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Determination of Free Solanesol Levels in Cigarette Filters by Liquid Chromatography–Mass Spectrometry

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Abstract

Solanesol, a naturally occurring constituent of tobacco, has been utilized as a good marker for environmental tobacco smoke particulate and as a noninvasive predictor of mainstream cigarette smoke tar and nicotine intake under naturalistic smoking conditions. A fast and accurate method for measuring free solanesol to assess tobacco smoke exposure is highly desirable. We have developed and validated a new environmentally friendly, high-throughput method for measuring solanesol content in discarded cigarette filter butts. The solanesol deposited in the used filters can be correlated with mainstream smoke deliveries of nicotine and total particle matter to estimate constituent delivery to smokers. A portion of filter material is removed from cigarette butts after machine smoking, spiked with internal standard solution, extracted and quantitatively analyzed using reverse-phase liquid chromatography coupled to a triple-quadrupole mass spectrometer. The new method incorporates a 48-well plate format for automated sample preparation that reduces sample preparation time and solvent use and increases sample throughput 10-fold compared to our previous method. Accuracy and precision were evaluated by spiking known amounts of solanesol on both clean and smoked cigarette butts. Recoveries exceeded 93% at both low and high spiking levels. Linear solanesol calibration curves ranged from 1.9 to 367 μg/butt with a 0.05 μg/butt limit of detection.

Introduction

Tobacco use, particularly smoking, remains one of the leading causes of preventable diseases, disabilities and death worldwide (1-3). Tobacco combustion generates a complex chemical mixture containing more than 7,000 constituents (4-8). Many of these constituents are responsible for a wide range of diseases, including lung diseases, cardiovascular

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Disclaimers

Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or by the U.S. Department of Health and Human Services. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Declaration of Interests

None declared.

diseases, lung cancer and other forms of cancer (9-14). Assessing smokers' exposure to cigarette smoke constituents is crucial to address health problems. Smoking machine regimens developed over the years by the Federal Trade Commission (FTC), International Organization for Standardization (ISO) and Health Canada (HC) have been useful tools for comparing and characterizing different cigarette brand smoke deliveries (15, 16). However, such measures do not directly reflect smokers' exposure or how product design impacts use. Thus, accurate exposure assessment using standardized testing has been a challenging problem. A smoker, unlike the smoking machine protocols, alters the inhalation of smoke constituents by changing the volume of his/her puff, the time between puffs, the number of puffs and the obstruction of the ventilation holes of the filter (deliberate or inadvertent) to capture the desired nicotine dose. In terms of assessing 'dose' per cigarette, two different smokers or even the same smoker may not smoke cigarettes consistently on a stick-by-stick basis (15-21).

Several approaches have investigated smokers' exposure to cigarette smoke constituents. The 'gold standard' has been measuring select exposure biomarkers, such as urinary nicotine metabolites (22-27), salivary cotinine (28-30), serum thiocyanate (31-34) and exhaled carbon monoxide (35, 36). However, these measures provide time-averaged information rather than describing individual cigarette consumption patterns. Sample collection for biomarker techniques may be invasive, require special storage and handling, volume or excretion corrections storage, and complex sample preparation. Alternatively, different approaches using spent cigarette filters for probing smoking behavior and exposure to smoke constituents have been developed. These methods include (a) visual analysis (37-40), (b) digital imaging of the filter cut surface (41, 42), and (c) analyses of filters for particulates (43, 44) and (d) nicotine (27, 45, 46). Our laboratory has used solanesol, a naturally occurring, long-chain, high-molecular-weight tobacco terpene, deposited in the cigarette filter during smoking as a means for estimating mainstream smoke constituents drawn through the filter (47-52). The solanesol levels can be correlated with nicotine, tar, nitrosamine and other constituent concentrations in cigarette smoke. These correlations can be used to estimate the 'mouth-level intake' for total mainstream cigarette smoke volume and the respective constituents' intake in natural settings noninvasively.

Over the last 20 years, our laboratory has developed several methods to measure mainstream cigarette smoke solanesol deposited in cigarette filter butts. Watson et al. (48) developed a high-performance liquid chromatography (HPLC)–tandem mass spectrometry (MS-MS) analytical method for determining solanesol content in cigarette butts using an isocratic normal phase separation with dichloromethane, hexane and methanol as the mobile phase. They demonstrated that the measured solanesol in the filter is a useful marker for estimating smoke uptake regardless of how the cigarette was smoked. Later, Polzin et al. (49) developed a method that combined LC using a normal phase column for analyte separation with a single-quadrupole mass spectrometer for detection. This method used hexane, ethyl acetate and methanol as the mobile phase, which resulted in a 70% reduction in solvent volume and incorporated automation in the sample preparation. Additionally, this method incorporated a surrogate internal standard, geranylgeraniol (GG), which is structurally similar to solanesol and improves reliability of cigarette-to-cigarette delivery estimates for nicotine and tobacco-specific nitrosamines (47, 51). Yan et al. (50) developed and validated a semiautomatic,

low-cost ultraviolet–visible (UV–Vis) method for the determination of solanesol in cigarette butts. The UV–Vis method showed consistent correlations between solanesol concentrations and cigarette constituents such as nicotine, benzene, two tobacco-specific nitrosamines (TSNAs), and four polycyclic aromatic hydrocarbons.

