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Determination of Humectants in Tobacco Filler by High Performance Chromatography/Single Quadrupole Mass Spectrometry *

by

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SUMMARY

Glycerol, and 1,2-propylene glycol are the humectants most commonly used by the tobacco industry. They are found in a variety of tobacco products and are often present at high levels (~2–5 % w/w). While humectants are generally considered safe, they may serve as precursors in the formation of harmful carbonyl compounds. A selective, precise, and sensitive method for the quantification of several humectants in cigarette filler was developed. The method's sample clean-up is a two-step process consisting of a mechanical extraction, followed by solid phase extraction. Individual humectants are separated, identified, and measured using liquid chromatography coupled to a single quadrupole mass spectrometer as the detector (LC/MS). Detection limits were 0.105, 0.575, and 0.039 mg/cigarette for glycerol, 1,2-propylene glycol and triethylene glycol, respectively. The quantification range for these analytes was 0.4–75.0 mg/cigarette. Twenty-seven brands of domestic commercial cigarettes were evaluated to assess typical levels of humectants in the tobacco filler. [Beitr. Tabakforsch. Int. 28 (2018) 170–178]

KEYWORDS

Humectants in tobacco filler; high performance chromatography/single quadrupole mass spectrometry; solid phase extraction

ZUSAMMENFASSUNG

Glycerin und 1,2-Propylenglykol sind die in der Tabakindustrie am häufigsten eingesetzten Feuchthaltemittel. Man findet sie in einer Vielzahl von Tabakprodukten und dort häufig in hohen Konzentrationen (~2–5 % w/w). Im Allgemeinen gelten Feuchthaltemittel als ungefährlich; sie können aber als Vorläufersubstanz bei der Bildung gefährlicher Carbonylverbindungen auftreten. Es wurde eine selektive, präzise und empfindliche Methode zur Quantifizierung mehrerer Feuchthaltemittel in Zigarettenfüllstoff entwickelt. Bei dieser Methode erfolgt die Probenreinigung in einem zweistufigen Prozess. Dieser besteht aus einer mechanischen Extraktion gefolgt von einer Festphasenextraktion. Einzelne Feuchthaltemittel wurden mithilfe der Flüssigchromatographie gekoppelt mit einem Single-Quadrupol-Massenspektrometer (LC/MS) als Detektor abgeschieden, bestimmt und gemessen. Die Nachweisgrenzen lagen für Glycerin, 1,2-Propylenglykol und Triethylenglykol jeweils bei 0,105, 0,575 und 0,039 mg/Zigarette. Der Quantifizierungsbereich für diese Analyten lag bei 0,4–75,0 mg/Zigarette. 27 inländische, im Handel erhältliche Zigarettenmarken wurden im Hinblick auf die typischen Konzentrationen an Feuchthaltemitteln im Tabakfüllstoff ausgewertet. [Beitr. Tabakforsch. Int. 28 (2018) 170–178]

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RESUME

La glycérine et le 1,2-propylène glycol sont les humectants les plus communément utilisés par l'industrie du tabac. Ils entrent dans la composition de divers produits de tabac où ils sont souvent présents dans des quantités élevées (~2–5% p/p). Alors que les humectants sont, en règle générale, considérés comme sûrs, ils peuvent servir de précurseurs dans la formation de composés carbonylés nocifs. Une méthode sélective, précise et sensible fut mise au point afin de quantifier divers humectants parmi les composants de remplissage des cigarettes. Cette méthode repose sur une épuration de l'échantillon en deux étapes, à savoir une extraction mécanique suivie d'une extraction en phase solide. Les humectants individuels sont séparés, identifiés et mesurés par le biais, en guise de détecteur, d'une chromatographie en phase liquide couplée à une unique spectrométrie de masse quadripolaire (LC/MS). Les seuils de détection s'élevèrent respectivement à 0,105, 0,575 et 0,039 mg/cigarette pour la glycérine, le 1,2 propylèneglycol et le triéthylèneglycol. La plage de quantification de ces analytes fut de 0,4–75,0 mg/cigarette. Vingt-sept marques de cigarettes commercialisées dans le pays furent testées afin d'évaluer les quantités types d'humectants dans le tabac de remplissage. [Beitr. Tabakforsch. Int.28 (2018) 170–178]

INTRODUCTION

Many types of tobacco products, such as cigars, cigarillos, pipe tobaccos, e-cigarettes, roll-your-own tobacco, and smokeless products, have been developed and promoted by tobacco companies; however, manufactured cigarettes remain the most popular form of tobacco used in the United States, with more than 293 billion cigarettes sold during 2011 alone (1). Cigarette filler consists of two major components: "tobacco constituents," which are substances naturally present in tobacco, and "tobacco additives," defined as substances added to tobacco during the manufacturing process. Tobacco additives could serve several different functions within the final tobacco product. For example, they may improve sensory properties, such as taste and aroma, enhancing the appeal of cigarettes for smokers (2–4); may heighten the addictive potency of nicotine by changing the pH of the gas phase or by producing synergistic effects with some pyrolysis products (5, 6); and, in the case of humectants, increase or stabilize the moisture-holding capacity of the tobacco (7–9).

