

# Development and Application of Portable Reflectometric Spectroscopy Combined with Solid-Phase Extraction for Determination of Potassium in Flue-Cured Tobacco Leaves

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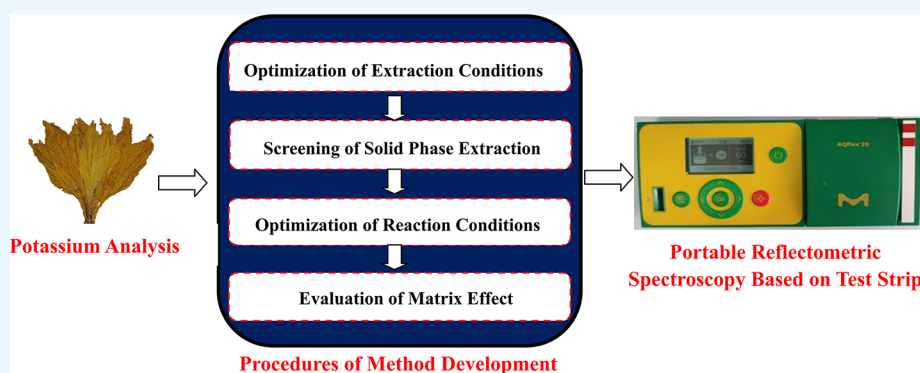
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**ABSTRACT:** Potassium (K) plays important roles in the energy and substance conversion of tobacco metabolism and is also regarded as one of the important indicators of tobacco quality evaluation. However, the K quantitative analytical method shows poor performance in terms of being easy-to-use, cost-effective, and portable. Here, we developed a rapid and simple method for the determination of K content in flue-cured tobacco leaves, including water extraction with 100 °C heating, purification with solid-phase extraction (SPE), and analysis with portable reflectometric spectroscopy based on K test strips. The method development consisted of optimization of the extraction and test strip reaction conditions, screening of SPE sorbent materials, and evaluation of the matrix effect. Under the optimum conditions, good linearity was observed in 0.20–0.90 mg/mL with a correlation coefficient >0.999. The extraction recoveries were found to be in the range of 98.0–99.5% with a repeatability and reproducibility of 1.15–1.98% and 2.04–3.26%, respectively. The sample measured range was calculated to be 0.76–3.68% K. Excellent agreement was found in accuracy between the developed reflectometric spectroscopy method and the standard method. The developed method was applied to analyze the K content in different cultivars, and the content varied greatly among the samples with lowest and highest contents for Y28 and Guiyan 5 cultivars, respectively. This study can provide a reliable approach for K analysis, which may become available on-site in a quick on-farm test.

## 1. INTRODUCTION

Potassium (K) is a necessary nutrient element and one of the three core macronutrients for tobacco growth. This element is very active in plants and plays important roles in the energy and substance conversion of tobacco metabolism.<sup>1</sup> The processes of osmoregulation, photosynthesis, enzyme activation, and the formation of carbohydrates, nucleic acids, and proteins are associated with K. For example, K promotes photosynthetic performance, which leads to increased metabolite and carbohydrate formation. K can strengthen the plant's resistance to diseases, pests, and frost, and severe potassium deficiency results in necrosis of the leaf edge.<sup>2</sup> The K content can also highly affect the quality of tobacco leaves, such as color, grade, body, texture, elasticity, fire-holding capacity, oil content, and aroma intensity.<sup>3,4</sup> For example, as

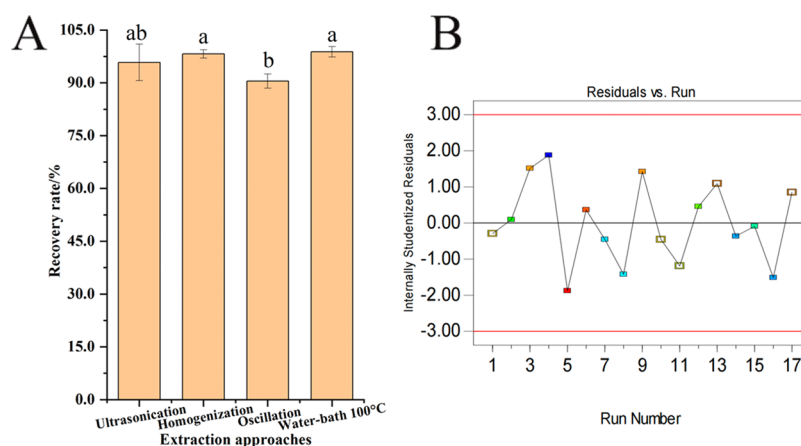
the K content increases, the sugar content in the leaves also increases, while the nicotine content decreases. This results in an optimal sugar/alkaloid ratio of 6–10, with 10 being the best.<sup>5</sup> A high sugar/alkaloid ratio is associated with mildness, whereas a low sugar/alkaloid ratio is associated with harshness.<sup>6</sup> Overall, tobacco as a special food is widely cultivated and consumed in China. K is regarded as an

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**Figure 1.** (A) Effect of extraction approaches on the recovery rate for K in flue-cured tobacco; different superscript letters denote significant differences ( $p < 0.05$ ); (B) RSM-BBD normal plot of studentized residual versus run number for the K extraction recovery.

important influencing factor of quality and yield. Hence, monitoring its content becomes very important to diagnose the quality of tobacco leaves and guide the rational use of potassium fertilizers.