Other research groups have explored using solanesol to estimate the intake concentration of the constituents of cigarette smoke (53-55). Feng et al. (55) described a methodology for simultaneous estimation of the retention of both selected particulate-phase (PP) and gas/vapor-phase (GVP) smoke constituents in the respiratory tract of smokers with facile monitoring of smokers' respiratory pattern during smoking. Exposure was estimated based on the difference between the amounts of chemical inhaled and exhaled. Solanesol cigarette butt analyses were used to estimate delivery of smoke constituents during smoking. To determine the amounts of smoke constituents exhaled, the smokers' expired breath was directed through a Cambridge filter pad (CFP), from which the exhaled PP constituents were measured quantitatively and the exhaled GPV constituents were measured using an infrared spectrometer.

An important aspect of the solanesol cigarette butts analyses is the noninvasive nature of the technique, which requires no specialized equipment or devices that could impede natural smoking behaviors. Collection of the discarded butts for solanesol analysis is straight forward as no special handling or storage conditions are required. Solanesol correlations provide information on a per-cigarette basis, and thus can be utilized to estimate individualized smoke dose on a stick-by-stick or situation-by-situation basis. Thus, the discarded butts provide information on per-cigarette doses of select constituents, or by summing results across the butts collected, time-dependent exposure or situation-dependent changes in smoking patterns can be ascertained. Several analytical methods, such as gas chromatography (GC) with flame ionization detection (FID) and/or mass spectrometry detection (56, 57) and HPLC with UV (58) and/or mass detectors (57, 59-62), have been also developed for the determination of solanesol levels in different matrices.

Green chemistry, a chemical concept of environmental awareness, has grown substantially since its inception over the last two decades. By definition, 'green chemistry is the design, development and implementation of chemical products and processes to reduce or eliminate the use and generation of hazardous substances for human health and the environment' (63). To reduce waste our laboratory developed a new, simple, selective, sensitive and robust method for accurate quantification of solanesol in cigarette butts using less toxic chemical reagents without sacrificing the selectivity and reproducibility of previous analysis. The new and fully validated solanesol method was providing reliable cigarette- to-cigarette mouth level intake for nicotine and the total particle matter (TPM). The new approach has been tested and validated using a range of filtered tobacco products including traditional cigarettes, modified reduced nicotine cigarettes and filtered little cigars.

Experimental Section

Reagents

Solanesol (purity > 93%) was purchased from TCI America (Portland, OR) and GG (purity > 95%) was purchased from A.G. Scientific, Inc. (San Diego, CA). Glacial acetic acid, ammonium acetate, anethole and (−)nicotine (purity > 99%) were purchased from Sigma-Aldrich Chemicals Co. (Saint Louis, MO). Deionized water, 2-propanol and methanol were purchased from Fisher Scientific (Hampton, NH). (−)Nicotine, >99%, and anethole were purchased from Sigma-Aldrich (Milwaukee, Wisconsin). Carbon monoxide reference solutions were purchased from Airgas (Atlanta, GA). All other chemicals were obtained through Sigma-Aldrich (St. Louis, MO) in the highest purity commercially available and used without further purification.

Smoking conditions

Cigarettes were conditioned at 22°C and 60% relative humidity for at least 48 hours to reach mass equilibrium before being smoked. Cigarettes were smoked using a Cerulean SM450 20-port linear smoking machine (Milton Keynes, UK). The TPM was collected on a 44-mm CFP produced by Whatman (Buckinghamshire, UK). The smoking machine was calibrated to maintain an average airflow velocity over the cigarette of 200±50 mm/s. The airflow velocity was measured using a Filtrona VMD 100 velocity measurement digitizer (Milton Keynes, UK) connected to a Schiltknecht ThermoAir2 thermoelectric anemometer equipped with an omnidirectional probe (Gossau, Switzerland). To guarantee the method's long stability and reproducibility of results, two quality control (QC) samples, one low and one high concentration (QCL and QCH), were analyzed with each sample set. Briefly, University of Kentucky (Lexington, KY) research cigarettes were smoked using two FTC machine regimes: ISO regime (35±0.1 ml puff volume, 2 s puff duration, 60 s puff interval and ventilation unblocked) to yield the QCL sample and HC regime (55±0.2 ml puff volume, 2 s puff duration, 30 s puff interval, and 100% ventilation blocked) to yield the QCH sample. After smoking, the cigarette filter was removed from the residual tobacco rod and stored in a cryovial (Nalgene, Rochester, NY) at −20°C until further processing.

