Nicotine content of tobacco leaf estimated by UV spectrum

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Abstract: The spectral data of different grades flue-cured tobacco sample have been determined by UV spectrometer. Their differences were compared. The spectral prediction models of nicotine content were established through correlation analysis between the content of nicotine and spectral data of flue-cured tobacco with the stepwise multivariate regression method. The statistical results showed that the nicotine content of flue-cured tobacco were very significantly correlated to their UV spectrum. There were also very significantly correlations between the predicted and experimental values. This indicates that it would be feasible to estimate the nicotine content in flue-cured tobacco by UV spectroscopy and UV spectroscopy can be used to determine conveniently and quickly nicotine content of flue-cured tobacco leave samples without pollution.

1. Introduction
The nicotine, one of the main chemical compositions in tobacco leaf, has the function of excited stimulation to the human body. Too much nicotine can harm the health of smokers. The near infrared (NIR) technology has been widely applied to rapidly analysis of tobacco chemical composition, tobacco leaf classification, production directions analysis, cigarette real false identification, etc [1-6]. But, the UV spectrum method is relatively less applied to study of tobacco leaf. Applying NIR to forecast cellulose content in the tobacco leaf [7] and flame atomic absorption spectrometer to continuous determination method of Cu, Fe contents in tobacco have been used [8]. UV spectrometer has been applied to measure nicotine content of tobacco leaf [9]. The near infrared reflectance spectroscopy (NIRS) has been used to analyze the protein content in flue-cured tobacco leaves. The mathematical model of NIRS for the protein content in flue-cured tobacco leaves has been established with stoichiometry method [10]. The near infrared spectroscopy has forecasted the tobacco leaf structure and oil [1]. The spectral method can forecast the tobacco leaf nicotine, K and total N, so as to establish spectra prediction models [11]. A mathematical prediction model of Ca in tobacco with FT-NIR spectrometry has been established [12-13]. 4,8,13-duvatriene-1,3-diol diastereoisomers have been identified in the cuticular wax of fresh burley tobacco leaves [14]. A reliable and rapid method
based on high-performance liquid chromatography (HPLC-UV) and positive ion electrospray-time of flight mass spectrometry (ESI-TOF/MS) have been developed for the characterization and quantification of solanesol in extracts of tobacco leaves from different sources [15]. A mathematical model of nicotine in total particulate matter has been established by scanning tobacco power with FT-NIR spectrometry [13]. A new dual-excitation fluorescence light detection and ranging (DE-FLIDAR) has been designed for remote quantitative assessment of epidermal UV absorption of tobacco leaves and canopies from chlorophyll (Chl) fluorescence (ChlF) measurements [16]. Nicotine has been extracted from tobacco by ultrasonic extraction method [17]. Determination of nicotine content in tobacco specifically by a reverse phase HPLC has been established [18]. Based on the linear relationship of nicotine content on the short wave absorption, the difference of different grades of tobacco leaf spectrum was compared, and the nicotine content of tobacco leaf was measured. A method of UV spectroscopy is discussed the feasibility of the determination of the content of nicotine in tobacco in this paper.

2. Materials and methods
The sample of flue-cured tobacco is K326 from the Tobacco Company of Guiyang in Guizhou Province (respectively, top, middle, next three part). The sun-cured tobacco came from market. The spectra and chemical component of samples were measured at Guizhou University.

The UV spectroscopy is American Agilent 8453E UV Spectroscopy system with wavelength from 190 nm to 1100 nm and slit width 1 nm. Before measurement, the tobacco leaf sample was dried to constant weight at 80 °C constant temperature in a constant temperature box. It was ground and sieved through 200 μm mesh. The 4 mg sample was placed in a 10 ml cuvette; which was joined 80% ethanol dissolution and vibrated approximately 1 min to dissolve completely. The solution was washed and filtered to a 25 ml capacitance flask with 80% ethanol. Then, the sample solution was put into quartz vessel to measure the UV spectrum from 190 nm to 1100 nm with Agilent 8543E UV spectroscopy. Before each spectral measurement, the instrument was adjusted with 80% ethanol. The characteristic absorption peak was in the spectrogram wavelength 210~360 nm. The nicotine contents were measured by chemical method.