To date, numerous studies for selective and sensitive K assays have been reported. These methods mainly include electrochemical, colorimetric detection, fluorescent, spectroscopic, and chromatographic methods.<sup>7–11</sup> Although most of them show high sensitivity, the complicated instrumentation limits the scope of their practical application.<sup>12</sup> These methods are only applicable to simple matrices (water, urine, and serum) and matrix effects are not evaluated properly.<sup>13</sup> In the analysis of tobacco K, ion chromatography-suppressed conductivity detectors, flow analysis-flame photometric detectors, inductively coupled plasma-optical emission spectroscopy, laser-induced breakdown spectroscopy, and instrumental neutron activation analysis are mainly used.<sup>14–17</sup> However, the easy-to-use, cost-effective, and portable nature of these instruments shows poor performance. The metrological parameters of the above methods are shown in Table S1. Therefore, it is necessary to develop a simple and accurate method to measure the K content in flue-cured tobacco leaves.

The reflectoquant analysis system allows ion (K, calcium, nitrate, ammonium, etc.) or organic (hydroxymethylfurfural, lactic acid, sucrose, ascorbic acid, etc.) concentrations to be determined quantitatively using specific test strips and a small portable reflectometric spectroscope, the RQflex.<sup>18–21</sup> The K-sensitive test strips are based on dipicrylamine to form an orange-colored complex. The principle is that the colored substance absorbs light at a certain wavelength, the light is reflected at the phase limit, and the portion of reflected light is measured with reflectometric spectroscopy.<sup>22</sup> This method has the following advantages: (1) high level of accuracy and precision; (2) easy-to-use, cost-effective, and portable instrumentation; (3) fast quantitative results in many cases; and (4) no waste and environmental issues and biodegradable test strips. However, the matrices are relatively simple in method application, such as water, soil, bee honey, etc.<sup>23–25</sup> The applicability of reflectometric spectroscopy in complex matrices deserves further study.

In the present study, the purpose of this study was to develop an easy-to-use, cost-effective, and accurate method for the rapid and simple quantification of K with the combination of a test strip and reflectometric spectroscopy in flue-cured

tobacco leaves. There were four main aims to (1) optimize the conditions of extraction in flue-cured tobacco and reaction in test strips, (2) screen suitable solid-phase extraction (SPE) sorbent materials for purification, (3) evaluate the matrix effect in complex matrices, and (4) compare with the reflectometric spectroscopy and classical continuous flow-flame photometry method and then apply for determination of K in different flue-cured tobacco cultivars.

## 2. RESULTS AND DISCUSSION

### 2.1. Optimization of K Extraction Conditions with Box–Behnken Design.

The minerals and trace elements in plant tissue are usually determined following wet digestion or high temperature (450 °C) ashing techniques involving digestion with single acid, triacid, or diacid mixtures (HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, and HClO<sub>4</sub>) or heating dissolution with HCl, but these methods are often costly, relatively dangerous, or time consuming due to the use of strong acid, large amounts of dry sample.<sup>26,27</sup> K in plant tissue is not bound to organic compounds and occurs in soluble forms, thus indicating the ease of its extractability.<sup>27</sup> Recently, a nondigestion extraction method was proposed for the estimation of plant K content using solution extraction with water, acetic acid, CaCl<sub>2</sub>, salt EDTA, or ammonium/magnesium acetate.<sup>28</sup> Compared with digestion and ashing techniques, more sample pretreatment can be handled in the same amount of time. Because the reaction of test strips is often disturbed by a high content of cations or anions, we chose water as the extraction solution to reduce the mask effect of the extraction agent. Furthermore, the influencing parameters, including different extraction approaches, liquid-to-material ratios, extraction temperature, and extraction time, were discussed. The experiments were performed in triplicate in several extraction approaches, i.e., ultrasonication; homogenization; oscillation; and water-bath 100 °C heating. Figure 1A indicates that water-bath heating and homogenization provided the maximum recovery rate. This is attributed to the greater mass transfer efficiency for K in these two extraction approaches. However, homogenization requires more sophisticated instruments. Hence, water-bath heating was selected for further optimization. The water extraction method is simple, rapid, and eco-friendly and virtually precludes the need for costly chemicals and equipment.

