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Quantitative Analysis of Humectants in Tobacco Products Using Gas Chromatography (GC) with Simultaneous Mass Spectrometry (MSD) and Flame Ionization Detection (FID)*

by

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SUMMARY

This paper describes the modification of an existing gas chromatographic (GC) method to incorporate simultaneous mass spectrometric (MSD) and flame ionization detection (FID) into the analysis of tobacco humectants. Glycerol, propylene glycol, and triethylene glycol were analyzed in tobacco labeled as roll-your-own (RYO), cigar, cigarette, moist snuff, and hookah tobacco. 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. The limits of detection for the FID detector were 0.5 µg/mL (propylene glycol and triethylene glycol) and 0.25 µg/mL (glycerol) with a linear range of 2–2000 µg/mL (propylene glycol and triethylene glycol) and 1–4000 µg/mL (glycerol). The limits of detection for the MSD detector were 2 µg/mL (propylene glycol and triethylene glycol) and 4 µg/mL (glycerol) with a linear range of 20–2000 µg/mL (propylene glycol and triethylene glycol) and 40–4000 µg/mL (glycerol). Significant improvement in the sensitivity of the MSD can be achieved by employing selective ion monitoring (SIM) detection mode. Although a high degree of correlation was observed between the results from FID and MSD analyses, marginal chromatographic resolution between glycerol and triethylene glycol limits the applicability of FID to samples containing low levels of both of these humectants. Utilizing MSD greatly improves the reliability of quantitative results because compensation for inadequate chromatographic resolution can be accomplished with mass selectivity in detection. [Beitr. Tabakforsch. Int. 25 (2013) 576–585]

ZUSAMMENFASSUNG

In dieser Arbeit wird die Modifizierung eines bestehenden Gaschromatographieverfahrens (GC) zur Erweiterung der Analyse von Tabakfeuchthaltemitteln um eine gleichzeitige massenspektrometrische Detektion (MSD) und Flammenionisationsdetektion (FID) beschrieben. Drehtabak (RYO), Zigarre, Zigarette, rauchloser Tabak und Wasserpfeifentabak wurden auf ihren Gehalt an Glycerin, 1,2-Propandiol und Triethylenglycol untersucht. Der Tabak wurde in Methanol, welches 1,3-Butanediol enthielt (interner Standard), extrahiert, gefiltert und auf einer 15 m langen Megabore-DB-Wax-Säule getrennt. Der Fluss nach der Trennsäule wurde mithilfe eines mikrofluidischen Splitters zur gleichzeitigen Detektion zwischen MSD und FID aufgeteilt. Die Nachweisgrenzen für den FID-Detektor betrugen 0,5 µg/mL (1,2-Propandiol und Triethylenglycol) bzw. 0,25 µg/mL (Glycerin) mit einem linearen Bereich von 2–2000 µg/mL (1,2-Propandiol und Triethylenglycol) bzw. 1–4000 µg/mL (Glycerin). Die Nachweisgrenzen für den MSD-Detektor betrugen 2 µg/mL (1,2-Propandiol und Triethylenglycol) bzw. 4 µg/mL (Glycerin) mit einem linearen Bereich von 20–2000 µg/mL (1,2-Propandiol und Triethylenglycol) bzw. 40–4000 µg/mL (Glycerin). Eine signifikante Verbesserung der Sensitivität der MSD kann durch die Verwendung des Detektionsmodus Selective Ion Monitoring (SIM) erreicht werden. Obwohl die Ergebnisse der FID- und MSD-Analysen in hohem Maße korrelierten, begrenzt eine marginale chromatographische Auflösung zwischen Glycerin und Triethylenglycol die Anwendbarkeit der FID auf Proben, die nur geringe Mengen dieser beiden Feuchthaltemittel enthalten. Der Einsatz von MSD verbessert die Zuverlässigkeit der quantitativen Ergebnisse enorm, da eine unzureichende chromatographische

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Auflösung mit massenselektiver Detektion kompensiert werden kann. [Beitr. Tabakforsch. Int. 25 (2013) 576–585]

RESUME

Le présent document décrit comment une méthode existante de chromatographie en phase gazeuse (CPG) a été modifiée pour intégrer une détection simultanée par spectrométrie de masse (DSM) et par ionisation de flamme (DIF) dans l'analyse des agents humectants présents dans le tabac. Le glycérol, le propylène glycol et le triéthylène glycol ont été analysés dans des tabacs étiquetés comme tabac à rouler " roll-your-own " (RYO), cigare, cigarette, tabac à priser ou à chiquer et narguilé. Le tabac a été extrait dans du méthanol contenant du butane 1,3 diol (standard interne), filtré et séparé sur une colonne en cire mégabore DB de 15 m. Le flux post-colonne a été réparti à l'aide d'un dispositif de séparation de micro-fluides entre la détection par spectrométrie de masse et la détection par ionisation de flamme pour une détection simultanée. Les limites de détection pour le détecteur par ionisation de flamme étaient de 0,5 µg/mL (propylène glycol et triéthylène glycol) et de 0,25 µg/mL (glycérol) avec un intervalle linéaire de 2–2000 µg/mL (propylène glycol et triéthylène glycol) et de 1–4000 µg/mL (glycérol). Les limites de détection pour le détecteur par spectrométrie de masse étaient de 2 µg/mL (propylène glycol et triéthylène glycol) et de 4 µg/mL (glycérol) avec un intervalle linéaire de 20–2000 µg/mL (propylène glycol et triéthylène glycol) et de 40–4000 µg/mL (glycérol). Une amélioration significative dans la sensibilité de la DSM peut être obtenue en employant le mode de détection par contrôle d'ion sélectif. Bien qu'un degré de corrélation élevé ait été observé entre les résultats des analyses DIF et DSM, une résolution chromatographique marginale entre le glycérol et le triéthylène glycol limite l'applicabilité de la DIF aux échantillons contenant de basses teneurs de ces deux agents humectants. L'utilisation de la DSM augmente considérablement la fiabilité des résultats quantitatifs car il est possible de compenser une résolution chromato-graphique inadéquate en utilisant la sélectivité de masse lors de la détection. [Beitr. Tabakforsch. Int. 25 (2013) 576–585]

INTRODUCTION

Humectants such as glycerol, propylene glycol, and triethylene glycol have been added to tobacco products for many years to facilitate processing of the cured tobacco leaf, retain moisture, and increase shelf life (1–5). Humectant concentrations vary greatly among different tobacco product types (cigarettes, hookah, etc.). For example, humectants in products such as cigarettes and pipe tobacco are added at levels that maintain moisture content without compromising the burn characteristics of the tobacco (2).

