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Determination of Sugars and Humectants in Cigarette by Solid Phase Extraction and Ion Chromatography with Pulsed Amperometric Detection

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The determination of sugars and humectants in cigarette by solid phase extraction and ion chromatography with pulsed amperometric detection (IC-PAD) was studied. The sugars (D-glucose, D-fructose, sucrose, D-galactose, maltose) and humectants (dglucitol, xylitol, glycerol, propylene glycol) were extracted from the cigarette samples with water. The extract was purified by solid phase extraction with C_{18} cartridge and analyzed by ion chromatography with pulsed amperometric detection. The recoveries of this method were ranging from 95-103 %. The relative standard derivation of overall intra-day variations were less than 1.8 % and the relative standard derivation of inter-day variations were less than 2.2 %. The detection limits were ranged from 30-80 ng/mL, respectively, for the different sugars and humectants. Results showed that the IC-PAD method provided a simple and powerful tool for the analysis of sugars and humectants in cigarette.

Key Words: Ion chromatography, Sugars and humectants, Cigarette, Solid phase extraction.

INTRODUCTION

During the manufacturing of cigarettes, up to 10 % by dry-weight of additives were added to cigarette to make them more attractive. As major components of tobacco additives, sugars (D-glucose, D-fructose, sucrose, D-galactose, maltose) and humectants (dglucitol, xylitol, glycerol, propylene glycol) are valuable for product characterization and differentiation. These compounds in cigarettes can influence the taste, flavor and moisture of cigarettes and can also affect sensory properties like harshness, smoothness and impact[1,2]. Therefore, the accurate determination of sugars and humectants in cigarette is important.

In general, sugars and humectants carbohydrates are usually hydrophilic, neutral and lack satisfactory chromophores for UV detection. The existing analytical methods for sugar compounds mostly fall into four categories: CE[3-5], GC-based methods[6,7], HPLC-based methods[8-10] and MS methods[6,11,12]. These effective methods were successfully applied for the analysis of many samples; however, there are still some limits or disadvantages in them. Nowadays, method of ion chromatography-pulsed amperometric detection (IC-PAD) was developed to analyze amino acids and sugars[13-15] because of its prominent advantages of performance. In IC-PAD, the use of alkaline eluents at high concentration causes most of the carbohydrate compounds with light acidic hydroxyl to be ionized, which allows separation by anion-exchange mechanisms. Then, detection occurs by measuring the current generated when the carbohydrate compounds are oxidized on a gold electrode. The optimized chromatography condition and the use of an appropriate detection potential make it a very selective technique for carbohydrates.

In this work, a new method for the determination of sugars (D-glucose, D-fructose, sucrose, D-galactose, maltose) and humectants (dglucitol, xylitol, glycerol, propylene glycol) in cigarette was studied. The 9 compounds have been determined with the IC-PAD. The linearity, repeatability and accuracy of this method have been determined with satisfactory results.

EXPERIMENTAL

All solvents, chromatography eluent and standards were prepared using deionized water, free of electrochemically active impurities. The 50 % sodium hydroxide solution used to make chromatography eluent was purchased from Fisher Scientific. The D-glucose, D-fructose, sucrose, D-galactose, maltose, dglucitol, xylitol, glycerol and propylene glycol were purchased from Fluka or Sigma-Aldrich, with 98-99 % purity. Packs of 8 different brands of cigarette were collected from Yunnan province.

The ion chromatographic analysis was performed on a Dionex ICS-3000 system. The system is composed of dual pump module (includes one isocratic pump and one gradient pump). Detection/chromatography module (contains injection valve, column and detector). The temperature in this part is maintained at 30 ºC. The electrochemical detector consisted of Au working electrode, Ag/AgCl reference electrode and Ti counter electrode. The Chromeleon 6.8 chromatography software (Dionex) was used for system control and data analysis. The anion exchange column (CarboPac MA1 (Dionex, 4 mm × 250 mm) was used. The electrochemical detector cell waveform was 0.1 V from 0.00-0.40 s, then -2.0 V from 0.41-0.42 s and a ramp from-0.2-0.6 during 0.42-0.43 s, followed by -0.1 V from 0.44-0.5 s (end of cycle). The integration period starts at 0.20 s and ends at 0.40 s. The mobile phase flow rate was 0.5 mL/min. The run time was 50 min for each sample.

Sample preparation: The samples were ground into homogenized power and 0.2 g of ground samples were accurately weighed into a 100 mL flask. To which, 50 mL of water was added and the sample was extracted with ultrasonic extraction for 0.5 h at room temperature. After the ultrasonic extraction, 5 mL of the upper layer solution was passed through the C_{18} cartridge at a flow of 10 mL/min to remove hydrophobic compounds. The first 2 mL solution was rejected to keep the uniformity of the sample solution and the following solution was collected. This solution was filtered through a 0.45 mm nylon filter and 5 µL of solution was injected for ion chromatography analysis.

RESULTS AND DISCUSSION

For ion chromatography analysis of sugars and humectants, a preliminary removal of low polar compounds (such as leaf pigment, polyphenols, alkaloid and others) must be performed because the low polar compounds can contaminate the chromatographic column (they cannot be eluted from the column by the mobile phase of sugars and humectants separation). In this paper, the purification of the sample solution by solid phase extraction with a C_{18} cartridge was studied. The sugars and humectants can be extracted from the cigarette sample with water and the water solution was passed through the cartridge at a flow rate of 10 mL/min. The low polar compounds can be retained when the sample solution passes through the cartridge, but the sugars and humectants can not. This procedure can remove the low polar compounds from sample solution quickly.