To further improve K extraction recovery, a multivariate strategy based on response surface methodology with a three-factor, three-level Box–Behnken design (BBD) was further employed to optimize important water-bath heating conditions, such as liquid-to-material ratio/w/w (A), extraction temperature/°C (B), and extraction time/min (C). The BBD is a class of rotatable or nearly rotatable second-order response surface designs based on a three-level incomplete factorial design.<sup>29</sup> An advantage of BBD over other response surface designs is that it is more efficient, does not have axial points, and may be experimentally more convenient and less expensive. The BBD of coded variables and uncoded variables and the response factors of extraction recovery are presented in Table 1. The coded variables were set as  $-1$ ,  $0$ ,  $+1$  and

**Table 1. Box–Behnken Design: Coded Variables, Real Variables, and Response Factors with K Extraction Recovery**

Nos	real-uncoded (coded) variables			response factors with extraction recovery/%
	A/w/w	B/°C	C/min	
1	42.5(0)	70(0)	10(0)	96.1
2	25.0(-1)	100(1)	10(0)	89.5
3	60.0(1)	70(0)	15(1)	97.5
4	25.0(-1)	40(-1)	10(0)	79.1
5	60.0(1)	100(1)	10(0)	101.1
6	42.5(0)	100(1)	15(1)	98.9
7	25.0(-1)	70(0)	15(1)	83.8
8	42.5(0)	40(-1)	15(1)	84.2
9	42.5(0)	100(1)	5(-1)	97.8
10	42.5(0)	70(0)	10(0)	95.9
11	42.5(0)	70(0)	10(0)	95.0
12	60.0(1)	70(0)	5(-1)	93.2
13	42.5(0)	70(0)	10(0)	97.8
14	42.5(0)	40(-1)	5(-1)	82.6
15	60.0(1)	40(-1)	10(0)	86.5
16	25.0(-1)	70(0)	5(-1)	82.5
17	42.5(0)	70(0)	10(0)	97.5
ANOVA	model <i>F</i> and <i>p</i> -value			47.06 and <0.0001
	lack of fit <i>p</i> -value			0.274
	coefficient of variation %			1.49%
	<i>R</i> <sup>2</sup>			0.984

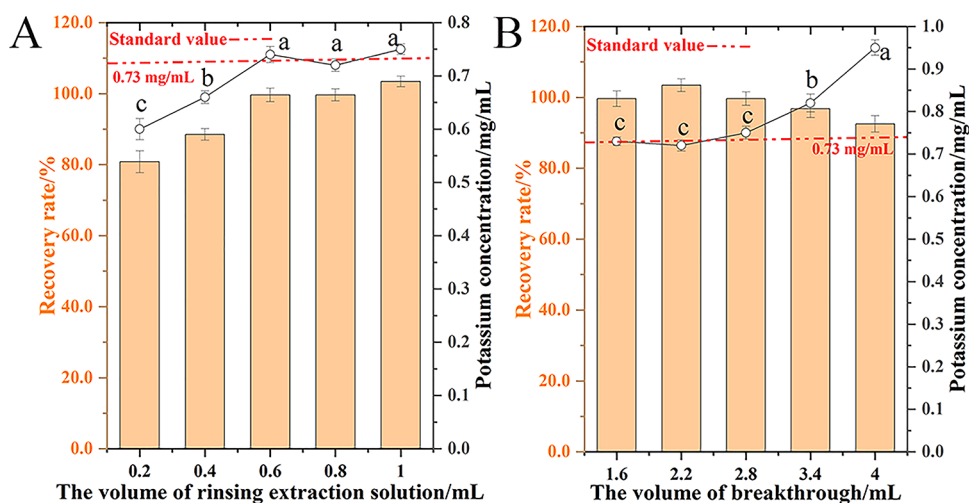
corresponded with the uncoded variables of A ( $-1 = 25$ ,  $0 = 42.5$ ,  $+1 = 60$ ), B ( $-1 = 40$ ,  $0 = 70$ ,  $+1 = 100$  °C), and C ( $-1 = 5$ ,  $0 = 10$ ,  $+1 = 15$  min). To evaluate how well the model fits the experimental results, the BBD was evaluated at a 5% level of significance and validated using analysis of variance (ANOVA), as shown in Table 1. The results showed that the model of K extraction recovery was highly significant, with *F* value = 47.06 and *p*-value < 0.0001. The coefficient of determination (*R*<sup>2</sup>) was 0.984. A lack of fit *p*-value = 0.274 greater than 0.05 indicated that the lack of fit was not significantly associated with the pure error. Additionally, a smaller coefficient of variation (CV) = 1.49% indicated that each model was reproducible. All these statistical parameters show the reliability of this model. To further predict the optimum conditions, the variables were set according to our specific requirements. The goals for variables of A, B, and C were set as in range, i.e., B and C were from the  $-1$  to  $+1$  level. Because the sensitivity of the method decreases with the increasing liquid-to-material ratio, the variables of A ranged from  $-1$  to  $0$ . The goal for K extraction recovery was set to maximize because the maximum value is the aim. Then, a number of solutions were generated by Design Expert software. The results showed that the highest K extraction recovery was 98.9%, and the optimum conditions were a liquid-to-material ratio of 40.0, an extraction temperature of 100 °C, and an extraction time of 10.75 min. The extraction recovery depends on plant tissue resistance in the form of a physical barrier to K release.<sup>27</sup> Heating extraction with high temperature can soften tobacco matrices and increase the solubility of K salt to improve extraction recovery.<sup>30</sup> The optimum conditions were round off for simplicity of operation, and the optimum extraction conditions were 40, 100 °C, and 11 min. Three experiments were performed to validate this result. The extraction recovery reached 99.5% ± 1.35%, which showed no significant difference between theoretical extraction recoveries. This model was adequate for reflecting the expected optimization. Figure 1B illustrates the curves of studentized residual versus run number for the K extraction recovery with the BBD model. The residual show a random scattering nearby zero with a change of less than ±2.0. This outcome also proves a satisfactory statistical model.