In 1963 FRIEDMAN and RAAB described a method for determining glycerol, diethylene glycol, and propylene glycol by gas chromatography (GC) (6). The multistep sample preparation involved Soxhlet extraction, reflux in acetone, evaporation, reconstitution in methanol, and filtration. The extracts were analyzed via GC with a 6 foot stainless steel DB-Wax packed column.

A collaborative study established in 1970 used GC with either thermal conductivity or FID for the determination of glycerol, propylene glycol, and triethylene glycol (7). Anethole was used as an internal standard. In contrast to the method developed in 1963, sample preparation was simple and involved shaking the tobacco in methanol and injecting the supernatant onto the GC. As written, the method described in the 1970 collaborative study was applicable to tobaccos containing 1 to 3.5% of a given humectant (7). In 1971, the collaborative study was continued using a modified method where 1,3-butanediol replaced anethole as the internal standard (8). This modification addressed coelution problems reported in the 1970 collaboration between the internal standard and triethylene glycol. Overall, the results of the 1971 study showed improvements in precision for the determination of propylene glycol and glycerol relative to the 1970 collaborative study.

It appears that there was no further research on analytical methods for the determination of humectants in tobacco products until the 1990's, when CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) carried out a series of collaborative experiments between 1993 and 1999 to study sample preparation, extraction procedures, and analytical parameters (9). These experiments, which focused on the analyses of glycerin and propylene glycol in tobacco and tobacco products, resulted in the publication of CORESTA Recommended Method (CRM) No 60 in 2005, which was modified in subsequent versions to include updated repeatability and reproducibility statistics (10). In this method, methanol is used as the extracting solvent and analyses are performed on a fused silica column with FID detection. It is important to point out that this method does not include the analysis of triethylene glycol in tobacco products. Furthermore, it is unknown if triethylene glycol was considered as an analyte at any point during method development.

Following passage of the Tobacco Act in 1997 by the Canadian government, HEALTH CANADA developed and published an analytical method for the determination of humectants in whole tobacco (11). Health Canada Official Method T-304 is similar to CORESTA CRM No 60 in that humectants are determined by analyzing methanolic extracts of tobacco via GC with flame ionization detection (FID). Method T-304 uses a DB-Wax fused silica column with 1,3-butanediol as the internal standard. Some improvement was shown over previous methods in that the analysis was completed in less than 10 min. However, the chromatographic conditions described in Method T-304 resulted in inadequate separation of the glycerol and triethylene glycol peaks (6.119 min and 6.220 min, respectively). Such poor resolution is particularly challenging when one of the humectants in question is formulated at a significantly higher concentration than the other. At best, the resulting chromatography would produce a shoulder peak for the less prominent humectant. Less favorably, the smaller peak could be completely assimilated into the larger peak. While triethylene glycol is not used as frequently as in the past, it can still be found in tobacco products. Therefore it is important to have a method that

can provide chromatographic separation and selective detection of glycerol and triethylene glycol.

Although FID is a sensitive detection technique, it is not selective and relies on chromatographic retention time to differentiate analytes. As was discussed in reference to Health Canada Official Method T-304, difficulties can arise in data analysis when peaks are not well resolved. MSD has the advantage of mass selectivity, which allows for peak identification that is not dependent on chromatographic resolution unless the component masses cannot be resolved. This paper describes a comparison of MSD and FID for GC analysis of humectants by post-column splitting of the column effluent prior to detection. The combination of MSD and FID with GC provides a rapid, sensitive and selective method for determination of humectants in tobacco products.

MATERIALS AND METHODS

Materials

Glycerol, propylene glycol, triethylene glycol, 1,3-butanediol, and methanol were purchased from Sigma-Aldrich. Three roll-your-own (RYO), thirteen cigar, eleven cigarette, ten moist snuff, and seven hookah tobacco products were purchased from tobacconists in Laurel, Maryland.

Standards for calibration

Standards of humectants were prepared according to the Health Canada method (11). Standards containing glycerol, propylene glycol, and triethylene glycol were prepared by dissolving the humectants in extraction solution (methanol containing 2.0 mg/mL 1,3-butanediol). Diluting from stock solutions, working standards were prepared containing glycerol (0.8, 1.6, 2.8, and 4.0 mg/mL) and both propylene glycol and triethylene glycol with concentrations of 0.4, 0.8, 1.4, and 2.0 mg/mL. Linear dynamic range and limits of detection and quantitation were determined using the calibrant solutions.

Tobacco extraction

Four grams of each tobacco product were extracted with 50 mL of extraction solution and shaken for 1 hour on a Burrell model 75 wrist action shaker. After the samples settled for approximately 30 min, the extracts were filtered through Whatman 30 µm filter paper. Since hookah tobacco contains as much as 65% humectants by weight, these samples were further diluted by a factor of 50 with extraction solution before injection (12). An aliquot of each extract was transferred to an autosampler vial and analyzed by GC-MS-FID. Each tobacco sample was extracted twice and each extract was analyzed three times.

Instrumental parameters

Tobacco extracts were analyzed using an Agilent 6890N GC with a split/splitless inlet. Simultaneous detection was achieved with an Agilent 5975 inert XL mass selective detector and flame ionization detector. Chromatographic parameters were chosen to mimic the Health Canada method for the determination of humectants in tobacco (11). Extracts were analyzed by splitless injection of 1 µL at 250 °C. Analytes were separated on a 15 m × 0.53 mm × 1 µm DB-Wax column (Agilent) with helium carrier gas at constant pressure of 14.5 psi. The GC oven was held at 120 °C for 2 min, then ramped at 15 °C/min to 180 °C and held for 4 min (total run time of 10 min).