Cigarette filter preparation

For sample preparation, cigarette filters were removed from the freezer and placed in a refrigerator 24 hours. Filter cigarette samples were allowed to equilibrate to room temperature for at least two hours prior to sample preparation. A 1-cm portion of the spent cigarette filter was removed from the mouth end; the tipping paper and paper overwrap was removed, and the bare filter was placed into a 48-well plate (Agilent, Billerica, MA) for extraction. The filter samples were spiked with 100 μL of GG internal standard. A 3 mL of isopropanol aliquot was added to each well followed by agitation on an orbital shaker for 2 h at 400 rpm using an automatic Caliper® liquid handler (PerkinElmer, Boston, MA) to ensure optimal extraction. An extract aliquot was diluted with isopropanol and methanol (1:1, v/v) to achieve a concentration of the internal standard of 0.8 μg/μL. The diluted extract was directly analyzed by reverse phase high-performance liquid chromatography (RP-HPLC) coupled to a triple-quadrupole mass spectrometer. To generate the solanesol calibration curve, solanesol stock solution (1 μg/μL) and GG stock solution (4 μg/μL) were prepared in

isopropanol. The GG stock solution was used as internal standard without further dilution (49). The solanesol stock solution was diluted with isopropanol to make 10 primary standard solutions containing solanesol concentrations from 12.5 to 1,000 μg/mL. An aliquot of each primary standard solution was spiked onto a 1-cm blank (unused) cigarette filter to prepare 10 calibration standards. Each calibration standard was spiked with 100 μL of GG internal standard. These calibration standards were prepared in tandem with the analytical samples. An aliquot of each standard was injected on the HPLC column to obtain a calibration curve range of 1.90–360 μg of solanesol.

Analysis of cigarette filter butt solanesol

The diluted extract was analyzed using an Agilent 1200 HPLC coupled to a Thermo Finnigan TSQ Quantum MS/MS® (ThermoFisher, San Jose, CA). The HPLC separation was achieved using a reverse-phase Waters Symmetry Shield RP18 (2.1 mm x 150 mm, 3.5 μm particle size) column. The column temperature was kept at 45° C and the injection volume was 5 μL. The mobile phase consisted of (A) 0.2% acetic acid (v/v) in methanol containing 5 mM ammonium acetate and (B) 100% isopropanol. The HPLC gradient conditions applied are listed in Table I. The HPLC eluents were ionized by atmospheric pressure chemical ionization in positive-ion mode using nitrogen as the sheath and auxiliary gases at pressures of 60 psi and 20 psi, respectively. Argon was used as the collision gas at pressure of 1 mTorr. The mass spectrometer was operated in the selected reaction monitoring mode as shown in Table II.

Determination of nicotine and TPM deliveries were performed as previously described (64, 65) and used to correlate with the corresponding filter butt solanesol levels. Although we used the analytical procedures previously published, smoking conditions in this study were modified to obtain a range of mainstream smoking deliveries that would more reflect or mimic the ranges obtained under normal human smoking (Table III). These solanesol values (n = 7) obtained for each of the smoking conditions listed in Table III were averaged and correlated to the corresponding nicotine and TPM level from the CFP analysis.

Results and Discussion

Solanesol was proposed by Tang et al. (66) as a potential marker for the cigarette smoke particulate matter dispersed in the environment and thus a measure of exposure to environment tobacco smoke (ETS). The physical properties of solanesol makes it a valuable marker for ETS and tobacco smoke as it is nonvolatile molecule and present only in the tobacco particle phase. Our research group developed analytical techniques for the analysis of total solanesol in cigarette butts to estimate an individual’s dosage of some smoke constituents such as nicotine, tar and tobacco-specific nitrosamines (47-51). Solanesol occurs in tobacco leaves and commercial tobacco products as both types: free alcohol and fatty acid esters. The free type is the more abundant form of solanesol, accounting for 70–95% of the total solanesol content depending on the tobacco type. The other, less abundant solanesol form exists as fatty acid esters. To release the solanesol esterified in tobacco, a saponification process using potassium hydroxide in methanol is required (67, 68). In this project, we explored the possibility of establishing correlations between free solanesol,

instead of total solanesol, and some of the tobacco smoke constituents such as nicotine and tar. The initial hypothesis was based on information previously reported. First, an increase in free solanesol during flue-curing process had been well documented and may be derived from the degradation of solanesyl esters. Second, the cured tobacco products contained only 10–17% more solanesol in the total solanesol concentration than the free solanesol concentration depending on tobacco type and the used extraction method (56). A group of 30 samples were prepared with and without potassium hydroxide (KOH) saponification step using the previous sample preparation method with hexane (49). Samples were analyzed by HPLC and a mass single detector. Figure 1 shows a bivariate normal distribution of the two independent methods ($n = 30$) with a Pearson's correlation of 0.951 and linear equation: $Y_{(\text{solanesol amount (μg) without KOH})} = 3.729 + 0.8797\ X_{(\text{solanesol amount (μg) with KOH})}$ ($R^2 = 0.905$). Based on experimental data, the amount of total solanesol versus the amount of free solanesol exhibits a linear relationship. Additionally, the amount of total solanesol was 12% higher than the amount of free solanesol as expected, according to previous data analysis by other research groups (56, 67, 68). These experimental observations demonstrate the utility of free solanesol, instead of total solanesol, to establish correlations with other tobacco smoke constituents. It is necessary to correlate free solanesol and mainstream smoke constituent on a per-brand basis to account for any differences in filter design, tobacco mass or tobacco type. Thus, for a brand the amount of free solanesol is internally consistent. Elimination of the saponification step to convert the less abundant fatty ester form greatly reduces harmful waste solvents.