Glycerol has been the most extensively used humectant in food, personal care and tobacco products. Other humectants, such as 1, 2-propylene glycol and triethylene glycol, in minor scale, have also been added to tobacco cigarettes with typical total humectants concentration ranging from 1% to 5% (10, 11). It should be noted that triethylene glycol is not used as frequently as in the past but it could still be found in some tobacco products. The majority of toxicological studies involving these popular humectants are mostly based on oral, and/or dermal adsorptions, and the results of these studies suggest these compounds are not harmful for use in food and consumer products (12). There are some experimental studies on the toxic effects of the pyrolysis products of these humectants, most of them using machine-smoking conditions (8, 10, 13–17). However, it is well known that none of the machine smoking regimens commonly used truly represent the human behavior of smoking (18, 19). Moreover, the results of these studies often conflict because the final pyrolysis products depend on the physical properties of cigarettes and the experimental conditions of the smoking process. Highly reactive species, such as acetaldehyde, acrolein, and formaldehyde, have been detected in small amounts in both mainstream and/or sidestream smoke as products of the pyrolysis of humectants (10, 20, 21).

Most of the used methods for detection and quantification of humectants in cigarette filler are based on gas chromatography (14–16) and only a few of them are based on high performance liquid chromatography (HPLC). Health Canada (14) and CORESTA recommended method N° 60 (22) are the most commonly used methods for the quantification of humectants in filler. These methods are based on a mechanical extraction (2 h) followed by a gas chromatographic separation with flame ionization detection (FID). In 2015, CORESTA published the recommended method N° 61 (23) "*Determination of 1,2-propylene glycol, glycerol, and sorbitol in tobacco and tobacco products by HPLC*". The recommended method N° 61 is based on a high-speed mechanical extraction with water (30 min) followed by HPLC separation with refractive index detection. SLANSKI and MOSHY (24) used a gas chromatograph equipped with a thermal conductivity detector (TCD). Briefly, a tobacco sample was extracted with methanol in a Soxhlet apparatus for at least 5 h. Next, the extract was evaporated by a dry nitrogen stream at room temperature, derivatized with a Tri-Sil reagent, and then injected into the GC/TCD system. RAINEY *et al.* (25) described a modified gas chromatographic method to incorporate simultaneously mass spectrometric (MSD) and FID into the analysis of tobacco humectants such as glycerol, 1,2-propylene glycol, and triethylene glycol in different tobacco matrices such as roll-your-own, cigar, cigarette, moist snuff, and hookah tobacco. In this method, tobacco was extracted in methanol containing 1,3-butanediol (internal standard), filtered, and separated on a 15-m megabore DB-Wax column. Post-column flow was distributed using a microfluidic splitter between the MSD and FID for simultaneous detection. While a high degree of correlation was obtained between the two used techniques, a minimal chromatographic problem was observed between glycerol and triethylene glycol, which restricts the applicability of FID to samples containing low levels of both of these humectants. Using MSD greatly improved the selectivity of the technique even though the FID showed a better sensitivity.

Some of these methods are labor intensive and have poor selectivity. Consequently, a fast and selective method without the extensive sample clean-up procedure and derivatization steps is desired. A new procedure that produces a relatively clean extract and uses a highly selective and sensitive detection technique for the determination of humectants in tobacco filler is described here. Concentrations of glycerol, triethylene glycol and 1,2-propylene glycol in cigarette filler from top-selling commercial cigarette brands of several major US tobacco companies were

Table 1. Summary of the method specifications. T_R: Retention time; LOD: Limit of detection; LDR: Linear dynamic range; Q_Ion: Quantification ion; C_Ion: Confirmation ion.

