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Simultaneous extraction of nicotine and solanesol from waste tobacco materials by the column chromatographic extraction method and their separation and purification

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ABSTRACT

The tobacco (*Nicotiana tabacum* L.) cultivation and cigarettes manufacture industries discard huge amount of waste tobacco materials that have strong nicotine smell and contaminate the environment. A high-efficient procedure was developed for simultaneously extracting nicotine and solanesol from the waste tobacco leaf vein through the column-chromatographic extraction (CCE), followed by automatically separating and simply purifying them. Dried material powder was loaded into columns and eluted with the optimal extraction solvent of petroleum ether (PE):95% alkali ethanol (4:6). Greater than 96% extraction efficiency for both nicotine and solanesol was obtained with a 2-fold excess solvent of the material (v/w) through a cyclic CCE procedure in small-scale and scaled-up experiments. The extraction solution was separated into an ethanol-aqueous phase containing 98% nicotine and an ether phase containing 96% solanesol at pH 2.0. The ethanol-aqueous phase was vacuum-concentrated to aqueous, and 99% purity nicotine was obtained by ether fractionation of the aqueous at pH 10.0. Solanesol in the ether phase was purified to 93.1% by one time silica gel column chromatography. All processes were completed at room temperature and all solvents used were completely recovered for reuse. This work provides an extensively simplified procedure to economically utilize the tobacco wastes.

1. Introduction

Tobacco (*Nicotiana tabacum* L.) is one of the most widely planted economic crops. China is the largest tobacco producer and consumer in the world with an annual tobacco production reached 400–500 million tons. In the tobacco cultivation and cigarette manufacture industries, more than 200 million tons of waste tobacco materials are produced annually. The wasters, including stem, leaf vein and roots of tobacco plants, low grade and defective tobacco leaves, have strong smell and cause serious environmental contamination [1]. The tobacco wastes contain high amount of nicotine and solanesol and are valuable resources for the extraction of the two bioactive compounds [2]. After extraction of nicotine and solanesol, the plant residues can be used for multiple purposes, such as fiberboards, pulps and organic fertilizers.

Nicotine is widely used in fine chemical, pharmaceutical and agriculture industries, and in the tobacco industry itself as an essential cigarette additive. Nicotine is reported to improve the health conditions of patients with diseases of dementia [3] and schizophrenia [4], dopaminergic neurons and axons [5], skin mild cognitive dysfunction [6], levodopa induced dyskinesia [7], and to reduce the intake of harmful substances of smokers in nicotine aided smoking cessation [8]. Nicotine has antimicrobial [2] and insecticidal activities and has been used as a natural insecticide with characteristics of easily degradable, non-toxic to human being and no environmental pollution [9]. Solanesol has useful medicinal properties and possesses anti-oxidant, anti-bacterial, anti-inflammation, and anti-ulcer activities [10]. Industrially, solanesol is used by the pharmaceutical industry as an intermediate in the synthesis of metabolically active quinones such as coenzyme Q10 and vitamin K analogues. The demand for solanesol continues to escalate since coenzyme Q10 entered the market as a dietary supplement [10].

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Tobacco is the main natural source for the isolation of nicotine and solanesol. Many protocols have been published for the extraction of nicotine and solanesol from tobacco leaves and wastes, which includes heat reflux extraction [11], solid phase extraction [12], ultrasound-assisted [11,13] or microwave-assisted extractions [14,15], and supercritical fluid extraction [16]. However, these reports are mainly for the extraction of a single compound. They need heating, or large volumes of organic solvents for the extraction. The separation and purification of solanesol used more than one time of chromatographic steps, including silica gel chromatography [17,18], Bio-Beads or Sephadex LH-20 gel chromatography [19], counter current chromatography [20], or their combinations. These methods are not cost-effective for comprehensive utilizing the tobacco wastes.

We have reported a highly efficient column-chromatographic extraction (CCE) method for the extraction of natural compounds from biological materials [21–23]. In this work, we report a unique procedure for simultaneously extracting nicotine and solanesol and hydrolyzing bound forms or ester forms of nicotine and solanesol using a minimum volume of the optimal solvent, followed by automatic separation and simple purification to economically produce highly-purified nicotine and solanesol using the waste tobacco materials.

2. Materials and methods

2.1. Waste tobacco material and reagents

The waste tobacco material (leaf vein with a few broken leaves) is a cigarette manufacture waste provided by Changsha Cigarette Factory. The material was dried in a 45 °C oven for 4 h, ground and passed through a 40-mesh sieve. Materials less than 40-mesh were used for the experiments.

