   A_s = corrected absorbance of standard

   The two values should agree to within ±2.5 % of the average.
3. Calculate the water content of the sample from the formula:

   $X \text{ NIR water} = F \times A_t \times V_t \times 100 \ / W_t$

   Where;
   F = average calibration factor,

   A_t = corrected absorbance of tobacco extract,

   V_t = volume (mL) of sample extract (normally 25 mL),

   W_t = weight (mg) of sample.

Method

Notes for the operator

1. The cells must be clean

   Immerse cells for 30 minutes in 5% warm Lipsol or Decon. This will remove most deposits.Wash with warm water to remove the detergent, followed by distilled or deionized water. Drain well, inverted on a tissue. Then immerse in two successive portions of dry methanol, draining well between immersions.

   Remember to clean the stoppers as well.

   The cells should not require frequent cleaning if handled correctly during use, and rinsed with methanol after a run, before storing in a desiccator.
2. When filling the cells from the solvent reservoir, dispense some of the solvent to waste and wipe the outer surface of the jet before filling the cells. Methanol evaporation from the surface results in condensation of water from the atmosphere.
3. It is good spectroscopic practice to place the cells in the spectrophotometer with the same orientation on each occasion, i.e. the same optical face towards the light source.
4. **Do not fill the cells to the level of the stopper**. This invariably results in extract, displaced by the stopper, contaminating the optical faces. Examination of the spectrophotometer cell carrier will show that the light path passes through the lower 2/3 of the cell.
5. A 100 mL beaker containing clean methanol can be used to wash accidental spillages from the outside of the cell by immersion, **before** wiping the surfaces with a tissue.

Discussion

The NIR method involves the extraction of tobacco with a suitable solvent and the measurement of the absorbance of the extract between 5600 and 4600 cm^{-1}. The water content is determined by correcting the measured absorbance at 5200 cm^{-1} (1.92 µm) for background absorbance and reference to a calibration curve.

A specific program was written using the Cary 5E Application Development Language (ADL) software capabilities. This program provides automation of measurements and calculations for the tobacco analysis (see Table 1).

Table 1. Report generated by Cary 5E ADL program

NIR Determination of Moisture Content
Using a Cary 5

Operator: Zafira Bilimis		**Date: 25 May 1994**		
Cell Blank	**A1943**	**A1845**	**Difference**	
1	0.00006	0.00006	0.00001	
2	-.00001	0.00002	-.00002	
Flask Blank	**A1943**	**A1845**	**Difference**	
1	0.00002	0.00004	-.00002	
2	0.00008	0.00008	0.00000	
	Wt.	**A1943**	**A1845**	**Fact**
Std 1	100.0	0.20069	0.06480	14.72
Std 2	100.3	0.19957	0.06369	14.76
Std 3	100.7	0.20194	0.06631	14.85
Std 4	101.0	0.20276	0.06897	15.10
Average Factor = 14.8571				
Sample No.	**Wt(mg)**	**A1943**	**A1850**	**%NIR H_2O**
Tobacco 1	500.1	0.2007	0.0648	10.09
Tobacco 2	500.3	0.1996	0.0637	10.09
Tobacco 3	499.9	0.2019	0.0663	10.08
Tobacco 4	500.5	0.2028	0.0690	9.93

Figure 1 displays the wavelength scans of four tobacco samples.

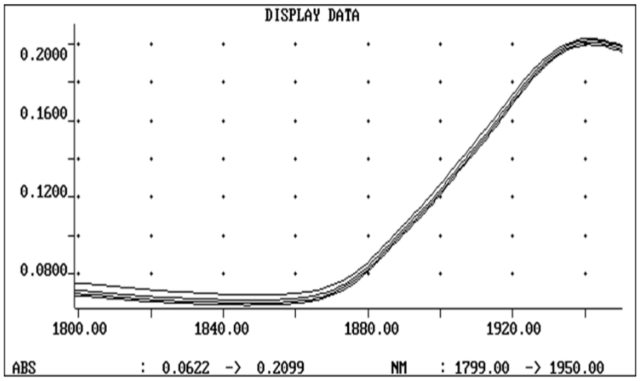

Figure 1. Wavelength scans of four tobacco samples

Choice of solvent

Several solvents were investigated including methanol, isopropanol, dimethylformamide, dioxan and a mixture of carbon tetrachloride and acetone. Methanol was found to be the most suitable solvent. It was the only solvent to give both fairly rapid extraction at room temperature and a reproducible calibration curve. Using methanol, quantitative extraction of water from tobacco at room temperature was obtained by allowing a mixture to stand for three hours or by shaking it gently for one hour.

Summary

Water is extracted from tobacco in dry methanol and the intensity of the combination band at 1943 nm, due to the -OH stretching and H-OH bending of the water molecule in the extract, is measured and compared to standard water solutions.

Conclusions

Various methods for the determination of water in tobacco have been examined. In terms of specificity and precision, the method based on the measurement of the absorption of water in the near infra-red (NIR) is to be preferred.

This method is dependent upon an efficient extraction of water from tobacco, and experiments using different solvents and temperatures of extraction indicate that the apparent moisture content varies with the temperature of extraction and can approximate to the value obtained by oven drying at a similar temperature.

It is suggested that the difference in the apparent moisture content for cold and hot extraction is a possible measure of water of "decomposition". This implication is by no means proven and further work with different tobaccos, etc, will be carried out

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