Analyte	T_R (min)	LOD (mg/cig)	LDR (mg)	R^2 (n = 11)	Q_Ion (amu)	C_Ion (amu)
Glycerol	3.26	0.105	3.13–52.20	0.9997	133.8	92.8
1,2-Propylene glycol	3.85	0.575	1.57–26.20	0.9998	117.8	58.8
Triethylene glycol	5.30	0.039	0.32–5.40	0.9997	150.8	151.9
Glycerol $^{13}C_3$	3.27	N/A	0.80	N/A	136.8	N/A
1,2 Propylene glycol-1,2-$^{13}C_2$	3.85	N/A	0.40	N/A	119.9	N/A

investigated and reported. To our knowledge, this is the first analytical method to analyze these compounds in tobacco filler which uses isotopically labeled internal standards for selective and accurate quantitation.

METHODS AND MATERIALS

Chemicals

All reagents used were analytical grade. HPLC grade acetonitrile and HPLC grade water were obtained from Fisher Scientific (Fairlawn, NJ, USA). Glacial acetic acid was purchased from JT Baker (Philipsburg, NJ, USA). OASIS HLB® 3cc (60 mg) mixed-bed polymeric cartridges used for solid phase extraction (SPE) were purchased from Waters Corporation (Milford, MA, USA).

Native analytical humectant standards, glycerol, 1,2-propylene glycol, and triethylene glycol, and isotopically labeled 1,2-propylene glycol-$^{13}C_2$ were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Isotopically labeled glycerol-$^{13}C_3$ was synthesized by Cambridge Isotope Laboratories (Andover, MA, USA). Both labeled standards had chemical and isotopic purities higher than 99%. Research cigarettes (3R4F) were obtained from the University of Kentucky (Lexington, KY, USA). Commercial cigarettes were purchased at retail outlets around the metropolitan Atlanta, Georgia area. The brands selected are not necessarily representative of the US market; they constitute a "convenience" sample, purchased solely to test validity of the method using commercial cigarettes.

Standard preparation

- *Isotopically labeled internal standard*

An isotopically labeled internal standard solution (ISTD) was prepared by weighing 200 mg of glycerol-$^{13}C_3$ and 100 mg of 1,2- propylene glycol-$^{13}C_2$ labeled standards into a 5-mL volumetric flask and dissolving with HPLC grade water. The resulting solution of 40 mg/mL glycerol-$^{13}C_3$ and 20 mg/mL 1,2-propylene glycol-$^{13}C_2$ was divided into 1-mL aliquots and stored at −20 °C until used. A 20 µL aliquot of the ISTD was added to all blanks, quality control samples (QCs), calibration standards, and unknowns directly on the tobacco filler solutions to achieve a final concentration of 0.80 mg/cig for glycerol-$^{13}C_3$ and 0.40 mg/cig for 1,2-propylene glycol-$^{13}C_2$.

- *Native standards and calibration plots*

A native standard stock solution of glycerol, 1,2-propylene glycol and triethylene glycol was prepared by weighing approximately 2.50, 1.25, and 0.25 g of the native standards into a 25-mL volumetric flask and dissolving with HPLC water to yield concentrations of 100, 50, and 10 mg/mL of the three analytes, respectively. Calibration standard solutions of the three target analytes were prepared daily by spiking 30, 60, 90, 120, 150, 300, and 500 µL aliquots of native standard stock solution into 20 mL volumetric flasks and dissolving with HPLC water giving a range of concentrations (see Table 1).

- *Quality control (QC) materials*

To ensure the method's long-term analytical stability and reproducibility of the results, two QC samples at low and high concentrations (QCL and QCH, respectively) were analyzed with each sample set. Briefly, a QCL was prepared by weighing 0.7 g of the filler from a 3R4F research cigarette and spiked with 100 µL of a stock solution with a concentration of 50 mg/mL 1,2-propylene glycol and 10 mg/mL triethylene glycol. A QCH was prepared by weighing 0.7 g of the 3R4F research cigarette filler and spiking with 100 µL of a stock solution with a concentration of 100 mg/mL glycerol, 98 mg/mL 1,2-propylene glycol and 20 mg/mL triethylene glycol. Twenty QCL and QCH samples were prepared and characterized over a 4-week period to establish individual analyte QC limits (mean, 95th and 99th confidence intervals). After establishing the control limits, the QC samples included within each analytical run were evaluated for validity using a modified Westgard multi-rule approach (26). Each analytical run contained two QCs, and a sample blank to monitor background levels and guard against contamination from sample carryover.