Flow from the analytical column was split using a microfluidic splitter (Agilent) with a 1 m × 0.32 mm uncoated deactivated fused-silica (UCDFS) restrictor tube at 12.5 psi to the FID and a 2 m × 0.18 mm UCDFS restrictor tube at 2 psi to the MSD. The FID was run at 300 °C with 30 mL/min hydrogen flow, 400 mL/min air flow, and 10 mL/min makeup flow. The MSD transfer line was maintained at 280 °C, MS source at 230 °C, and MS quadrupole at 150 °C. The MSD was run in scan mode with mass range between 30–300 daltons.

A post run that included reversing the flow from the electronic pneumatics control (EPC) at 20 psi was conducted at the end of each analysis for 5 min at an oven temperature of 220 °C. This backflush was employed to prevent carryover and to allow any retained analytes to exit the column through the split vent of the inlet. Agilent ChemStation software (D.02) was used for data acquisition and data analysis.

RESULTS AND DISCUSSION

Separation of humectants via GC-MS-FID

The purpose of this study was to modify an existing method for the determination of humectants in tobacco to provide sufficient selectivity and sensitivity to resolve analytes of interest. Figure 1 shows typical chromatograms of a standard solution using MS and FID detection. Similar to results observed using Health Canada Official Method T-304 (11), the data in Figure 1 show marginal chromatographic resolution of the glycerol and triethylene glycol peaks (R=1.03). While peak overlap in Figure 1b appears to be minor, low resolution can convolute quantitative results particularly when using non-selective detection techniques such as FID. It is important to note that previous method development (7–11, 13) focused on the quantitation of humectants in cigarette tobacco, which are relatively low in total humectant concentration. This is reflected in the scope of application for the Health Canada humectants method (11), which describes the expected range of individual humectants to be 0.5% to 4.0% on an "as received" basis. This range is applicable to cigarettes, roll-your-own, and most conventional pipe tobaccos. However, the levels observed in hookah-type tobaccos are substantially higher, as shown in the results presented here. The effect of substantially increased levels of humectants on the analysis by GC-FID has been observed in this study. Difficulties arose when a tobacco product contained a large amount of glycerol, which produced a broad peak around 9 min (results not shown). Since the method used FID, it was impossible to determine if it was glycerol, triethylene

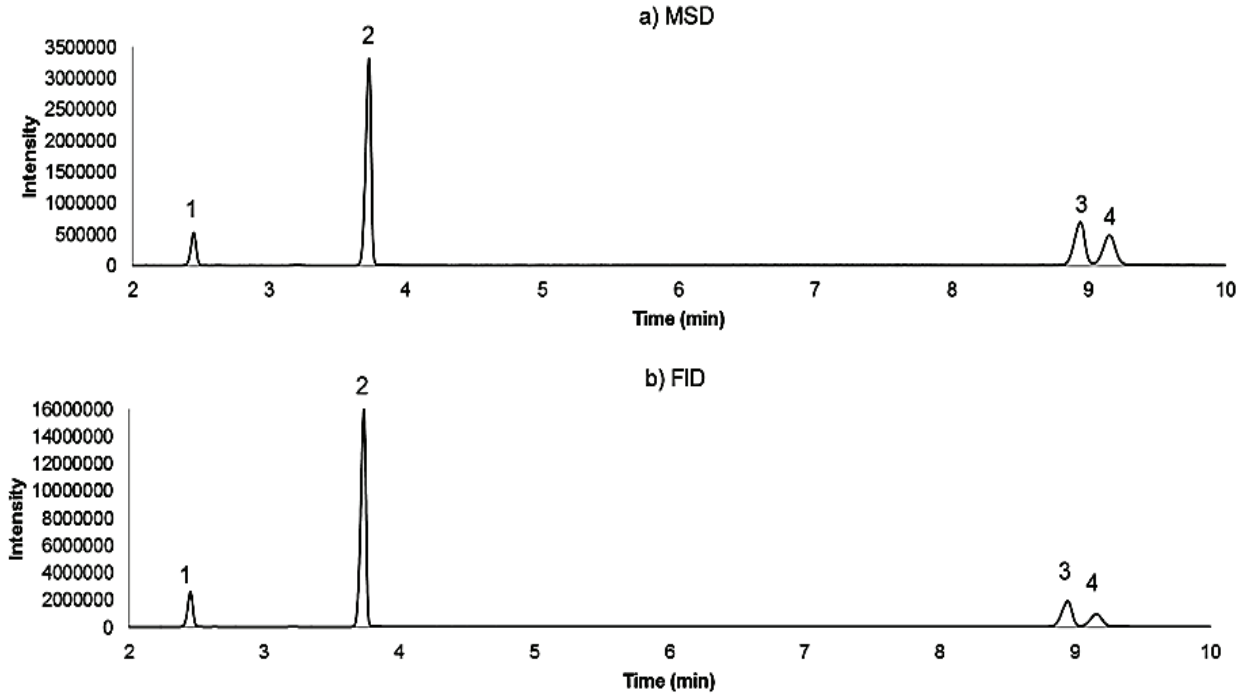

Figure 1. Comparison of chromatograms using a) MSD and b) FID of a humectant standard. Peak labels 1: propylene glycol, 2: 1,3-butanediol (IS),3:glycerol, 4:triethylene glycol.

