Gas Chromatographic-Selected Ion Monitoring-Mass Spectrometric Determination of Cigarette Mainstream Smoke Components with Sensory Attributes*

by

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SUMMARY

A new method has been developed that detects significant quantitative differences in the amounts of pyrazines, pyridines, furfurals, carboxylic acids, β -damascenone, sclareolide, and megastigmatrienones in the mainstream smoke of a series of five commercial cigarettes. This new quantitative method is based on the gas chromatographic-selected ion monitoring-mass spectrometric (GC-SIM-MS) determination of the selected smoke constituents. The accuracy and precision of the approach were well within acceptable parameters with the majority of cases relative standard deviation (RSD) values consistently around 5%. Sample preparation was simple requiring only the dissolution of the trapped particulate material in a known volume of methanol followed by injection of this clear dark colored solution into the gas chromatograph. This approach represents an advance in the technology in terms of higher sample throughput and less sample workup. Certain products demonstrated consistent trends in concentration of specific chemical classes. The mainstream smoke from a University of Kentucky reference cigarette, 2R4F, was included for reference purposes. These results are applicable in the overall evaluation of the components responsible for the taste associated with cigarette products. [Beitr. Tabakforsch. Int. 21 (2004) 175–183]

ZUSAMMENFASSUNG

Eine neue Methode wurde entwickelt, mit der sich quantitativ signifikante Unterschiede bei den Mengen an Pyrazinen, Pyridinen, Furfuralen, Carbonsäuren, β -Damascenone, Sclareoliden und Megastigmatrienonen im Hauptstromrauch von fünf handelsüblichen Zigaretten erfassen lassen. Diese neue quantitative Methode basiert auf einer gaschromatographisch-massenspektrometrischen Bestimmung mit „Selected ion monitoring“ (GC-SIM-MS) der

ausgewählten Rauchbestandteile. Die Richtigkeit und Präzision des Ansatzes bewegen sich in einem annehmbaren Schwankungsbereich, wobei die relative Standardabweichung (RSD) überwiegend bei 5% liegt. Die Probenaufbereitung war einfach und erforderte lediglich die Lösung der aufgefangenen Partikel in einer vorgegebenen Methanolmenge und nachfolgende Einspritzung dieser klaren, dunklen Lösung in einen Gaschromatographen. Dieser Ansatz stellt wegen des höheren Probendurchsatzes und geringerer Probenaufbereitung einen Entwicklungsfortschritt dar. Bei einigen Zigarettentypen wurden konsistente Trends bezüglich der Konzentration spezifischer chemischer Substanzklassen festgestellt. Der Hauptstromrauch der Referenzzigarette 2R4F der University of Kentucky wurde zum Vergleich herangezogen. Die Ergebnisse sind für die allgemeine Bewertung der Rauchkomponenten von Bedeutung, die für den Geschmack der Zigaretten verantwortlich sind. [Beitr. Tabakforsch. Int. 21 (2004) 175–183]

RESUME

Une nouvelle méthode analytique a été développée pour détecter des différences quantitatives significatives dans la teneur des pyrazines, pyridines, furfurals, acides carboxyliques, β -damascénone, sclariolide et mégastigmatrièneones dans la fumée principale d'une série de cinq marques de cigarettes du marché. Cette nouvelle méthode quantitative est basée sur le dosage des composants de la fumée de tabac par chromatographie en phase gazeuse-spectrométrie de masse en mode de suivi d'ions sélectionnés (GC-SIM-MS). La précision et la fiabilité de cette approche sont acceptables avec des valeurs d'écart type relatif pour la plupart des composants d'environ 5%. La préparation des échantillons est simple, nécessitant seulement la dissolution

des particules piégées dans du méthanol dans un volume connu, suivi de l'injection de cette solution clair foncée dans le chromatographe à gaz (GC). Cette approche constitue une avance technologique en augmentant la quantité des échantillons traités et en réduisant la préparation des échantillons. Chez certains produits une concentration de substances de classes chimiques spécifiques a été observée. La fumée principale de la cigarette de référence Kentucky 2R4F a été évaluée pour des raisons de comparaison. Ces résultats peuvent être appliqués pour l'évaluation des composants responsables du goût associés aux produits de cigarettes. [Beitr. Tabakforsch. Int. 21 (2004) 176–183]