Several steps in the analytical procedure were investigated to achieve the use of environmentally 'friendlier' mobile phases without sacrificing performance and potentially improving the analytical quality of the previous method. The composition of the extraction solvent, the separation mode, the column temperature and the injection volume were studied to achieve this goal. Previous extraction process used a combination of 1 M KOH and hexane as an extraction solvent (49). Samples were agitated for 2 hours at 200 revolutions per minute (RPM) and then diluted with hexane and injected into the HPLC-MS (XoldM). The new method used isopropanol to extract the free solanesol. Samples were agitated for 2 hours at 400 RPM using an orbital shaker (LiCONiC LPX44, LiCONiC AG, Industriestrasse 8–12, 9,493 Mauren, Principality of Liechtenstein, Europe), and then diluted 1:1 to methanol and injected to the HPLC–MS-MS system. Both extraction processes presented a linear relation as previously noted. The previous liquid chromatographic separation method (49) was reached using a normal-phase HPLC with a Luna silicate (Phenomenex, Torrance, CA) column (150 mm × 2 mm × 3 μm). The HPLC was operated in an isocratic mode using a mobile phase of 75% hexane, 24.5% ethyl acetate and 0.5% methanol at 0.7 mL/min and room temperature. The older extraction method (49) required the HPLC system to be located in a fume extraction system to minimize environmental contamination and reduce exposure to the analyst. The new method eliminates practically all the noxious chemicals of the previous approach.

A set of 120 samples ($n = 120$) of commercial cigarettes were smoked and analyzed using both methods: total solanesol by HPLC–MS and free solanesol by HPLC–MS-MS. Figure 2 shows a bivariate normal distribution of the two independent methods with a Pearson

correlation of 0.948. These methods present a linear relationship with an equation: $Y_{(\text{Free Solanesol})} = 0.9728 \times X_{(\text{Total Solanesol})} - 0.6378$ ($R^2 = 0.900$).

The method was fully validated to ensure the best quality of the results obtained in the detection and quantification of free solanesol in cigarette filter butts. Accuracy, dynamic range, linearity, detection limit, precision and ruggedness (including stability) were assessed. Matrix effects were also determined to confirm the use of solvent-based calibrators was appropriate.

A calibration curve was constructed for each analytical solanesol run using the response factors of 10 calibrators covering the linear dynamic range (LDR) from 1.9 to 367 μg/butt. The LDR was selected such that the lowest standard concentration was lower than or at the limit of detection (LOD) and the highest calibrator concentration was higher than the concentrations measured for a few representative domestic products smoked using HC regime. Analysis of the calibration curves ($n = 7$) indicated that a linear regression with ($1/x$) weighting resulted in an optimal distribution of residuals, and calibration curves displayed a coefficient of determination (R^2) greater than 0.995, indicating appropriate linearity for the analysis.

The LOD was estimated by evaluating the signal-to-noise (S/N) ratio for a low concentration (0.153 ug/butt) solanesol standard injected three times into the instrument over the course of 6 days ($n = 18$). The LOD was extrapolated for an S/N value of 3 based on the mean S/N value of the 21 measurements. The LOD calculated in this manner was 0.0459 μg/butt.

The absolute accuracy of the method was impossible to determine because no reference smoked samples were available for evaluation. Accuracy was assessed by determining the percentage recoveries in smoked research cigarette butts. Forty research cigarettes (University of Kentucky 3R4F) were smoked using ISO regime and divided into four groups of 10. A 1-cm portion of each of the spent cigarette filter was removed from the mouth end samples were prepared as previously described. All 40 samples were spiked with the same amount of internal standard, the first group of 10 samples (blank) was not spiked with solanesol (reference material solution). The other three groups were spiked with a known concentration of solanesol (reference material solution), creating three different levels of solanesol (low, medium and high concentration, $n = 10$). Analytical recovery was calculated as the percentage difference between the response of analyte spiked on the 3R4F and the 3R4F blank divided by the theoretical value. The averaged calculated percentages for the low, medium and high recovery samples were 103%, 105% and 93%, respectively. Additionally, reproducibility was estimated as the relative standard deviation of the replicate measurements. The relative standard deviations for the low, medium and high recovery samples were 11.6%, 6.6% and 4.7%, respectively.

To guarantee the method's long-term analytical stability and reproducibility of the results, a blank sample and two QC samples at low and high concentrations (QCL and QCH, respectively) were analyzed with each sample set. The intermediate precision was calculated by analyzing filter cigarette butt samples of QC materials (3R4F smoked under ISO regime (QCL) and 3R4F smoked under HC regime (QCH)) collected over the course of 20

separate collection runs (n = 20), each conducted on a separate day 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 (69). A solvent, blank and QC samples were analyzed to monitor background levels and guard against contamination from sample carryover. Analysis of intermediate precision yielded relative standard deviations of 17.9% for QCL and 14.7% for QCH.