Sample preparation

Cigarettes were conditioned for at least 48 h at 22 °C and 60% humidity before use, following the ISO 3402:1999 standard operating procedure (27). A 0.7 g sample of tobacco filler was weighed and placed into an extraction vial, followed by 20 mL of HPLC water. Analytes were extracted using an orbital shaker (VWR, West Chester, PA, USA) at 160 rpm for 60 min. After removal from the shaker, the vial contents were allowed to settle for 30 min until the supernatant was clear. Solid phase extraction was

then carried out to reduce non-polar interfering components. Oasis HLB® 3cc SPE cartridges were conditioned with 600 µL methanol followed by 600 µL HPLC water. A 600-µL aliquot of the supernatant was passed through the conditioned SPE cartridge, followed by 1 mL of 40% methanol in HPLC water, and the eluate was collected in a test tube. A 200 µL aliquot of eluate was transferred to an auto sampler vial and capped prior to analysis using HPLC-MSD.

Instrumental analysis

Chromatographic separation was performed on a Zorbax SB-C3 reversed-phase column (5 µm particle size, 100 Å pore size, and 150 mm length × 4.6 mm inner diameter; Agilent Technologies, Santa Clara, CA, USA). The flow rate was 0.5 mL/min, and the injection volume was 10 µL. The column temperature was kept at 30 °C during the analysis. The mobile phase consisted of 0.2 % acetic acid in HPLC water (solvent A) and 100% acetonitrile (solvent B). Chromatographic separation was achieved using gradient conditions. The initial mobile phase composition of 80:20 (solvent A:solvent B) was kept constant for 2 min and then the composition was changed linearly to 40% B until 7 min and held for 1 min at that composition. After 8 min, the column was equilibrated to initial conditions for 6 min. The total run time per sample, including equilibration time, was 15 min.

Humectant extracts were analyzed quantitatively using a Surveyor® LC system (Thermo Electron Corporation, San Jose, CA, USA) coupled to an MSQ+ ® single quadruple mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA) equipped with an atmospheric pressure chemical ionization (APCI) source. The optimum APCI source temperature was 350 °C with nebulizing gas at 45 psi. The mass spectrometer was operated in the positive-ion mode using selective ion monitoring. Mass spectral data were collected in centroid mode (see Table 1) which is characterized by collection of continuum data processed to display a single, centered point for each distribution of ions in a spectrum. Peak integration and data processing were performed using Xcalibur software (Version 1.4). All automatically integrated peaks were reviewed by the analyst and manually corrected as necessary. Peak areas and other pertinent data were exported into a Microsoft Excel file and loaded into a Microsoft Access database for permanent storage. All statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA).

Daily operating protocol

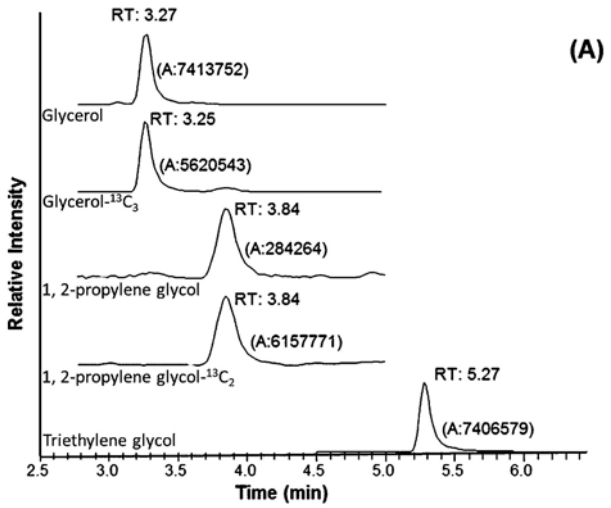
A typical sample batch included one reagent blank, 38 unknown samples, one QCL, one QCH, and seven standards. Before daily instrumental analysis, a known standard was analyzed to confirm acceptable chromatographic resolution and mass spectral sensitivity. The CDC tobacco products laboratory requires that the reagent blank be free of analytes and that both QCH and QCL pass the Westgard multi-rule criteria before approval as valid results.

RESULTS AND DISCUSSION

A key goal of the CDC tobacco products laboratory is developing analytical techniques to measure toxic and addictive substances in tobacco products, smoke, and other emissions. In this project, University of Kentucky 3R4F (Lexington, KY, USA) research cigarettes were used as control samples during the development and validation processes of the method. Several sample preparation and extraction procedures were evaluated and optimized. Initially, the water extraction time was optimized to maximize sensitivity of the humectants compounds using a series of experiments that were conducted with 30-, 45-, 60-, and 90-min extraction times at room temperature. Results indicated that a 60-min extraction time was optimal. OASIS HLB® 60-mg cartridges and Strata X® 60-mg cartridges were used to reduce background and matrix effects. Both cartridges are macro-porous copolymers made from a balanced ratio of two monomers, the lipophilic divinylbenzene and the hydrophilic *N*-vinylpyrrolidone. Several water-methanol solutions (10, 20, 30 and 40% methanol) were tested as eluents for the extraction; the 40% methanol solution resulted in the best recoveries. Both cartridges produced similar recovery results.