Standard compounds of nicotine and solanesol were purchased from the National Institute for Food and Drug Control of China. HPLC solvents were purchased from Burdick & Jackson Inc. (Muskegon, MI, USA). Food-grade ethanol (95%) was used in all extractions and purifications. Other analytical- or biochemical-grade organic solvents and chemical reagents were purchased from local suppliers.

2.2. Analysis of nicotine and solanesol by TLC and HPLC

Silica gel G TLC plates (10 × 10 mm, Merck Co., Germany) were used for qualitative analysis of nicotine and solanesol. The developing solvents were chloroform:methanol (10:1, v/v) for nicotine analysis, and 1,2-dichloroethane for solanesol analysis. All TLC plates were stained in iodine vapor after developed.

A Shimadzu SPD-20A HPLC system (Shimadzu, Japan) with LC-20AT UV detector and YMC-packed ODS column (250 mm × 4.6 mm, 5 μm) was used for qualitative and quantitative analyzes of nicotine and solanesol. Nicotine was analyzed using a mobile phase of methanol:aqueous solution of 0.2% triethylamine (4:6, v/v) at a flow rate of 0.8 mL/min and detected at 254 nm. Solanesol was analyzed using a mobile phase of 100% methanol at a flow rate of 0.8 mL/min and detected at 215 nm. All samples for HPLC analysis were filtered through 0.45 μm membrane filters before injection. Nicotine and solanesol in samples were identified by the retention times and co-injection tests with their corresponding standard compounds. Their amounts were determined by their peak areas based on the standard curves.

Fig. 1 shows the results of optimized HPLC analyzes of nicotine (Fig. 1A and B) and solanesol (Fig 1C and D) of standard compounds and the extracts from the waste tobacco material. The nicotine content had good linear relationship with the peak area in the range from 5 μg to 35 μg (Fig. 1E). Solanesol content had good linear relationship with the peak area in the range from 0.2 μg to 35 μg (Fig. 1F).

The analytic methods had high precision with RSDs (relative standard derivation, $n = 5$) of 0.75% for nicotine and 0.42% for solanesol. Repeatability tests showed that the RSDs ($n = 5$) were 1.01% and 0.12%, with recovery rates of 99.81% ± 0.52% and 99.81% ± 0.59% for nicotine and solanesol, respectively. Nicotine (in 95% ethanol) and solanesol (in PE) were stable within 24 h at room temperature, and the RSDs of their amounts were 1.70% for nicotine and 0.55% for solanesol at 24 h.

2.3. Selection of extraction solvents

To select the extraction solvent that best dissolves nicotine and solanesol in the waste tobacco material, 0.5 g of dried material was added into 5 mL extraction solvents (petroleum ether or PE:ethanol from 10:0, 8:2, 6:4, 4:6, 2:8 or 0:10) and incubated at room temperature (30 °C water bath) for one hour with 20 rpm/min shaking. After centrifugation at 5000*g* for 10 min, nicotine and solanesol in the solutions were analyzed by HPLC.

2.4. Determination of minimum solvent volume and minimum dissolution time

Dried material (0.5 g) was added into 5 mL extraction solvent and incubated at room temperature (30 °C water bath) for different hours (1–5 h) with 20 rpm shaking. The solution and tobacco residue were then separated by centrifugation at 5000*g* for 10 min. The solvent volume required for the tobacco material fully imbibed was determined as the minimum volume (MV). MV is a basic volume unit in the extraction. The contents of nicotine and solanesol in the solutions were determined by HPLC, and the minimum times for the two compounds fully dissolved were determined.

2.5. Column-chromatographic extraction

CCE was performed in glass chromatographic columns [21–23]. For non-cyclic CCE, 8 g tobacco material was loaded into a column with one MV solvent at a bed height to diameter ratio of 10:1 or 5:1, through the wet column preparation method. After remained for 3 h until nicotine and solanesol dissolved, the column was eluted with the extraction solvent at a flow rate of one MV/h. The eluent was collected in fractions (one MV each) and the contents of nicotine and solanesol in each fraction were analyzed by HPLC.

For cyclic CCE, two MVs eluent was collected from the column, the first MV was collected as the extraction solution, and the second MV was used to extract the next batch material. The volume of extraction solution was one MV.

2.6. Separation of nicotine and solanesol

The extraction solution was adjusted to pH 2.0 (with 1 mol HCl for all pH adjustment) and separated into two phases. The ethanol aqueous phase was washed with 1/4 volume PE, and the PE phase was washed with 1/4 volume of 90% acidic ethanol (pH 2.0). The nicotine aqueous solution was obtained after vacuum-concentration of the combined ethanol aqueous phase. Crude solanesol was obtained after vacuum-concentration of the combined PE phase.

2.7. Purification of nicotine

The nicotine aqueous solution was adjusted to pH 10.0 and fractionated with PE for three times with equal volume for first time