Determination of matrix effects was required due to the absence of a blank cigarette filter butt (solanesol-free). Matrix effects between smoked cigarette filter butt and blank cigarette filter butt of 3R4F research cigarettes were assessed by comparing the slopes of two sets of calibrators prepared in smoked filter butt extract (matrix) solution and isopropanol (non-matrix) solution. Ten-point calibration curves were constructed in smoked filter butt extract and in isopropanol alone, equivalent to smoked samples and calibrators, respectively. Least-squares slopes were calculated for five independent calibration curves, averaged for the matrix-based and non-matrix-based samples, and the averaged slopes were compared for both sample sets. Both matrix-based and non-matrix-based calibrators demonstrated linearity with a $R^2 > 0.99$ and matrix effects were minimal with an average difference of 5.0% between slopes.

The specificity of this method increased over previous methods (48-50) with the addition of two confirmation ions for solanesol (Table II). For QC, the ratios of the quantification ion over confirmation ions of solanesol are calculated and evaluated versus pre-established values. The use of the ion ratios allows us to identify interferences in the chromatograms, thus ensuring the reporting of high-quality data. Additionally, specificity was clearly demonstrated by the chromatographic resolution of smoked matrix-based samples. No chromatographic interferences were observed (Figure 3).

Sample stability was assessed using three different smoked solution samples (two low and one high concentrations) under two different conditions: bright lights at room temperature (24±2)°C and in the dark freezer at (−20 ±2)°C. To avoid confounding issues resulting from simultaneous degradation of standard and internal standard, the concentrated internal standard ‘stock solutions’ were kept separately at (−20 ±2)°C. On days of analysis, a single vial of internal standard and vials corresponding to each sample from each environment were equilibrated to room temperature. Then, each vial was spiked with internal standard solution and vortexed. Results following 30 days under the specified conditions were determined as a percentage of the original response for the sample. After 30 days all samples, calibration and matrix-based samples exhibited less than a 10% change in apparent concentration. Therefore, samples were stored in the dark at −20°C. Smoked samples were typically analyzed the day they were generated, but if storage was required, they were stored for no longer than 30 days.

The ruggedness of the method was assessed by changing five method parameters using three different conditions including the final method parameters. Column temperature, sample dilution, mobile phase ratio, extraction solvent volume and extraction time were parameters

studied under ruggedness evaluation. All of these modifications had negligible impact on the results, demonstrating that the technique is robust.

Table IV shows the linear regression results between the amount of free solanesol deposited in the cigarette filter and the deliveries of nicotine and TPM. R^2 values were higher than 0.800 and ranged for nicotine and TPM from 0.8187 to 0.9618 and from 0.8873 to 0.9605, respectively. In this project, the correlation curves were designed to cover a wide range of smoking conditions, to better reflect human smoking conditions or to incorporate potential compensatory smoking. Because of the differences in tobacco composition and physical properties among brands of cigarettes and small cigars, the slopes of the correlation curves vary by product, creating the need to develop individual correlations for each of the cigarette brands to be studied. However, products designed with the same characteristics have very close slopes. In our laboratory, we are taking advantage of this situation and we are creating a database for the most common brands of cigarettes on the market, which would speed up the analysis time in future studies. Of note, no statistical differences were observed on the slope of correlation curves as well as estimated data for nicotine and TPM for cigarette filter butts from menthol cigarettes and flavorless cigarettes from the matched reduced nicotine Spectrum cigarettes. Table V shows the estimated nicotine values of the cigarette filter butts collected in different studies and the amount of nicotine in the filler of each product. The estimated amount of nicotine and TPM for each tobacco product was calculated using the linear relationship in Table IV and averaged across all the brand's respective butts.

Although the idea of estimating the delivery of tobacco components using the determination of solanesol in cigarette butts originated in the early 1990s, we continue to modify analytical techniques to achieve more environmentally friendly methods without losing the selectivity and sensitivity of the original analysis. The data from Tables IV and V suggested that the measured level of free solanesol in the filter provides a useful marker for estimating mouth level intake independent of how it was smoked. We have also demonstrated that in addition to cigarettes, other types of filtered tobacco products, such as little cigars, and reduced nicotine content cigarettes can be evaluated. We are confident that the method discussed in this manuscript could be used for two primary purposes: to establish new correlations between solanesol and other constituents of mainstream of smoke such as volatile organic compounds phenols and polyaromatic compounds and to investigate new emerging tobacco products with filters like IQOS (data not shown), a new device that heats tobacco rather than burns it. The IQOS companies claim the device heats tobacco at a lower temperature than traditional cigarettes, resulting in fewer toxins released to the body; therefore, solanesol analysis could be a useful to establish and quantify reductions relative to cigarettes.