**2.2. Optimization of SPE Procedures.** The dark yellow aqueous extract solution of K in flue-cured tobacco may affect the quantitative detection of reflectometric spectroscopy. To

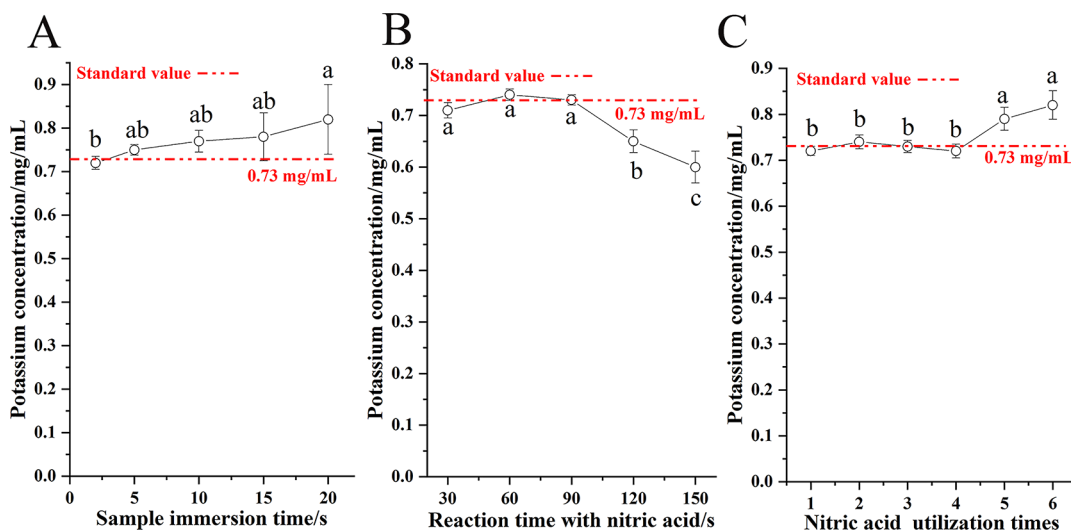
**Table 2. Comparison of Different SPE Sorbent Materials with Purification Effect and Extraction Efficiency**

SPE cartridge	AC 250 mg/3 mL	C18 500 mg/3 mL	CARB 250 mg/3 mL	CARB 500 mg/6 mL	NH <sub>2</sub> 500 mg/3 mL	PSA 500 mg/3 mL	CARB/NH <sub>2</sub> 500/500 mg/6 mL	CARB/PSA 500/500 mg/6 mL
type of sorbent <sup>a</sup>	AC	C18	GCB	GCB	NH <sub>2</sub>	PSA	GCB/NH <sub>2</sub>	GCB/PSA
solution color	orange	dark orange	colorless	colorless	dark yellow-green	yellow-green	colorless	colorless
standard reference value/mg/mL					0.73			
spiked content/ mg					0.50			
measured value in sample/mg/ mL	0.85	0.95	0.74	0.72	0.91	0.88	0.70	0.72
measured value after spiked/ mg/mL	1.07	1.15	1.00	0.98	1.11	1.09	0.95	0.96
recovery rate	85.1%	77.7%	99.7%	99.7%	77.7%	81.4%	96.1%	99.7%

<sup>a</sup>AC, activated carbon; C18, octadecyl silane; GCB, graphitized carbon black; NH<sub>2</sub>, aminopropyl silane; PSA, *N*-propyl ethylenediamine.



**Figure 2.** Effect of the volume of rinsing extraction solution (A) and breakthrough (B) on the recovery rate and K concentration in SPE purification. Different superscript letters denote significant differences ( $p < 0.05$ ).