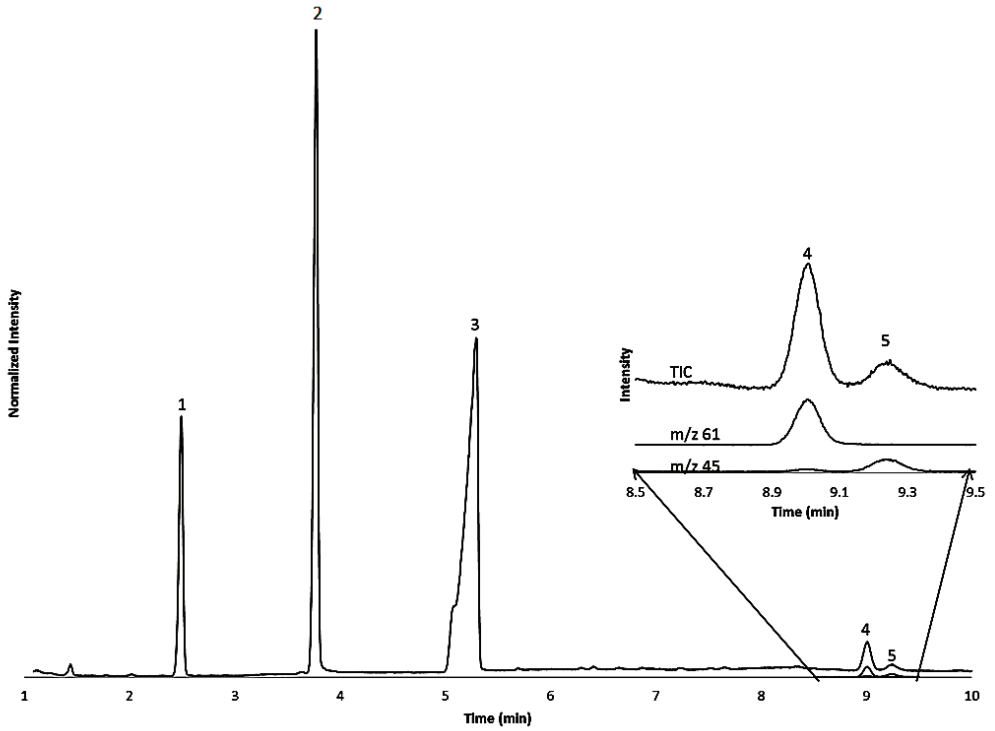

Figure 2. Chromatogram of tobacco sample #5. Inset demonstrates the added benefit of using MSD to ensure chromatographic separation of glycerol and triethylene glycol. Peak labels 1: propylene glycol, 2: 1,3-butanediol (IS), 3: nicotine, 4: glycerol, 5: triethylene glycol.

Table 1. Retention time, limit of detection, linear range, and calibration curve R^2 values for humectants.

Humectant	RT (min)	Limit of detection (µg/mL)		Linear range (µg/mL)		R^2	
		FID	MSD	FID	MSD	FID	MSD
Propylene glycol	2.4	0.5	2	2–2000	20–2000	0.9998	1.0000
Glycerol	8.9	0.25	4	1–4000	40–4000	0.9999	1.0000
Triethylene glycol	9.1	0.5	2	2–2000	20–2000	1.0000	0.9999

glycol, or a combination of glycerol and triethylene glycol. Ultimately, the sample was diluted substantially to bring the level of humectant(s) within the range of the method. As a result, the peak was identified as glycerol but, given the lack of specificity in the detection technique (FID) and the level of dilution, this laboratory was unable to determine if a minor level of triethylene glycol was present in the undiluted sample.

Although chromatographic resolution might have been improved with the selection of another stationary phase, significant modifications to Health Canada Official Method T-304 were not among the goals of this study. The addition of MS detection is an enhancement to the method that does not require alterations to the sample preparation, stationary phase, or chromatographic conditions. MSD can provide the mass selectivity to distinguish glycerol and triethylene glycol and the broad peak around 9 min could have been deconvoluted through the use of extracted ion chromatograms (EIC) as seen in Figure 2. Using MS detection, poor chromatographic resolution can be nullified as a limitation of the method.

Analytical parameters

Once the method was developed, linearity, linear range, limit of detection, and carryover were evaluated. Standard solutions of glycerol, propylene glycol, and triethylene glycol were analyzed to determine these parameters. Table 1 shows the limit of detection, linear range, and correlation coefficient (R^2) values determined using the calibration curves taken from MSD and FID data. It should be noted that these parameters were determined based on the injected concentrations. The amount of analyte that actually reached each detector was dependent on the microfluidic splitter, which provides a split ratio of approximately 15:1 with the majority of the column effluent going the FID.

Carryover was evaluated as a potential source of error and was eliminated by implementing a 5 min post-run backflush. The post-run conditions involved an increase in the oven temperature to 220 °C (40 °C hotter than the ending temperature of the GC method) and a pressure from the EPC of 20 psi, keeping the inlet pressure at 14.5 psi. This reverses column flow, which allows any retained analytes to exit the column through the split vent of the inlet. Use of this post-run step eliminated any carryover from the previous tobacco sample, as evidenced in Figure 3 where vanillin and ethyl vanillin were identified by spectral matching with the NIST mass spectral library.

Quantitative analysis of tobacco samples

Figure 4 shows chromatograms that are characteristic of each tobacco type. The humectants were confirmed by retention time and mass spectra. These chromatograms show that in general RYO, cigarettes, cigars and moist snuff contain relatively low levels of glycerol. It is also evident from Figure 4 that hookah tobaccos contain a large amount of glycerol. In such cases, the mass spectral data was useful in demonstrating that there was no co-elution of glycerol and triethylene glycol in the FID results. It should be noted that triethylene glycol was found in six of the 44 tobacco product samples analyzed. In all six of these samples, it was possible to resolve the triethylene glycol and glycerol peaks in the FID chromatograms. This is because, in each case, both peaks were small and did not have sufficient peak width to interfere significantly with one another. The detection of triethylene glycol in the six samples was further confirmed using extracted ion chromatograms as demonstrated in Figure 2.

The concentration of humectants in each sample was quantified using data from MSD and FID. The average concentrations (percent by weight) from three injections of two extractions of each tobacco sample are presented in Table 2. These concentrations are based on "as received" weight for all tobacco products. Results shown in these tables were calculated from both MSD and FID data. Correlation between MSD and FID data was also evaluated by plotting concentration of humectants (% by weight) from MSD results versus FID results as seen in Figure 5. An R^2 of 0.9999 was calculated from linear regression analysis and demonstrates a high degree of correlation between the results from FID and MSD. Figure 6 focuses on the results for RYO, cigarette, cigar, and snuff tobaccos and shows that, although there is some clustering of data, these tobacco products overlap with respect to humectant content. As was discussed previously, hookah tobaccos contain significantly greater levels of humectants. This is observable in both Table 2 and Figure 4.