Conclusions

We developed a sensitive, selective, efficient and environmentally friendly analytical technique for the measurement of solanesol in cigarette filter butts, taking advantage of several recent technological advances, mainly in sample preparation and mass spectrometry. This method could be used to establish correlations between solanesol and a range of other mainstream constituents of cigarette smoke such as phenols, volatile organic compounds, polycyclic aromatic compounds and metals that are harmful or potentially harmful. Our

previous method had focused exclusively on traditional filtered cigarettes. In this new approach, we have demonstrated the utility for measuring additional filtered products including reduced nicotine content cigarettes and filtered little cigars. The advantages of analyzing the filters of discarded combustible products is the ability to examine smoking intake in a noninvasive manner for nicotine and other constituents on a stick-by-stick basis or to sum the results over a day work of butts to determine daily intake.

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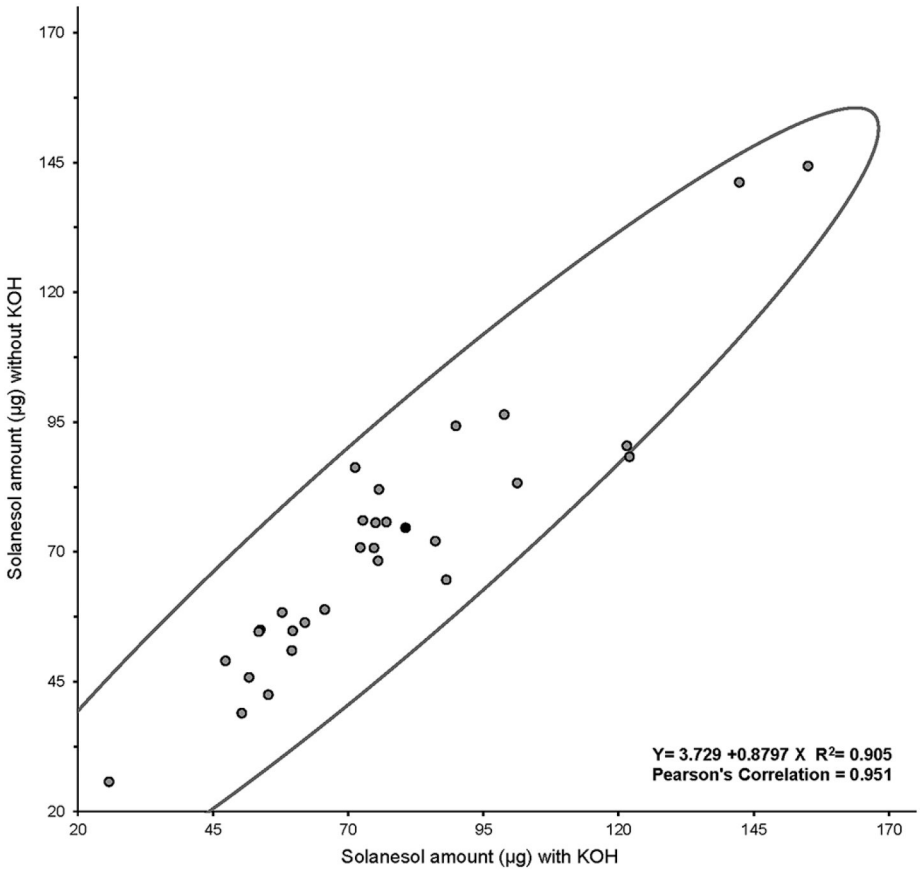

Figure 1.

Scatterplot of (Total) solanesol analysis with KOH vs. (Free) solanesol without KOH analysis. The ellipse element shows a bivariate normal density ellipse of 95%. Samples obtained from both sample preparation methods were analyzed using HPLC–MS.