Measurement of low molecular weight compounds is a challenge using a single quadruple mass spectrometer because of potential interferences from high solvent backgrounds and other isobaric ions present in the extracts. Initially, we did attempt to optimize the ($[M+H]^+$) ion of glycerol, 1,2-propylene glycol, triethylene glycol and their corresponding isotopically labeled internal standards by adding low concentrations of methanol to the mobile phase, but the signal for 1,2-propylene glycol decreased and we could not find other confirmation ions. We decided to use acetonitrile (ACN) as our organic mobile phase since an acetonitrile cluster was produced ($[M+H+ACN]^+$). The ACN cluster was sufficiently stable and intense for the analysis and provided a readily detectable confirmation ion ($[M+H]^+$) upon loss of the acetonitrile molecule. Typical reconstructed ion chromatograms (RIC) of the spiked 3R4F tobacco filler (A) and an unknown tobacco sample (B) are shown in Figure 1. The analytical run time, including equilibration was 15 min, and all of the analytes were chromatographically resolved. The retention times for glycerol, 1,2-propylene glycol, and triethylene glycol were 3.25, 3.84 and 5.27 min, respectively. Chromatograms generated using the method parameters given here showed no known chemical interferences. The chromatographic resolution enhances the method's ability to yield accurate determinations of peak areas.

The limits of detection (LOD) were calculated as $3S_0$, where S_0 is the standard deviation of the analyte signal at zero concentration (28). S_0 was estimated as the y-intercept of a linear regression analysis of a plot of the standard deviation (in units of concentration) *versus* the concentrations of the four lowest standards. The highest LOD was observed for 1,2-propylene glycol (0.575 mg/cig) followed by glycerol (0.105 mg/cig) and the lowest one was triethylene glycol (0.039 mg/cig). The cause of the higher LOD for 1,2-propylene glycol is not entirely clear but likely results from the lower ionization efficiency of the acetonitrile adduct, which although more selective, could



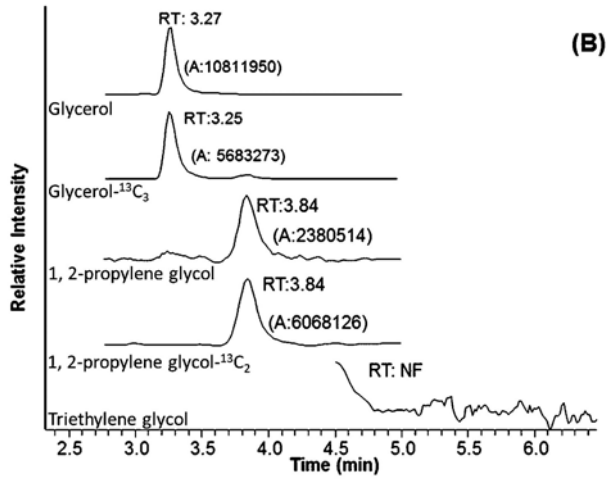


Figure 1. Typical chromatogram of the spiked 3R4F tobacco filler (A) and unknown tobacco filler sample (B). Panels (A and B) show trace for each native analyte and internal standard. glycerol-13C3 was used as internal standard for glycerol and 1, 2-propylene glycol-13C2 was used as internal standard for the rest of the analytes. RT: retention time, A: area counts, RT: NF (peak not found).

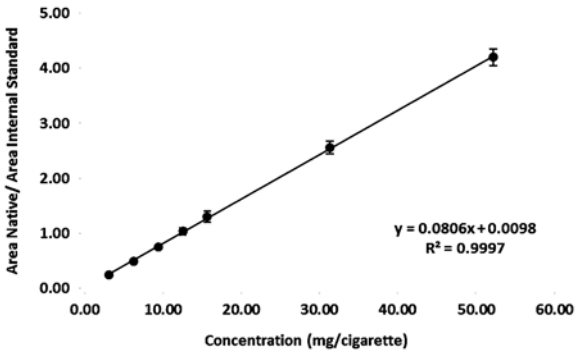


Figure 2. An overlay of typical standard averaged calibration curve for glycerol (n = 8 per each calibration point). The calibration curve shows linearity over the entire calibration range, with a correlation coefficient higher than 0.999.