In this study, the results for the MSD and FID were very similar in accuracy and precision. The use of mass spectrometric detection adds a level of selectivity to the analytical method that is not provided by FID detection. It should be noted that, on comparison to the current MSD results (in scan mode), FID detection does have the advantage of lower LODs (limit of detection) and extended calibration range to lower concentrations. The detection limits for the individual humectants by MSD are in the 2 to 4 ppm range while the detection limits by FID are in the 0.25 to 0.5 ppm range. Even though the MSD has slightly higher LODs than the FID, MSD is well suited for measurement of humectant in the products of interest and provides confirmation of the chemical identity of the humectant compounds measured. In a head-to-head comparison, this study shows that a GC/MSD method is comparable to GC/FID approach in the accuracy/precision of measured values and offers adequate

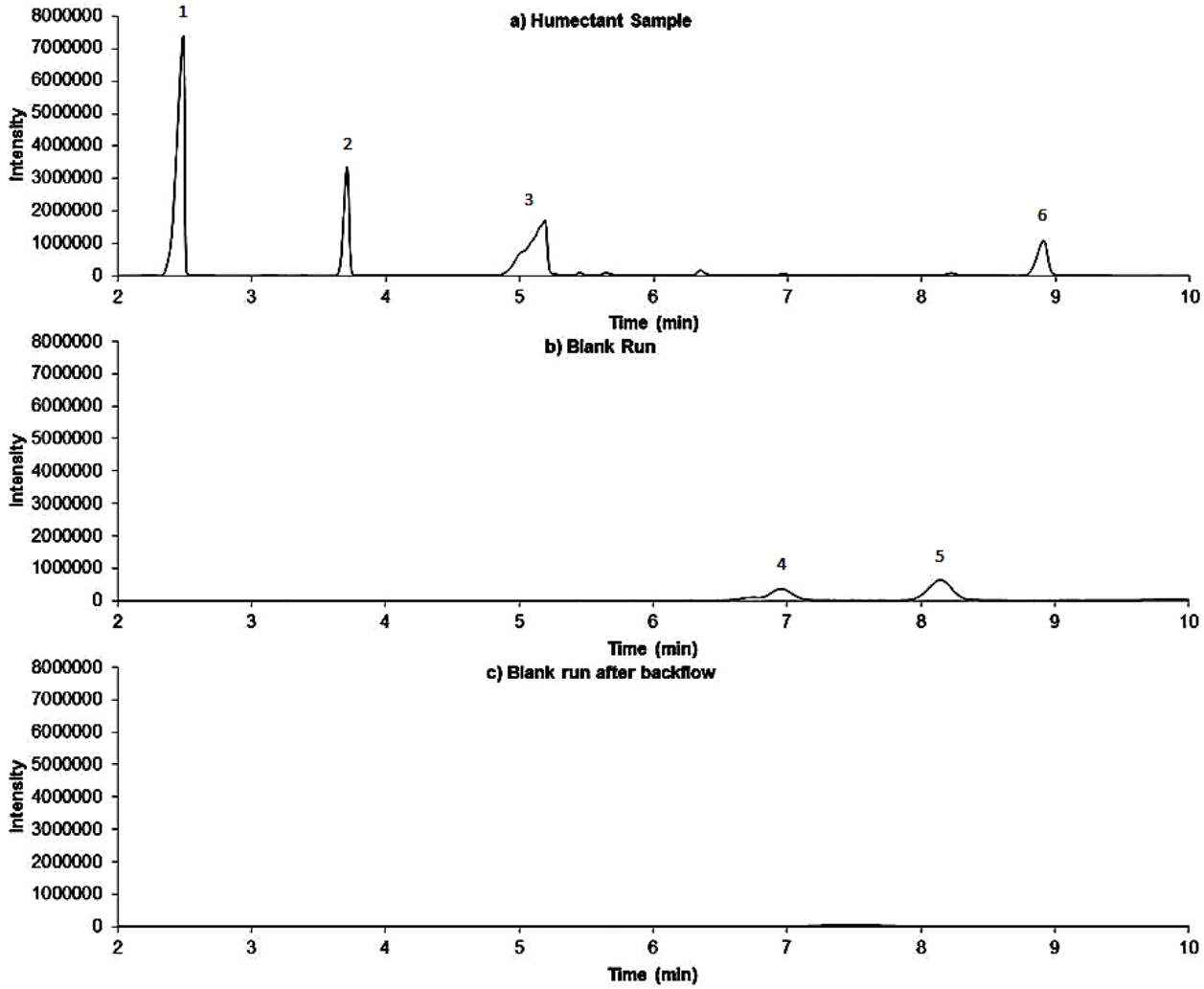

Figure 3. Evaluation of a backflush post-run. a) chromatogram of a tobacco sample b) chromatogram of a methanol blank run after the tobacco sample with no post-run c) chromatogram of a methanol blank run after a tobacco sample with post-run. Peak labels 1: propylene glycol 2: 1,3-butanediol (IS) 3: nicotine 4: ethyl vanillin 5: vanillin 6: glycerol.

detection limits and chemical specificity not offered by the GC/FID approach. This study demonstrates that GC/MSD method present here provides the appropriate calibration range, accuracy and chemically specificity needed for measuring % humectants in a wide range of tobacco product types. In addition, the determination of humectants was improved using a post-run backflush to eliminate carry-over and late eluting compounds.

Statistical analysis of the results of this study shows good correlation between results calculated from FID and MSD data. The high degree of correlation between the data sets might suggest that the added resolving power of the MSD is unnecessary. Such a conclusion is unwarranted based on the limited scope of the product analysis. In samples containing triethylene glycol, the levels of humectants were so low that the separation of the triethylene glycol and glycerol peaks was sufficient for quantitation. Since triethylene glycol was not found in samples that contained high levels of glycerol, chromatographic resolution under these conditions could not be investigated. It is important to point out that, without MSD, verification that triethylene glycol was absent from samples with high glycerol content would have been difficult. It is certain that application of the existing method (11) to hookah-type products will produce ambiguous results if the samples contain large amounts of glycerol. For the analyst attempting to identify the humectants present in the product using FID alone, dilution may be required to resolve glycerol and triethylene glycol, if one or both are thought to be present in the sample. Given that a minor component could be diluted to a level below the LOD, a limitation to the application of the existing method (11) is exposed.