INTRODUCTION

Commercial cigarettes are made by blending various types of tobacco leaves and reconstituted/processed tobacco materials. Flavor ingredients are sometimes used, generally in the sub ppm range, to complement the subjective characteristics of the tobacco smoke. In a number of cases, flavor compounds transfer unchanged into the mainstream smoke (1). In addition, tobacco smoke generated from a tobacco burning cigarette is a very complex matrix containing hundreds or even thousands of individual compounds each having an associated aroma/flavor characteristic and a corresponding aroma or taste threshold value (2–9). A number of the components identified in tobacco smoke have well established aroma characteristics and aroma thresholds (10,11). The pathways by which these compounds are formed have been the subject of a variety of investigations some having specific foci (9–20). Classes of compounds found in tobacco smoke having powerful sensory attributes at relatively low levels include pyridines, pyrazines, furfurals, carboxylic acids, and carotenoid degradation products such as β -damascenone and megastigmatrienone isomers (10,11). The approximate levels of these classes of compounds in tobacco smoke have concentration ranges in the area of multiple $\mu\text{g}/\text{cig}$ (1–10,12). With aroma and flavor threshold values for some of these compound classes in the ppm to ppb range, these classes of compounds possess the capability of having significant sensory impact on cigarette products.

Thus, an analytical method, having a simple sample preparation protocol, has been developed that has the capability to provide quantitative determination of numerous targeted compounds of differing functionality in the mainstream smoke condensate. In this work the details of the sample collection and analytical protocol will be presented. The method is based on the gas chromatographic-selected ion monitoring-mass spectrometric (GC-SIM-MS) determination of selected mainstream smoke constituents having sensory attributes. The targeted compound classes include pyridines, pyrazines, carboxylic acids, furfurals, and carotenoid degradation products such as megastigmatrienones and β -damascenone. For reference purposes, a University of Kentucky reference cigarette, 2R4F, was included in the study. This product also serves as a quality control sample in the analysis scheme in that multiple analyses of the smoke from the 2R4F product were conducted throughout the entire timeframe of the experiments.

EXPERIMENTAL

Sample description and preparation

The five products, Products A through E, were obtained commercially and the reference product, 2R4F, was obtained from the Tobacco and Health Research Institute of the University of Kentucky. The products were conditioned under Federal Trade Commission (FTC) parameters (20). The mainstream smoke condensate of 40 cigarettes from three repetitive smokings of each product was captured on a glass tube by electrostatic precipitation (ESP) by means of a Borgwaldt 20 port rotary smoking machine. The products were smoked to a butt length of 37 mm using a 35 cc puff, every 60 s of 2-s duration. The condensate from the ESP tube was collected by washing with 20 mL of methanol (Burdick and Jackson, Muskegon, MI). The samples were placed in GC autosampler vials and stored at -80°C until ready for analysis. Most often the samples were analyzed immediately after smoking. The smokings were performed on three separate days. Wet total particulate matter (WTPM) was gravimetrically determined by accurately determining the mass of particulate matter collected on the ESP tube due to the smoking of 40 cigarettes.

Standards preparation

Standards were prepared by dissolving known amounts of each compound in known volumes of methanol. Pyrazine, methylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 2,6-dimethyl-3-ethylpyrazine, and tetramethylpyrazine, were obtained from Pyrazine Specialties, Inc., Atlanta, GA. The acids, 2-methylpropionic acid, 3-methylbutyric acid, and 3-methylpentanoic acid, the pyridines (pyridine, 2-methylpyridine, 2,6-dimethylpyridine, 2-ethylpyridine, 3-methylpyridine, 4-methylpyridine, 2,5-dimethylpyridine, 2,4-dimethylpyridine, 2,3-dimethylpyridine, 2,4,6-trimethylpyridine, 3-ethylpyridine, 4-ethylpyridine, 5-ethyl-2-methylpyridine, 2,3,5-trimethylpyridine, 3-butylpyridine), cyclotene, furfural, and 5-methylfurfural were obtained from Aldrich Chemical Company, Milwaukee, WI. Beta-damascenone was obtained from Firmenich Inc., Princeton, NJ and the mixture of four megastigmatrienones was obtained from IFF, Inc., Dayton, NJ. Sclareolide was obtained from the Avoca Division of R.J. Reynolds Tobacco Company. All reagents were used as received and purities were greater than 95% in all cases.