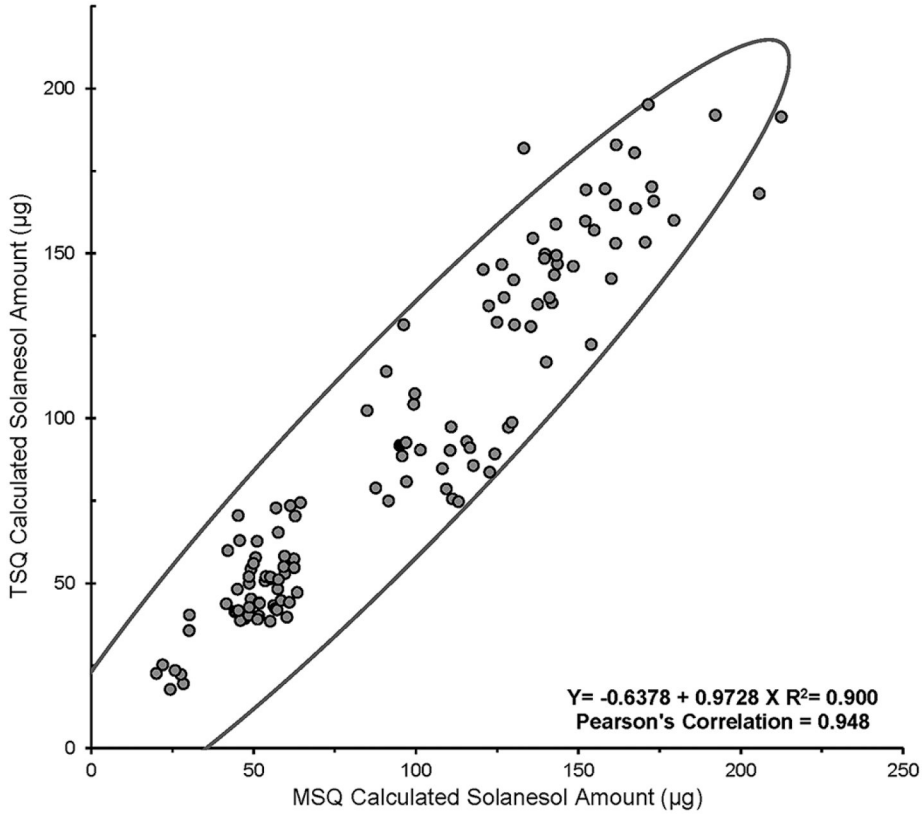

Figure 2.

Scatterplot of total solanesol by HPLC–MS (MSQ) vs. free solanesol by HPLC–MS-MS (TSQ). The ellipse element shows a bivariate normal density ellipse of 95%.

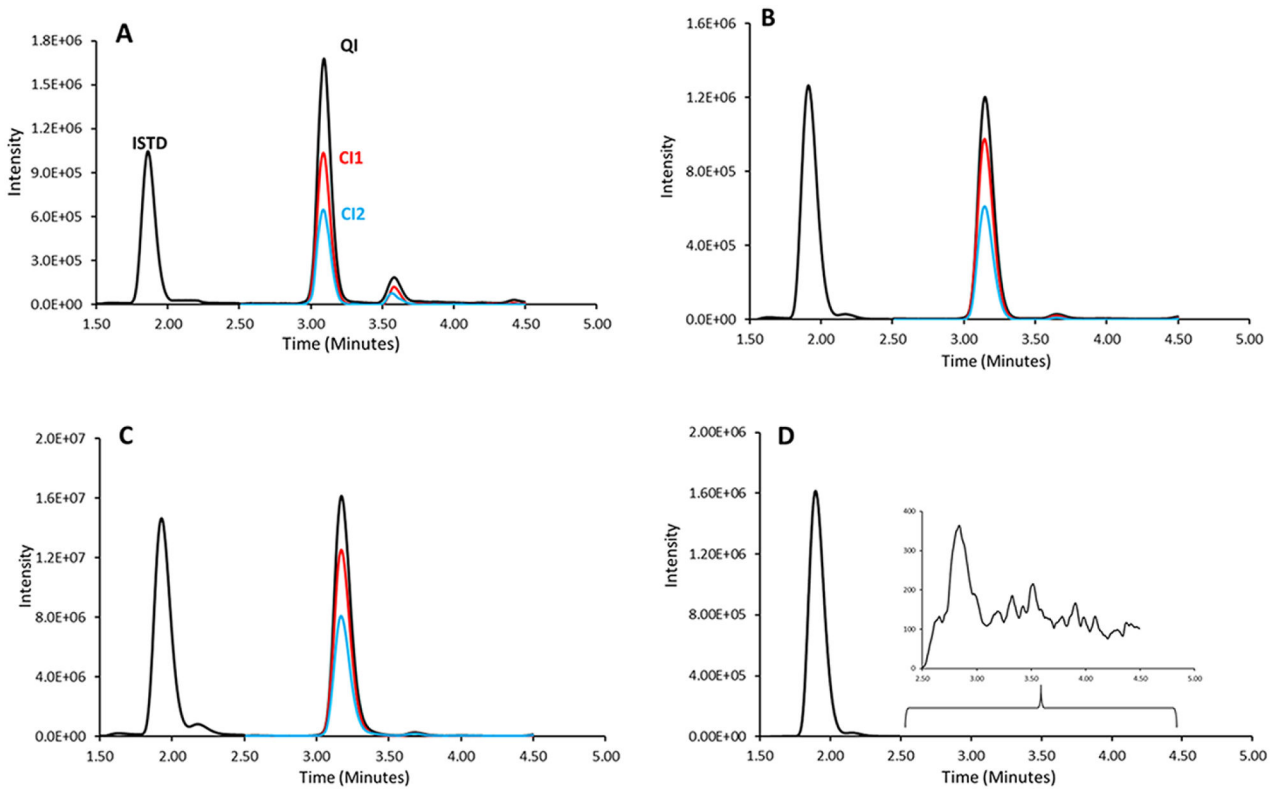

Figure 3.

Multiple reaction monitoring traces for the quantification ion , and two confirmation ions (CI1 and CI2) for solanesol (Retention time = 3.2 min ± 0.3) and the surrogate internal standard (ISTD), GG (Retention time = 1.9 min ± 0.3) in (A) Commercial cigarette sample; (B) Low QC cigarette sample; (C) Little cigar sample and (D) Blank sample with inset showing the amplified region for solanesol. To present all peaks in the same scale, all solanesol confirmation ions intensities were increased by a factor of 20.

Table I.