Regardless of the product analyses shown here, mass spectrometry is the detection method of choice when using the GC conditions described in this report to provide the chemical selectivity not offered by flame ionization detection. Furthermore, utilization of selective ion monitoring further enhances the sensitivity of MSD and potentially erases the apparent sensitivity advantage of FID.

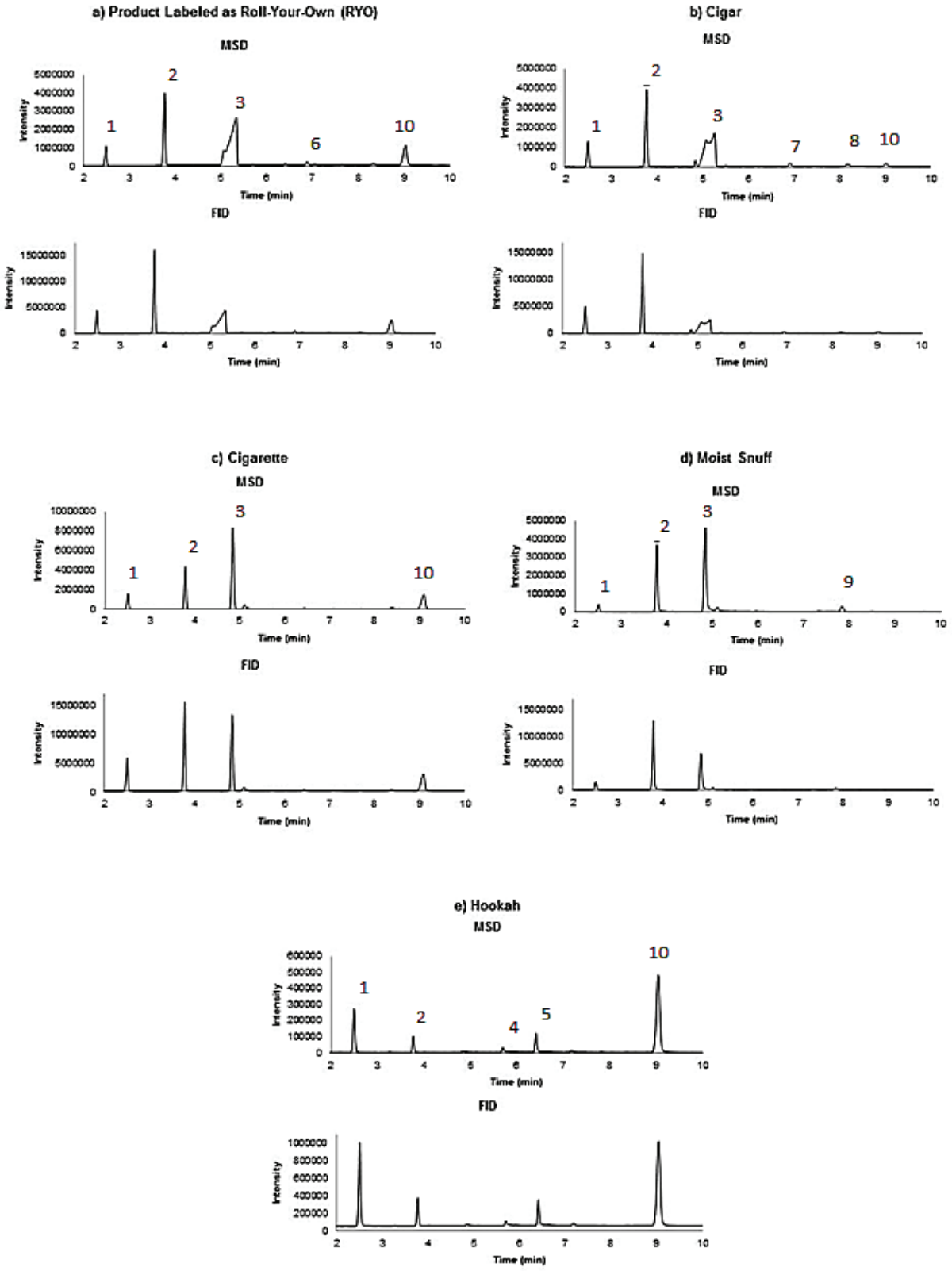

Figure 4. MSD and FID chromatograms of tobacco samples analyzed. a) product labeled as RYO b) cigar c) cigarette d) moist snuff e) hookah. Peak labels 1: propylene glycol 2: 1,3-butanediol 3: nicotine 4: glyceraldehyde 5: dihydroxyacetone 6: sorbic acid 7: decalactone 8: piperonal 9: undecalactone 10: glycerol

Table 2. Humectant concentrations detected in different tobacco types by GC with MSD and FID. BQL and ND indicate results that were below quantitation limit or below the limit of detection. n = 2, three injections per replicate.