**Figure 3.** Effect of sample immersion time (A), reaction time with nitric acid (B) and nitric acid utilization times (C) on K concentration in test strip reaction conditions. Different superscript letters denote significant differences ( $p < 0.05$ ).

eliminate the matrix interference to the maximum extent, herein, SPE is a widely used technique for purifying impurities.<sup>31</sup> Optimization of the SPE procedures was performed with the aim of achieving satisfactory recoveries. Different parameters that affect the recovery (type of sorbent, volume of rinsing solvent, and breakthrough volume) were studied. In the SPE procedure, sorbent materials are primarily important influencing factors for extraction and purification.<sup>32</sup> First, the extraction efficiency of seven cartridges (AC, C18, CARB, NH<sub>2</sub>, PSA, CARB/NH<sub>2</sub>, and CARB/PSA) was tested using the 2.5 mL aqueous extract solution spiked with 0.50 mg K. As shown in Table 2, the maximum recovery efficiency was obtained by using CARB, CARB/NH<sub>2</sub>, and CARB/PSA. These sorbent materials have no adsorption capacity for K and are mainly used to remove pigments, organic compounds, and partial inorganic ions.<sup>33</sup> In addition, high recovery in CARB/PSA and CARB/NH<sub>2</sub> also demonstrated that PSA and NH<sub>2</sub> have no adsorption capacity for K. It is possible that the low recovery of AC, C18, PSA, and NH<sub>2</sub> is due to the poor adsorption to matrix interference. The solution color also showed that the pigments cannot be removed completely with

AC, C18, PSA, and NH<sub>2</sub>, as shown in Table 2. Although the recovery is low, the measured value of the standard sample is high. The color of the reaction product between hexanitrodiphenylamine and potassium is orange-yellow. The pigments may increase the color background values and then cause high measured results. This result was also verified by adding the pigment lutein to the potassium standard, which showed a 38% increase in the measured value. This indicates that the influence of tobacco solution color on the assay is greater than the matrix effect. Considering simplification and cost reduction, CARB 250 mg/3 mL was chosen.

The volume of rinsing extraction solution and breakthrough of the cartridges were further optimized. As shown in Figure 2A, the volumes of rinsing extraction solution were 0.2, 0.4, 0.6, 0.8, and 1 mL respectively. A high recovery rate was obtained between 0.6 and 1.0 mL. The dilution effect of residual water resulted in a lower recovery rate between 0.2 and 0.4 mL. As shown in Figure 2B, the breakthrough volumes were 1.6, 2.2, 2.8, 3.4, and 4.0 mL, respectively. The measured value significantly increased between 2.8 and 4.0 mL. This may be due to pigment breakthrough resulting in an increase in the

**Table 3. Recovery, Repeatability, and Reproducibility of K Analysis in Flue-Cured Tobacco Leaves with the Developed Method**

K	content/%	added/%	found/%	recovery	repeatability	reproducibility
low spiked	2.45%	1.00%	3.43%	98.0%	1.15%	2.04%
high spiked	0.95%	2.00%	2.94%	99.5%	1.98%	3.26%

measured value. In consideration of reducing extraction solution consumption, the volume of rinsing extraction solution and breakthrough for 0.6 and 2.8 mL was chosen.

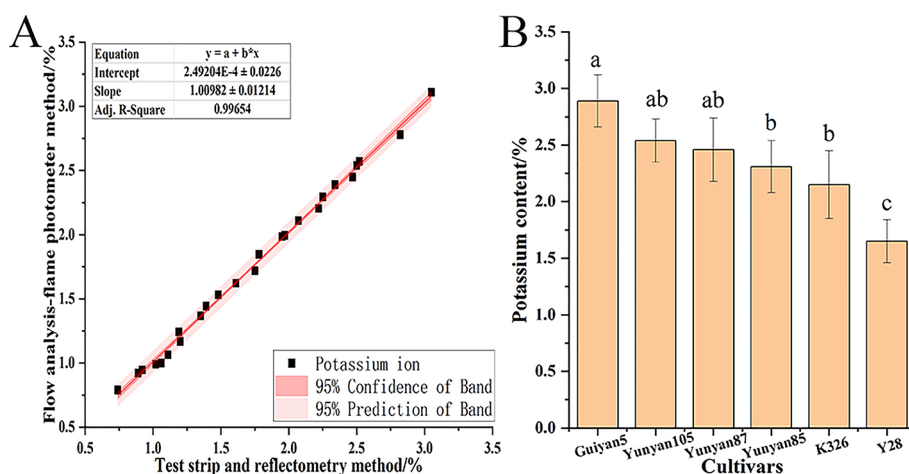
**2.3. Optimization of Test Strip Reaction Conditions.** The principle of test strips is K in the sample react with the hexanitrodiphenylamine to generate an orange-red potassium salt, and then the hexanitrodiphenylamine reacts with acid to turn pale yellow, while the potassium hexanitrodiphenylamine salt preserves the orange color.<sup>22</sup> Hence, the K concentration is directly proportional to the depth of the orange-red color.<sup>34</sup> Although reaction times, etc., in aqueous matrices are described in the instructions for use, the complex matrices need to optimize the sample immersion time, reaction time with K-1 reagent solution (HNO<sub>3</sub>) and the number of K-1 reagent solution utilization to obtain optimum conditions. Figure 3A shows that the sample immersion time was optimized at 2, 5, 10, 15, and 20 s. As the immersion time in the sample solution increased, the measured value tended to increase slightly, and the deviation increased significantly. Figure 3B shows that the reaction time was tested at 30, 60, 90, 120, and 150 s. As the reaction time with the K-1 reagent solution increased, the measured value slightly increased and then significantly decreased. This result may be attributed to the fact that the hexanitrodiphenylamine fixed on the test strip was dissolved during the longer reaction. Figure 3C shows that the number of K-1 reagent solution utilizations were performed with 1, 2, 3, 4, 5, and 6-times. The measured value of the fifth utilization significantly increased, probably because the reaction solution decreases the acidity, which prevented hexanitrodiphenylamine from changing to a light yellow color. In summary, the sample immersion time, reaction time and number of K-1 reagent solutions utilization were selected as 2–5 s, 60 s and 4-times, respectively.