3. Experimental results and analysis
3.1. The different grade flue-cured tobacco and sun-cured tobacco spectral analysis
In wavelength 210~360 nm, the information of the flue-cured tobacco was very abundant, but signal intensity was quite high. The spectral difference of different grade flue-cured tobacco sample was shown in Figure 1. The spectra of the same leaf position for flue-cured tobacco were also very different. The spectra represent the information of pyridinium, pyrrole, adenine, etc. Different grade sun-cured tobacco sample has different spectrum, showed in Figure 2. This explains what was flue-cured tobacco or sun-cured tobacco, because different grade sample had different nicotine content. The highest absorbance, the most nicotine content the tobacco contains. From Figure 2 and Table 1, we may see differences of nicotine content and spectral curve in different sun-cured tobacco samples. The SY-1 indicated the high nicotine content, while the SY-2 indicates the low nicotine content.
Table 1. Nicotine content of flue-cured tobacco and sun-cured tobacco.

| Tobacco leaf grade | Flue-cured tobacco | Sun-cured tobacco |
|--------------------|--------------------|------------------|
|                    | B3F  | B3L  | C2F  | C3F  | C4F  | X2F  | X2L  | X3F  | X3L  | SY-1 | SY-2 |
| Nicotine content   | 3.01 | 2.00 | 2.68 | 1.81 | 1.83 | 1.77 | 1.75 | 0.84 | 1.33 | 1.23 | 4.47 | 2.55 |
| (10%)              |      |      |      |      |      |      |      |      |      |      |      |      |

3.2. Correlation analysis of ultraviolet spectrum and nicotine content of flue-cured tobacco

It has been separately calculated for correlation coefficient between the spectrum of flue-cured tobacco of different grade and its nicotine content (%). The conclusion indicated that there is a significant correlation between the spectra of flue-cured tobacco of different grades and their nicotine content (%). By using stepwise regression equation in multivariate analysis, the best wavelength band and variable dimension of regression equation were chosen to fit the best mathematical model. The best wavelength band was 210–360 nm, and the best regression equation variable was two variables. From Table 2, the correlation coefficient was 0.8978.

![Figure 1](image1.png)  ![Figure 2](image2.png)

**Figure 1.** Ultraviolet absorption of 10 kinds of different samples flue-cured tobacco.  **Figure 2.** Ultraviolet absorption of 2 kinds of different samples sun-cured tobacco.

| Sample            | Regression model             | $R^2$  | F(=MSR/MSE) |
|-------------------|------------------------------|--------|-------------|
| Flue-cured tobacco| $Y = -2.5365 + 2.9584x_1 - 3.3598x_2$ | 0.8978** | 30.7359     |

Note: ** indicates that $p < 0.01$ significance test has been passed.
Thirteen samples were extracted in flue-cured tobacco leaf samples. This UV spectral examination model was carried out to examine their nicotine content. These values were compared to UV spectral calculated value. The conclusion demonstrated that two sets of data were very identical, showing that UV accuracy approaches the chemical method very much. From Table 3, it can be seen that the nicotine content of flue-cured tobacco can be predicted by multivariate stepwise regression method according to their ultraviolet absorption spectra. The prediction accuracy is more than 90%.

Table 3. Comparison of the predicted value of the ultraviolet absorption spectrum and measured value of nicotine content (%) in flue-cured tobacco.

| Sample number | Measured value | Predicted value | Absolute error | Relative error (%) |
|---------------|----------------|-----------------|----------------|--------------------|
| 1#            | 2.29           | 2.41            | 0.12           | 5.24               |
| 2#            | 2.02           | 2.31            | 0.29           | 14.36              |
| 3#            | 3.64           | 3.57            | 0.07           | 1.92               |
| 4#            | 3.32           | 3.26            | 0.06           | 1.81               |
| 5#            | 3.70           | 3.66            | 0.04           | 1.08               |
| 6#            | 4.66           | 3.91            | 0.75           | 16.09              |
| 7#            | 3.13           | 3.27            | 0.14           | 4.47               |
| 8#            | 2.46           | 2.56            | 0.10           | 4.07               |
| 9#            | 2.84           | 2.94            | 0.10           | 3.52               |
| 10#           | 2.23           | 2.46            | 0.23           | 10.31              |
| 11#           | 1.49           | 1.66            | 0.17           | 11.41              |
| 12#           | 2.47           | 2.47            | 0.00           | 0.00               |
| 13#           | 2.68           | 2.73            | 0.05           | 1.87               |
Absolute error = $|predicted\ value - measured\ value|$, 
Relative error = $\frac{predicted\ value - measured\ value}{measured\ value} \times 100\%$

4. Conclusions
The UV spectroscopic analysis of was developed to set up the spectral examination model of nicotine content of flue-cured tobacco. The results of the model were consistent with the experimental data. UV spectroscopic analysis for nicotine content of flue-cured tobacco sample has advantages of simple operating and quick survey speed for massive samples.

The spectral detection model proposed in this paper can accurately predict the nicotine content in flue-cured tobacco, but it requires more samples to improve its accuracy.

Acknowledgments
This work was supported by the National Natural Science Foundation of China (Grant numbers 11164004), Guizhou industrial breakthrough project (Grant numbers GY [2012]3060) and Nomarch Foundation of Guizhou Province.

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4th International Conference on Agricultural and Biological Sciences IOP Publishing
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