ID	Humectants (% wt/wt)							
	MSD				FID			
	Propylene glycol	Glycerol	Triethylene glycol	Total	Propylene glycol	Glycerol	Triethylene glycol	Total
RYO (roll your own)								
1	1.23 ± 0.03	1.30 ± 0.05	ND	2.53 ± 0.03	1.22 ± 0.04	1.26 ± 0.04	ND	2.48 ± 0.03
2	0.83 ± 0.01	1.04 ± 0.05	ND	1.87 ± 0.04	0.82 ± 0.01	1.01 ± 0.02	ND	1.83 ± 0.02
3	0.87 ± 0.01	1.45 ± 0.03	ND	2.32 ± 0.03	0.858 ± 0.003	1.39 ± 0.03	ND	2.25 ± 0.03
Cigar								
4	1.59 ± 0.01	0.274 ± 0.005	ND	1.86 ± 0.02	1.56 ± 0.01	0.274 ± 0.005	ND	1.83 ± 0.01
5	1.33 ± 0.01	0.275 ± 0.007	BQL	1.63 ± 0.01	1.30 ± 0.01	0.27 ± 0.01	0.050 ± 0.001	1.62 ± 0.01
6	1.40 ± 0.02	0.277 ± 0.001	ND	1.68 ± 0.02	1.37 ± 0.02	0.28 ± 0.01	0.040 ± 0.001	1.69 ± 0.02
7	0.9 ± 0.4	0.16 ± 0.04	ND	1.1 ± 0.5	0.9 ± 0.4	0.18 ± 0.04	ND	1.1 ± 0.4
8	0.77 ± 0.02	0.112 ± 0.004	0.117 ± 0.002	1.00 ± 0.01	0.75 ± 0.02	0.13 ± 0.01	0.140 ± 0.006	1.02 ± 0.01
9	0.30 ± 0.03	0.092 ± 0.001	0.109 ± 0.004	0.50 ± 0.03	0.32 ± 0.02	0.111 ± 0.001	0.140 ± 0.004	0.57 ± 0.03
10	2.1 ± 0.1	1.31 ± 0.03	ND	3.4 ± 0.2	2.1 ± 0.1	1.28 ± 0.03	ND	3.4 ± 0.2
11	0.96 ± 0.06	0.134 ± 0.001	ND	1.09 ± 0.06	0.94 ± 0.06	0.151 ± 0.001	ND	1.09 ± 0.06
12	1.08 ± 0.01	1.71 ± 0.02	ND	2.79 ± 0.03	1.08 ± 0.01	1.63 ± 0.02	ND	2.71 ± 0.02
13	1.14 ± 0.01	0.247 ± 0.001	ND	1.39 ± 0.01	1.12 ± 0.01	0.258 ± 0.001	ND	1.38 ± 0.01
14	1.05 ± 0.02	0.118 ± 0.003	0.39 ± 0.01	1.56 ± 0.01	1.02 ± 0.02	0.140 ± 0.005	0.390 ± 0.004	1.55 ± 0.01
15	1.70 ± 0.06	0.129 ± 0.002	0.37 ± 0.01	2.20 ± 0.04	1.67 ± 0.06	0.153 ± 0.001	0.37 ± 0.01	2.19 ± 0.04
16	0.96 ± 0.02	0.158 ± 0.002	ND	1.12 ± 0.02	0.96 ± 0.02	0.183 ± 0.001	ND	1.14 ± 0.02
Cigarette								
17	1.00 ± 0.01	2.61 ± 0.04	ND	3.61 ± 0.03	0.989 ± 0.005	2.49 ± 0.04	ND	3.48 ± 0.03
18	0.91 ± 0.01	1.83 ± 0.02	ND	2.74 ± 0.03	0.90 ± 0.01	1.73 ± 0.02	ND	2.63 ± 0.03
19	0.61 ± 0.01	2.15 ± 0.07	ND	2.76 ± 0.08	0.607 ± 0.003	2.03 ± 0.07	ND	2.64 ± 0.07
20	0.646 ± 0.004	1.61 ± 0.02	ND	2.26 ± 0.03	0.641 ± 0.003	1.51 ± 0.02	ND	2.15 ± 0.02
21	1.049 ± 0.005	3.66 ± 0.05	ND	4.71 ± 0.06	1.031 ± 0.004	3.54 ± 0.06	ND	4.57 ± 0.06
22	ND	0.134 ± 0.002	ND	0.134 ± 0.002	ND	0.152 ± 0.002	ND	0.152 ± 0.002
23	1.238 ± 0.004	1.819 ± 0.009	ND	3.06 ± 0.01	1.219 ± 0.003	1.712 ± 0.006	ND	2.931 ± 0.007
24	1.314 ± 0.005	1.922 ± 0.006	ND	3.24 ± 0.01	1.293 ± 0.005	1.812 ± 0.006	ND	3.105 ± 0.007
25	0.292 ± 0.002	2.75 ± 0.09	ND	3.04 ± 0.09	0.310 ± 0.001	2.63 ± 0.09	ND	2.94 ± 0.09
26	0.732 ± 0.002	2.66 ± 0.02	ND	3.39 ± 0.02	0.736 ± 0.004	2.554 ± 0.009	ND	3.290 ± 0.006
27	0.96 ± 0.01	1.37 ± 0.03	ND	2.33 ± 0.04	0.95 ± 0.01	1.29 ± 0.03	ND	2.24 ± 0.04
3R4F	BQL	2.52 ± 0.02	ND	2.52 ± 0.02	0.04 ± 0.02	2.40 ± 0.02	ND	2.44 ± 0.03
Moist snuff								
28	0.181 ± 0.001	0.01 ± 0.03	ND	0.19 ± 0.03	0.200 ± 0.002	ND	ND	0.200 ± 0.002
29	0.344 ± 0.002	0.03 ± 0.04	ND	0.37 ± 0.03	0.357 ± 0.002	ND	ND	0.357 ± 0.002
30	0.002 ± 0.003	ND	ND	ND	ND	ND	ND	ND
31	0.354 ± 0.001	ND	ND	0.354 ± 0.001	0.370 ± 0.003	ND	ND	0.370 ± 0.003
32	0.384 ± 0.001	ND	ND	0.384 ± 0.001	0.410 ± 0.002	ND	ND	0.410 ± 0.002
33	ND	ND	ND	ND	ND	ND	ND	ND
34	BQL	ND	ND	BQL	ND	ND	ND	ND
35	BQL	ND	ND	BQL	ND	ND	ND	ND
36	BQL	4.19 ± 0.03	ND	4.19 ± 0.03	ND	4.11 ± 0.02	ND	4.11 ± 0.02
37	ND	ND	ND	ND	ND	ND	ND	ND
38	ND	40.2 ± 0.5	ND	40.2 ± 0.5	ND	40.2 ± 0.6	ND	40.2 ± 0.6
39	1.56 ± 0.03	43.3 ± 0.9	ND	44.9 ± 0.9	1.69 ±0.003	43.3 ± 0.9	ND	45 ± 1
40	3.2 ± 0.1	34 ± 2	ND	37 ± 2	3.3 ± 0.01	34 ± 2	ND	37 ± 2
41	9.37 ± 0.04	19.5 ± 0.2	ND	28.9 ± 0.2	9.62 ± 0.06	19.3 ± 0.2	ND	28.9 ± 0.2
42	10.35 ± 0.06	21.2 ± 0.2	ND	31.6 ± 0.2	10.69 ± 0.02	21.2 ± 0.1	ND	31.9 ± 0.1
43	9.7 ± 0.2	23.5 ± 0.4	ND	33.2 ± 0.5	10.10 ± 0.03	23.6 ± 0.3	ND	33.7 ± 0.2
44	10.1 ± 0.1	20.4 ± 0.3	ND	30.5 ± 0.4	10.31 ± 0.09	20.2 ± 0.1	ND	30.5 ± 0.2