also result in ion suppression. Note that the lowest detection limit was achieved for the compound analyzed as the protonated molecule (triethylene glycol), not the acetonitrile adducts. Probably this factor could increase the ionization of the protonated molecular ion and/or minimize the possible chemical interference in the detection of triethylene glycol (Table 1). One of the major advantages of atmospheric pressure chemical ionization is high ionization efficiency and low fragmentation of the protonated species, perhaps allowing for lower limits of detection. When proton affinity of the solvent is higher than the proton affinity of water, the solvent is protonated and can react with other compounds forming stable adducts, which although more selective, could also result in ion suppression.

Calibration curves were constructed for each analytical run using the response factors of seven calibrators covering the linear range for each analyte, with seven analyte concentrations (concentrations ranging from 0.4 to 75.0 mg/cig). Response factors were calculated as the area of the native analyte ion divided by the area of the labeled internal standard ion. A linear regression analysis of the calibration curve provided a slope and intercept from which unknown sample concentrations were determined. Calibration curves for glycerol were linear in a concentration range of 3.13–52.20 mg/cig (Figure 2) which is adequate for cigarettes. The R^2 value for the linear regression analysis was 0.9997. Similar results were obtained for the other analytes (Table 1).

The absolute accuracy of this method was difficult to determine because no reference materials were available for evaluation. However, results were compared to a reference literature method from Kentucky Tobacco Research & Development Center for determination of glycerol in a 3R4F cigarette (29). Assuming the concentration of glycerol published in the literature (2.34%) is the "true" value, and that our experimentally determined value was 2.34%, the percent accuracy of this method is 100% for glycerol. Triethylene glycol and 1,2-propylene glycol in 3R4F were not detected either by using the reference method and/or this method.

The accuracy for each analyte was assessed by spike recovery at high and low concentrations and yield percentage values of 91.3 and 102.9 % for glycerol, 102.5 and 99.7 % for 1,2-propylene glycol and 95.8 and 100.3% for triethylene glycol, respectively. Initially, 10 samples (5 for each group) of 0.7 g 3R4F reference tobacco were spiked with 20 µL of ISTD and 100 and 200 µL of the stock solution to produce the low and high concentration samples, respectively. Then, five blank 3R4F (spiked only with 20 µL of ISTD) were also analyzed. Analytical recovery was calculated as the percent difference between the response of analyte spiked on the 3R4F and the 3R4F blank divided by the theoretical value. For the low concentration spike samples, the average analytical recoveries for all analytes were higher than 91%. The high concentration spike yielded average recoveries higher than 99.0%. Additionally, reproducibility was estimated as the relative standard deviation of the replicate measurements (Table 2). The relative standard deviations for the low and high standard addition were lower than 2.1%.

The method precision was determined by calculating the

Table 2. Accuracy (aka: spike recovery extraction) of analytes at low and high concentrations (n = 5).

Analyte	Low spiking level			High spiking level		
	Amount (mg)	Accuracy (%)	Precision (%)	Amount (mg)	Accuracy (%)	Precision (%)
Glycerol	9.9	91.3	0.6	19.8	102.9	2.0
1,2 Propylene glycol	9.8	102.5	0.3	19.6	99.7	0.5
Triethylene glycol	2.4	95.8	0.1	4.7	100.3	0.1

Table 3. Total coefficient of variation (CV) for each analyte generated over a one-month period for the study (n = 40).

Analyte	QC_{low}		QC_{high}	
	Mean	% CV	Mean	% CV
Glycerol	17.9	4.0	36.4	5.1
1,2 Propylene glycol	5.4	9.7	20.3	6.0
Triethylene glycol	1.0	9.1	4.1	7.1

coefficient of variation (CV) of repeated measurements (20 days) of the two QC materials, prepared daily over a one-month period. CVs ranged from 4.0–9.7% (Table 3). Quality control charts such as the one shown for glycerol (Figure 3) were generated for all analytes and were used to determine the validity of each analytical run and to guarantee appropriate analytical precision.

Robustness, which refers to how an analytical procedure can be influenced by small experimental modifications during its performance, was also evaluated for this project. Chromatography flow rate, extraction time, extracted sample amount, and sample injection volume were varied ±15% from the final values. These modifications had negligible impact on the results, demonstrating that the technique is robust.