**2.4. Matrix Effect Evaluation.** Matrix effects often occur in spectrum and chromatography when targeted compounds are analyzed in complex sample matrices.<sup>35</sup> Although invisible in the reflectometric spectroscopy, they will have deleterious impacts on the accuracy and sensitivity of the determination. Many methods have been used to reduce matrix effects, such as diluting the samples, sample cleanup, internal standard calibration, injecting smaller volumes and optimization of chromatographic parameters.<sup>36</sup> The decreased matrix effect in the reflectometric spectroscopy is only applicable to sample dilution and purification. In this paper, to ensure sensitivity, SPE purification coupled with graphitized carbon black (GCB) was used to reduce matrix effects. The potassium standard was spiked into the extraction solution and deionized water. The matrix effects were calculated using the following equation: matrix effect % =  $\{[(\text{matrix-blank})/\text{water}] \times 100\}$ , where [matrix] is the measured value in the spiked extraction solution, [blank] is the measured value in the non-spiked extraction solution, and [water] is the measured value in the spiked water. A matrix effect of 100% means that no matrix effects are present, while a ratio above or below 100% means matrix enhancement effect or suppression effect, respectively.<sup>37</sup> The matrix effect was evaluated with an extraction solution

before and after purification with CARB SPE. The experimental results showed that the matrix effect before purification was a significant suppression effect with a ratio of 72.5%, while the matrix effect after purification was a slight suppression effect with a ratio of 98.6%. These results demonstrated that the matrix effect can be ignored after purification. The CARB SPE with GCB sorbent material has strong adsorption capacity, which can absorb not only pigments but also partial metal ions and water-soluble organic compounds such as nicotine, polyphenols, sugars, and other water-soluble substances in tobacco.<sup>38</sup> Low-matrix effects also indicate no interference for unadsorbed metal ions. It has been clearly stated that the K test strip has a very strong anti-interference ability to common anions and cations in the instructions, which can reach 1000 mg/L. Based on the above data, matrix effects can be ignored after CARB SPE cleanup, and a solvent matched-calibration curve could be used to quantify real samples.

**2.5. Method Validation with Analytical Parameters.** The developed method was validated with linearity (external standard calibration curve), sensitivity (measuring range, limit of quantification), accuracy (extraction recovery), and precision (repeatability and reproducibility) under optimum conditions. The measured range of K test strips was between 0.25 and 1.20 mg/mL in the instructions. The solvent matched-calibration curves were constructed using weighted (1/X) least-squares linear regression models, by plotting the measured concentration (y) versus the gradient real concentration (x). Linearity was evaluated using regression analysis in the 0.20–0.90 mg/mL and was expressed as the correlation coefficient (R). The linear curve was  $y = 1.364x - 0.01199$  with  $R = 0.9992$ . The measured range was corrected by a linear curve to 0.19–0.92 mg/mL. The sample measured range was calculated as 0.76–3.68% K based on the sample weight, dilution ratio of the sample and recoveries and the limit of quantification was 0.76%. These measured K values covered the major range of tobacco leaf tissue K contents (0.50–3.88%) reported in the literature.<sup>27</sup> If few samples are below or above the range, the extract should be concentrated or diluted.

Accuracy was evaluated through the recovery (%) of six replicate samples spiked with low- or high-levels of the analytes in high or low content samples. The standard K solution was spiked, incubated overnight at 4 °C, and then extracted. Recovery % =  $\{[(\text{Concentration of the spiked sample} - \text{Concentration of the unspiked sample})/\text{Spiked concentration}] \times 100\}$ . The precision was evaluated through repeatability and reproducibility. Repeatability (within-day precision) was calculated by analyzing five independent samples within a single day, while reproducibility (between-day precision) was calculated by analyzing five independent samples over three consecutive days. Table 3 shows that the mean recoveries for K in the flue-cured tobacco ranged from 98.0 to 99.5%. The repeatability and reproducibility varied from 1.15 to 1.98% and 2.04 to 3.26%, respectively. It is evident that the proposed water extraction following the test strips and reflectometric spectroscopy method proved high accuracy and precision,