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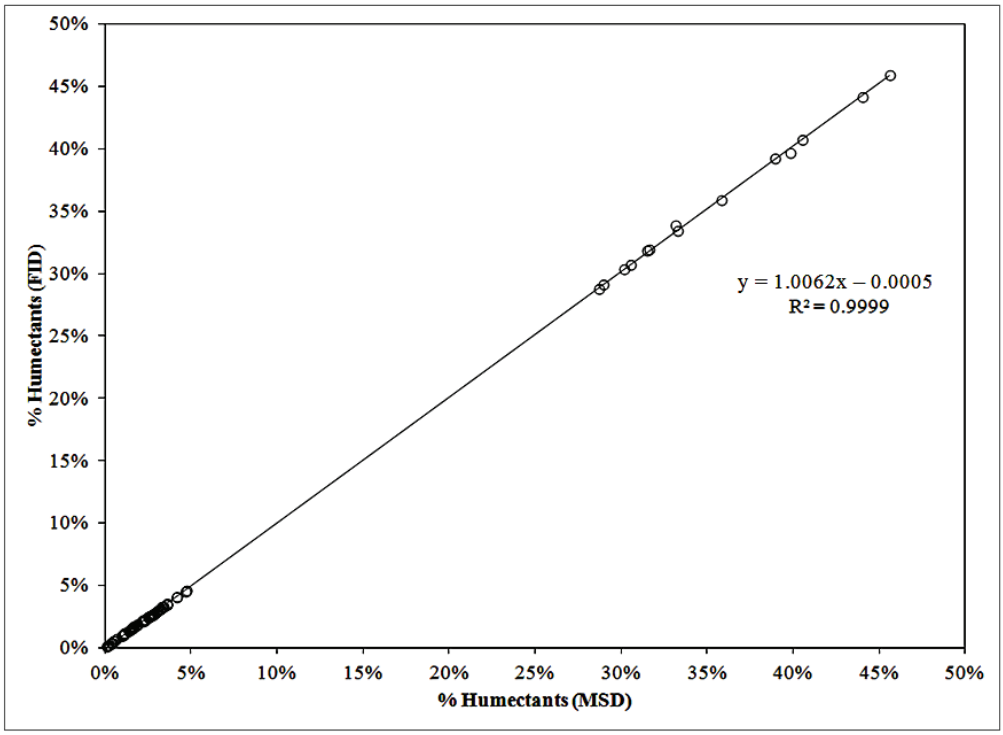

Figure 5. Correlation of total % humectants by GC-FID vs. total % humectants by GC-MSD measured in various tobacco products.

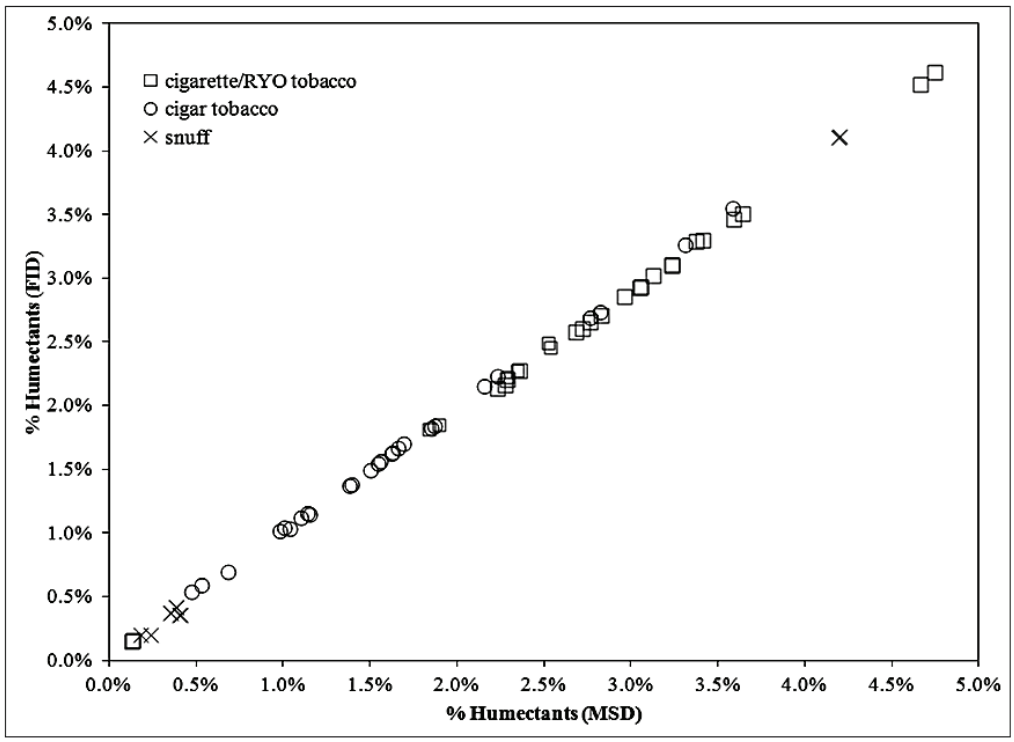

Figure 6. Correlation of total % humectants by GC-FID vs. total % humectants by GC-MSD measured in RYO, Cigarette, Cigar, and Snuff tobaccos.

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