The CarboPac MA1, CarboPac PA1, CarboPac PA10 and CarboPac PA100 column were routinely used as analytical column for ion chromatography analysis of sugars and humectants. The four columns performed differently for sugars and humectants. In present studies, the CarboPac MA1 had the highest capacity and best selectivity. Therefore, the CarboPac MA1 was selected as analytical column in this experiment.

The sodium hydroxide solution was usually used as mobile phase for CarboPac MA1 column. The concentration of the sodium hydroxide was optimized from 0.2-0.6 mol/L. The results show that the retention times of these compounds (except for propylene glycol and glycerol) decrease with increase in the concentration of sodium hydroxide. The retention time of propylene glycol remains constant while that of glycerol increases slightly with increase in the concentration of sodium hydroxide. Meanwhile the resolution of all compounds is acceptable at the concentration of 0.48 mol/L. So we select 0.48 mol/L sodium hydroxide solution as mobile phase to separate these compounds. In this condition, the chromatograms of the standard and the cigarette sample are shown in Fig. 1.

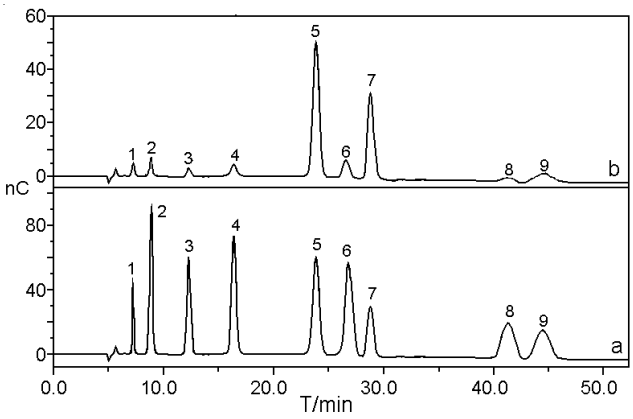

Fig. 1. Chromatogram of standard (a) and cigarette sample (b) (1) propylene glycol, (2) glycerol, (3) xylitol, (4) dglucitol, (5) glucose, (6) galactose, (7) fructose, (8) maltose, (9) sucrose

Under the optimum conditions, the regression equations of sugars and humectants were established based on the standard samples injected and their peaks area. The limits of detection are calculated by the ratio of signal to noise (S/N = 3). The results were shown in Table-1. The reproducibility of this method was also examined. The relative standard deviations (n = 7) were shown in Table-1 too.

The recovery tests were carried out by adding sugars (D-glucose, D-fructose, sucrose, D-galactose, maltose) and humectants (dglucitol, xylitol, glycerol, propylene glycol) to the samples (three different concentrations of markers: 10, 20

TABLE-1
REGRESSION EQUATION, COEFFICIENT AND DETECT LIMIT

Components	Linearity range (µg mL^{-1})	Coefficient	Regression equation C (µg mL^{-1})	Detect limits (ng mL^{-1})
Propylene glycol	0.4-60	0.9994	A = 5.27 C – 0.055	50
Glycerol	0.2-50	0.9993	A = 21.2 C +0.184	30
Xylitol	0.3-70	0.9995	A = 13.5 C – 0.122	45
D-Glucitol	0.5-60	0.9992	A = 10.2 C – 0.321	40
D-Glucose	0.3-120	0.9997	A = 7.22 C + 0.232	60
D-Galactose	0.3-50	0.9996	A = 6.87 C – 0.175	50
D-Fructose	0.5-150	0.9995	A = 3.06 C – 0.082	60
Maltose	0.3-120	0.9997	A = 4.82 C + 0.153	50
Sucrose	0.6-150	0.9994	A = 4.57 C – 0.212	80

and 50 µg). The sample was prepared as above "sample preparation" procedure and injected for HPLC analysis to calculate the amount of the sugars and humectants founded. The results shown that the recoveries (n = 5) were ranged from 95-103 %. This method has high recovery.

The measurements of intra-day and inter-day variability (determination of the same samples for seven times) were utilized to determine the precision of the developed method. The results showed that the relative standard derivation of overall intra-day variations were less than 1.8 % and the relative standard derivation of inter-day variations were less than 2.2 %. This method has high precision.

Conclusion

The proposed ion chromatography method enables simultaneous determination of 9 sugars and humectants because of good separation and resolution of the chromatographic peaks, which provides a powerful tool for the analysis of sugars and humectants. Furthermore, this chromatographic system with PAD provides a highly simple, fast, selective and reproducible method to analysis of cigarette. To the optimized method of HPAEC-PAD, not only sugars but also alditols and alcohols in cigarette products could be analyzed in one run. The solid phase extraction with C_{18} cartridge was also used to purify the sample. This procedure can remove the low polar comounds from sample solution quickly. The linearity, repeatability and accuracy have been determined with satisfactory results. This work confirmed that the assessment of carbohydrate levels is significant for the determination of cigarette.

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