**Figure 4.** (A) Relationship analysis between the test strip-reflectometric spectroscopy method and classical flow analysis-flame photometer method; (B) comparison of K content in different cultivars. Different superscript letters denote significant differences ( $p < 0.05$ ).

which meets the requirements of the International Council for Harmonization (ICH) guidelines.<sup>39</sup>

**2.6. Method Comparison and Application.** To further verify the accuracy, the developed method needs to prove the comparability across standard methods (YC/T 217-2007) using the same samples. Linear relationships of the developed method with standard methods for K content in flue-cured tobacco are depicted in Figure 4A. The K content obtained with the reflectometric spectroscopy method was significantly correlated ( $p < 0.01$ ) with the flow analysis-flame photometric detector method with Pearson  $R = 0.998$ . The linear regression equation was  $y = 1.0098x + 0.000249$  with  $R^2 = 0.996$ , showing comparable accuracy, following the 1:1 line. Similarly, researchers have compared reflectometric spectroscopy RQflex10 with ion chromatography and high performance liquid chromatography methods for nitrate and 5-hydroxymethylfurfural content analysis. The results indicated that the  $R^2$  between the two methods for nitrate was 0.996, and RQflex 10 was found to be more accurate.<sup>40</sup> Vrzal demonstrated that HPLC can be replaced by reflectometry for 5-hydroxymethylfurfural content analysis with a  $R^2$  of 0.912.<sup>20</sup> All these results indicated that the reflectometric spectroscopy RQflex 20 is comparable in accuracy with other methods. Furthermore, the RQflex instrument was easy-to-use, cost-effective and portable and may become available on-site in a quick on-farm test. The developed method was further applied to analyze the K content of different cultivars. Flue-cured cultivars with different genetic backgrounds are an important inherent factor that affects the K content. Figure 4B shows that the content of K varied greatly among the six cultivars. The content followed the order Guiyan5 > Yunyan105 > Yunyan87 > Yunyan85 > K326 > Y28. Previous studies have shown that Yunyan 5 is a high potassium-rich cultivar, while Y28 is a hybrid F1 generation of flue- and sun-cured tobacco with a relatively low potassium enrichment ability.<sup>41,42</sup> Potassium deficiency is a common problem in China's major tobacco growing industry, and the content of K in leaf tissue is a key factor that influences the yield and quality of tobacco.<sup>4</sup> Therefore, the developed method can be routinely used to monitor the K status of crops to develop an appropriate fertilization strategy and assess the leaf quality.

### 3. CONCLUSIONS

This study presents a water extraction, CARB SPE purification followed by test strips and a reflectometric spectroscopy method for accurate and precise quantitation of the K content in flue-cured tobacco leaves. Water extraction was optimized with BBD and serves as an inexpensive alternative to the most commonly used standard triacid digestion method for routine analysis. The purification with GCB sorbent materials can completely remove the matrix effect and pigment interference. The optimum conditions were suitable to match the complex flue-cured tobacco matrices. The method was fully validated using analytical parameters and was found to have excellent performance, with excellent accuracy, outstanding precision and minimal matrix effect. The sample analysis further demonstrated that this developed method is comparable in accuracy to the standard method. The developed method serves as a simple, accurate, cost-effective, and eco-friendly alternative and may become available on-site in a quick on-farm test. However, further investigations are needed to assess the suitability of this method for determining the K content in diverse matrices, including the plant materials, industrial foods, etc.

### 4. MATERIALS AND METHODS

**4.1. Chemicals and Reagents.** All chemicals were of analytical grade unless otherwise indicated. Potassium nitrate (purity  $\geq 99.0\%$ ) was purchased from Sinopharm (Beijing, China). Lutein (HPLC  $\geq 90\%$ ) and methanol (HPLC grade) were purchased from Solarbio (Beijing, China) and Fisher Chemicals (Loughborough, UK). Ultrapure water was produced by a Milli-Q purification system. The SPE cartridge ProElut AC 250 mg/3 mL, ProElut C18 500 mg/3 mL, ProElut CARB 250 mg/3 mL, ProElut CARB 500 mg/6 mL, ProElut NH<sub>2</sub> 500 mg/3 mL, ProElut PSA 500 mg/3 mL, ProElut CARB/NH<sub>2</sub> 500/500 mg/6 mL and ProElut CARB/PSA 500/500 mg/6 mL were purchased from Dikma (Beijing, China).