The suitability of the method was evaluated in the analysis of 27 types of domestic cigarettes, including 11 brands from the 4 major cigarette manufacturers. These brands were randomly selected and are not representative of the US marketplace. However, included in this "random" convenience selection are brands with different flavors (e.g., menthol) and packaging styles. Measurements of these 27 brand variants of the 4 leading US manufacturers showed fairly consistent total humectants levels in most brands (Table 4). These compounds were detected in approximately 80% of the analyzed brands. Triethylene glycol was not detected in any of the analyzed brands.

Of the 27 brands of cigarettes tested in this study, only 4 brands showed no detectable amounts of the humectants analyzed. The other 23 brands showed the presence of glycerol and/or 1,2-propylene glycol with concentrations ranging from 1.66 to 3.57% and from 0.23 to 1.35% for glycerol and 1,2-propylene glycol, respectively (Figure 4). In general, all four companies use more glycerol than 1,2-propylene glycol as a humectant agent.

Although most methods in the literature use GC and/or HPLC with a wide range of detectors such as flame detector (22, 25), refractive index detector (23), and thermal conductivity detector (24), we took advantage of the specificity that HPLC/MS coupled with isotopically labeled internal standards can offer. While one of the most recent manuscripts (25) also reports the use of GC/MS for measuring the analytes of interest, the authors did not use isotopically labeled internal standards. In our method, for an analyte to be positively detected, two conditions are required: 1) the analyte must coelute on the HPLC column with the labeled standard, which is often the case when isotopically labeled internal standards are used, and 2) it must have a least two specified ions which allow us to add one step of confirmation for the identity of the analytes (ratio of the quantification ion/confirmation ion). Additionally, our method provides a simple, fast, and specific tool for the analysis of humectants in tobacco samples. It has a two-step sample clean-up process involving mechanical extraction, followed by solid phase extraction. Individual humectants are separated, identified,

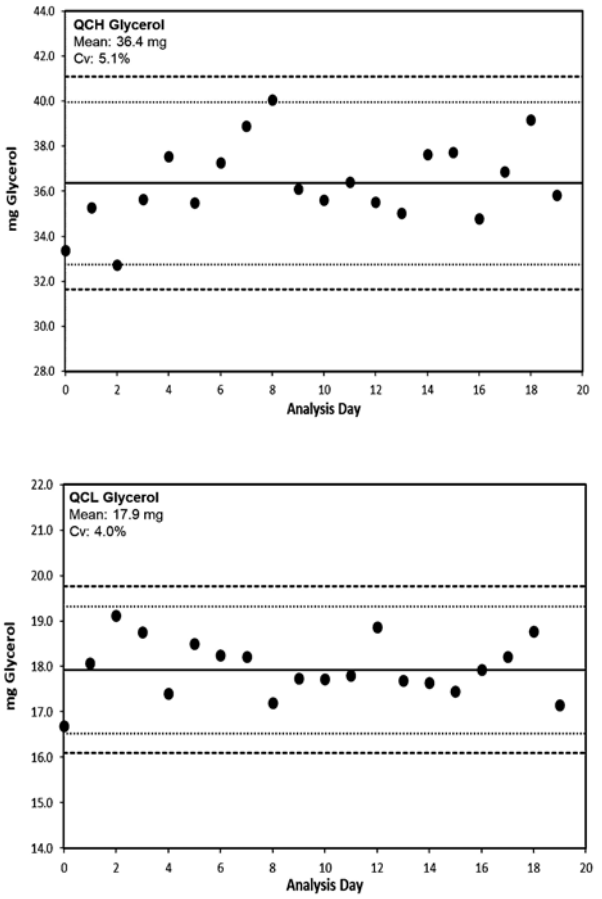


Figure 3. Quality control charts of glycerol from the present study (n = 2 per day). Top quality control chart represents high QC data and bottom quality control chart represents low QC data. The solid line represents the mean, the dotted and dashed lines represent the upper and lower 95th and 99th percentile control limits, respectively.