HPLC conditions for solanesol analysis.

Run time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.0–1.5	0.25	60	40
3.0–4.0	0.40	15	85
4.5–6.0	0.50	60	40
6.0–7.0	0.25	60	40

Mobile Phase A: 0.2% acetic acid, (v/v) in methanol containing 5 mM ammonium acetate; Mobile Phase B: 100% isopropanol.

Table II.

Segments used in the mass spectrometer during the solanesol analysis.

Segment	Divert valve	Time (min)	Precursor mass (*m/z*)	Product mass (*m/z*)	Collision energy (eV)	Retention time (min)	Ions
1	Waste	0.0–1.5	NA	NA	NA	NA	NA
			NA	NA	NA	NA	NA
2	Detector	1.5–2.5	273.6	273.6	5	1.9 ±0.3	ISTD_Q1
			273.6	149.1	17	1.9 ±0.3	ISTD_CI2
3	Detector	2.5–4.5	613.6	613.6	11	3.2 ±0.3	SOL_Q1
			613.6	95.2	36	3.2±0.3	SOL_CI1
			613.6	107.2	39	3.2±0.3	SOL_CI2
4	Waste	4.5–7.0	NA	NA	NA	NA	NA
			NA	NA	NA	NA	NA

NA: Not applicable; ISTD: Internal standard (GG); Q: Quantification ion; CI: Confirmation ion; SOL: Solanesol.

Table III.

Smoking machine conditions under which the tobacco products were smoked.

Regime name	Description
ISO 3 puffs	• 3 puff count • 35 mL puff volume, 60 s puff interval, 2 s duration • unblocked vent
ISO 5 puffs	• 5 puff count • 35 mL puff volume, 60 s puff interval, 2 s duration • unblocked vent
Regular ISO	• smoke to 3 mm length from filter • 35 mL puff volume, 60 s puff interval, 2 s duration • unblocked vent
HC 4 puffs	• 4 puff count • 55 mL puff volume, 30 s puff interval, 2 s duration • 100% blocked vent
Regular HC	• smoke to 3 mm length from filter • 55 mL puff volume, 30 s puff interval, 2 s duration • 100% blocked vent
Blocked vent 65/20	• smoke to 3 mm length from filter • 65 mL puff volume, 20 s puff interval, 2 s duration • 100% blocked vent

Table IV.

Linear regression data for the relationship of solanesol vs nicotine and total particle matter (TPM).

		Solanesol vs. nicotine			Solanesol vs. TPM		
Brand	**Type**	**Slope**	***y*-Intercept**	***R*²**	**Slope**	***y*-Intercept *x* (−)**	**R²**
NRC102	RN	0.0007	0.0038	0.9006	0.S729	18.9050	0.9299
NRC103	RN-Men	0.0008	0.0021	0.9412	1.0642	24.1120	0.9524
NRC600	RN	0.0133	0.0870	0.9793	0.5524	19.4810	0.9283
NRC601	RN-Men	0.0137	−0.0019	0.8187	0.5435	19.6720	0.9338
Cheyenne	LC-Men	0.0246	0.0236	0.9363	1.1450	12.7380	0.8873
Santa Fe	LC	0.0195	0.0870	0.9387	0.9513	10.0060	0.9308
Swisher Sweet	LC-CF	0.0140	0.1510	0.9269	0.5407	9.5337	0.9166
Newport Green	Cig	0.0243	0.0217	0.9342	0.7205	9.1471	0.9025
Marlboro Light	Cig-L	0.0173	−0.0031	0.9618	0.5379	9.1471	0.9553
Marlboro Red 100	Cig	0.0174	−0.0199	0.9613	0.5276	9.9036	0.9605

RN: Reduced nicotine content cigarette; Men: Menthol flavor product; LC: Little cigar; Cig: Cigarettes; CF: Cherry flavor; and L: Light.

Table V.

Estimated nicotine values of the cigarette filter butts collected in three different human studies and the amount of nicotine in the filler of each tobacco product.

Brand	Type	Number of samples (*n*)	Nicotine_filler (mg/Cig)	Estimated nicotine* (mg/Cig)
NRC102	RN	150	0.31±0.01	0.041±0.010
NRC103	RN-Men	150	0.28±0.01	0.045±0.009
NRC600	RN	150	15.7±0.3	1.31±0.35
NRC601	RN-Men	150	17.3±0.6	1.19±0.28
Cheyenne	LC-Men	50	12.4±0.5	0.90±0.41
Santa Fe	LC-U	50	11.6±0.5	1.00±0.40
Swisher Sweet	LC-CF	50	16.6±1.0	1.50±0.34
Newport Green	Cig	50	19.4±0.2	0.9±0.3
Marlboro Light	Cig-L	50	19.1±0.1	0.9±0.3
Marlboro Red 100	Cig	50	19.7±0.4	1.2±0.4

* Nicotine amount estimated from linear regression from Table IV; RN: Reduced nicotine content cigarette; Men: Menthol flavor product; LC: Little cigar; Cig: Cigarettes; CF: Cherry flavor; and L: Light.