**4.2. Preparation of Standard Solutions and Samples.** A total of 207.2 mg of potassium nitrate was weighed, and standard stock solutions were prepared at 2.072 mg/mL with ultrapure water, which was equal to 0.80 mg/mL potassium. Working standard solutions were prepared freshly before use by transferring 2.5, 4, 5.5, 7, and 8.5 mL in a 10 mL volumetric

flask and then diluting with water. The concentrations of potassium were 0.20, 0.32, 0.44, 0.56, and 0.68 mg/mL. To compare the accuracy between the reflectometric spectroscopy method and the classical standard method, a total of 25 flue-cured tobacco samples representing different K contents were selected. These samples were expected to have a broad range of K concentrations, which included relatively low (<1%), medium (1–2%), and high (>2%) K contents. Furthermore, six cultivars (Guiyan5, Yunyan105, Yunyan87, Yunyan85, K326, and Y28) with different potassium levels were selected for analysis. These were grown in field plots at a tobacco planting base in Anshun city, Guizhou province. The field design was a completely randomized block with each cultivar. Seedlings were grown in a greenhouse, transplanted to the field on April 20, 2021, and subsequently grown according to standard tobacco cultivation protocols. The mature leaves (leaf position: from bottom to top in 11th leaves) were later harvested and cured in a barn with circulating heated air. All flue-cured tobacco samples were oven-dried at 60 °C for 48 h and finely ground to pass through a 0.5-mm sieve prior to analysis. The standard reference sample (cultivar and grade: Yunyan87 & C3F) was used for the experimental optimization and the method validation, which was kindly gifted from the Zhengzhou Tobacco Research Institute. The potassium content (2.45% w/w) was certified with CANS testing and certification laboratories.

**4.3. Extraction and Cleanup of K in Flue-Cured Tobacco.** The flue-cured tobacco samples (100 mg) were weighed into a 10 mL glass centrifuge tube, and then 4 mL of ultrapure water was added for extraction. After shaking the tube in a Vortex 1 mixer (IKA, Staufen, Germany) at 4000 rpm for 30 s, the extraction was performed with water-bath heating at 100 °C for 11 min. The sample was further vortexed at 4000 rpm for 30 s and then centrifuged at 3000 rpm for 5 min. The supernatant was further purified using ProElut CARB SPE. The GCB SPE was preconditioned sequentially with 5 mL of methanol and 5 mL of water at a flow rate of 5 mL/min. Then, 2.5 mL (less than 2.8 mL breakthrough volume) of supernatant was loaded onto the cartridges at a flow rate of 2 mL/min. Approximately 0.6 mL of solution was discarded, and then the residual solution was collected in a 2 mL plastic centrifuge tube. The elute was analyzed with reflectometric spectroscopy.

**4.4. Reflectometric Spectroscopy Conditions and Calculation.** The quantitative analysis was carried out with a RQflex (RQflex 20, Merck Company, Darmstadt, Germany), which is a portable and small handheld reflectometric spectroscope. RQflex 20 is powered on and warmed up for 10 min. Following the manufacturer's instructions, the bar-code strip was used for calibration. First, both the reaction zones of the test strips were immersed in the sample solution for 2–5 s. The excess liquid was allowed to run off via the long edge of the strip onto an absorbent paper towel. Then, the test strips were immersed in K–1 reagent solution (HNO<sub>3</sub>) for 60 s, and excess liquid was also allowed to run off. Immediately, the test strips were inserted into the strip adapter with reaction zones facing the instrumental display. The measured value of the potassium concentration was displayed in mg/mL. The K–1 reagent solution can be reused four-times. The RQflex 20 was cleaned and recalibrated every 20 samples. The K concentration of the extract solution was obtained with the solvent matched-calibration curves. The K content in flue-cured tobacco was calculated according to

the following equation:  $C (\%) = (C_e \times V_e) / W$ , where  $C_e$  is the potassium concentration in the extraction solution (mg/mL),  $V_e$  is the volume of the extraction solution (mL), and  $W$  is the weight of the flue-cured tobacco (mg).

**4.5. Flow Analysis-Flame Photometric Detector Conditions.** The K content of flue-cured tobacco was analyzed by the classical standard method. Briefly, 0.25 g of flue-cured tobacco was subjected to 25 mL 5% acetic acid solution for 30 min oscillation extraction. The extraction solution was analyzed with a flow analysis-flame photometric detector. All instrument parameters and analytical methods can be found in standard of YC/T 217-2007.

**4.6. Statistical Analysis.** The extraction recovery and K standard concentration were used as the optimization criteria in the certified standard reference sample. ANOVA of BBD was conducted with Design-Expert version 8.0.6 software using response surface methodology (Stat-Ease Inc., Minneapolis, MN, USA). ANOVA was performed with the response surface quadratic model to justify the adequacy. To measure how well the proposed model fit the experimental data, parameters such as model  $F$ - and  $p$ -values, lack of fit, CV, and  $R^2$  were used for evaluation. The model  $p$ -value and CV less than 0.05 and 10%, lack of fit and  $R^2$  greater than 0.05 and 0.90 showed that a reliable model was obtained. Student's  $t$  test with Fisher's least significant difference test was applied with  $p < 0.05$  among different conditions using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Pearson correlations and simple linear regressions were performed to evaluate the relationship between the K contents determined by the reflectometric spectroscopy method and classical standard methods. All diagrams were plotted with Origin 2021 (OriginLab Corp., Northampton, MA, USA).

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c01326>.

The comparison of metrological parameters for the traditional determination method of K ([PDF](#))

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### Notes

The authors declare no competing financial interest.

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