Table 4. Triplicate measurements of glycerol and 1,2-propylene glycol from the four leading US manufacturers. STD: Standard deviation of three determinations; % CV: Coefficient of variation; Total_Hum: Addition of glycerol and 1,2-propylene glycol concentrations, R: regular, M: menthol. NF: not found, NA: not applicable

Manufacturer	Brand	Flavor	Glycerol			1,2-Propylene glycol			Total_Hum (%)		
			Mean	STD	% CV	Mean	STD	% CV	Mean	STD	% CV
A	B_01	R	1.91	0.03	1.83	0.78	0.01	0.93	2.69	0.04	1.57
	B_02	R	2.98	0.14	4.57	0.87	0.05	5.76	3.85	0.19	4.84
	B_03	M	3.49	0.27	7.70	0.71	0.07	9.79	4.20	0.34	8.05
	B_04	M	3.57	0.39	10.95	0.89	0.06	6.64	4.47	0.45	10.09
B	B_05	R	2.76	0.26	9.39	0.75	0.03	4.35	3.50	0.29	8.31
	B_06	M	2.38	0.02	0.89	0.48	0.04	8.87	2.86	0.06	2.23
	B_07	M	2.44	0.09	3.55	0.48	0.01	2.50	2.92	0.10	3.38
	B_08	M	2.63	0.14	5.19	0.68	0.05	7.39	3.31	0.19	5.64
C	B_09	R	1.66	0.02	1.35	0.87	0.11	12.84	2.53	0.13	5.29
	B_10	R	2.46	0.08	3.07	0.66	0.08	11.76	3.12	0.15	4.91
	B_11	R	1.88	0.06	3.34	1.07	0.04	3.94	2.94	0.10	3.56
	B_12	R	1.94	0.12	6.42	1.02	0.02	2.31	2.97	0.15	5.00
	B_13	R	1.78	0.07	4.01	0.72	0.06	7.98	2.50	0.13	5.16
	B_14	R	2.12	0.06	2.81	0.78	0.05	5.76	2.91	0.10	3.60
	B_15	M	1.68	0.05	3.24	1.16	0.02	2.01	2.85	0.08	2.74
	B_16	M	2.10	0.05	2.42	1.32	0.05	3.55	3.43	0.10	2.86
D	B_17	R	2.63	0.29	10.92	0.73	0.10	13.20	3.36	0.38	11.42
	B_18	R	2.95	0.11	3.77	0.67	0.03	5.00	3.61	0.14	4.00
	B_19	R	2.94	0.54	18.39	0.89	0.02	2.03	3.84	0.56	14.59
	B_20	M	2.54	0.10	3.99	0.24	0.02	8.53	2.78	0.12	4.39
	B_21	M	2.79	0.33	11.90	0.27	0.03	10.09	3.07	0.36	11.74
	B_22	M	2.59	0.22	8.53	0.23	0.03	13.09	2.82	0.25	8.90
	B_23	M	2.72	0.06	2.39	NF	NA	NA	2.72	0.06	2.39
	B_24	R	< LOD	NA	NA	NF	NA	NA	NA	NA	NA
	B_25	R	< LOD	NA	NA	NF	NA	NA	NA	NA	NA
	B_26	R	< LOD	NA	NA	NF	NA	NA	NA	NA	NA
	B_27	R	< LOD	NA	NA	NF	NA	NA	NA	NA	NA

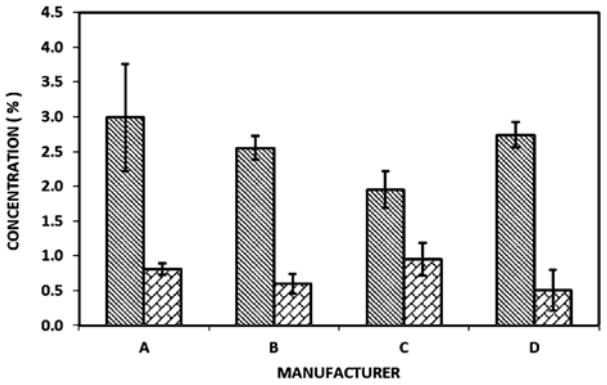


Figure 4. A bar graph of concentration of glycerol (diagonal plot) and 1,2-propylene glycol (brick plot) in the 23 brands from the 4 leading US tobacco companies.

and measured using liquid chromatography coupled to a single quadruple mass spectrometer as the detector (LC/MS). The biggest limitation of this method lies in that we did not test it with different tobacco matrices such as smokeless samples, e-liquids, etc. We believe that this method could be used with many other tobacco matrices without major modifications to increase the potential application of the method.

CONCLUSIONS

An analytical method for determination of 3 humectant agents in tobacco filler using mechanical extraction followed by a simple solid phase extraction sample preparation step coupled with isotope-dilution HPLC/MS was developed and validated. The new method was applied to the analysis of 27 commercial brands from 3 major US manufacturers. This method is characterized by its straightforward sample preparation, sensitivity, selectivity, and precision. The stability and precision of the measurement system over several months has demonstrated the robustness of the method. The above features demonstrate the suitability of this method for the routine analysis of humectants in cigarette filler.

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