Safety note

Most of the compounds employed in this study are toxic in the neat form and may enter the body by inhalation or absorption through the skin. Effects of over exposure include nausea, irritation, and burning. Caution must be taken when working with the neat compounds or concentrated solutions. All solution preparations should be performed in an approved fume hood. Appropriate chemical-resistant gloves should be worn.

Table 1. Gas chromatography-mass spectrometry operating conditions

Parameters	Conditions
System configuration	Hewlett Packard (HP) 6890 GC equipped with a 5973 mass selective detector and an autosampler
Column	DB-WAXetr, 30 m, 0.25 mm i.d., 0.25 µm film thickness
Injection port temp.	250 °C
Injection	Split 10:10
Inlet pressure	Constant flow, 1 mL/min
Column oven initial temp.	45 °C
Column oven initial time	0.50 min
Column oven ramp rate	5 °C/min
Column oven final temp.	250 °C
Column oven final time	8.5 min
MS transfer line temp.	250 °C
Mass spectral databases	NBS, Wiley
Mass spectrometer configuration	Selected ion monitoring, 70 eV

Table 2. Compounds, selected ions and retention times

Compound	SIM ions	RT, min
Pyrazine	80	7.86
Methylpyrazine	94	9.09
2,6-Dimethylpyrazine	107, 108	10.56
Ethylpyrazine	107, 108	10.74
2,3-Dimethylpyrazine	107, 108	11.01
2-Ethyl-6-methylpyrazine	121, 122	11.97
2-Ethyl-5-methylpyrazine	121, 122	12.12
Trimethylpyrazine	121, 122	12.39
2,5-Dimethyl-3-ethylpyrazine	121, 135, 136	13.29
2,6-Dimethyl-3-ethylpyrazine	121, 135, 136	13.69
Furfural	95, 96	14.06
5-Methylfurfural	109, 110	16.69
2-Methylpropionic acid	73, 88	16.79
3-Methylbutyric acid	60, 87	19.01
3-Methylpentanoic acid	60, 87	21.82
β-Damascenone	69, 121, 190	22.20
Megastigmatrienone I	147, 175, 190	28.89
Megastigmatrienone II	147, 175, 190	29.67
Megastigmatrienone III	147, 175, 190	30.92
Megastigmatrienone IV	147, 175, 190	31.41
Sclareolide	123, 235, 95	42.61

Gas chromatography-mass spectrometry analysis conditions

The gas chromatography-selected ion monitoring-mass spectrometry parameters listed in Table 1, were applied to the analysis of each sample. The average response of one injection of each sample from three separate smokings was used to calculate concentration and percent relative standard deviation (%RSD).

Two separate GC-MS systems under the same conditions and same column type were employed in the overall approach. This tactic was employed to facilitate assignment

Table 3. Compounds, selected ions and retention times

Compound	SIM ions	RT, min
Pyridine	79, 52	6.79
2-Methylpyridine	93, 78	7.55
2,6-Dimethylpyridine	107, 106	8.30
2-Ethylpyridine	106, 107	9.12
3-Methylpyridine	93, 92	9.34
4-Methylpyridine	93, 92	9.50
2,5-Dimethylpyridine	107, 106	10.13
2,4-Dimethylpyridine	107, 106	10.36
2,3-Dimethylpyridine	107, 106	10.97
2,4,6-Trimethylpyridine	121, 120	11.17
3-Ethylpyridine	92, 107	11.48
4-Ethylpyridine	107, 106	11.77
5-Ethyl-2-methylpyridine	106, 121	12.28
2,3,5-Trimethylpyridine	121, 120	13.79
3-Butylpyridine	92, 135	16.32
Cyclotene	112, 69	21.96

and collection of selected ions within relatively narrow retention time windows as well as to improve overall sample analysis time.

Calibration and component yield calculations

Calibration curves for each compound or in the case of the megastigmatrienones (a four isomer mixture) were prepared from serial dilutions of three concentrations in the general range of 100, 10 and 1 µg/mL. A minimum of four standard values was employed for the generation of each calibration curve. The mass spectrum of each component was obtained and from the spectrum was taken the major ion and at least one confirmatory ion whenever possible. To confirm the identity of those components having only one selected ion, standard additions to the smoke matrix were performed periodically and response factors calculated. In addition, the chromatograms of smoke samples fortified with standards were closely examined for the appearance of peaks not associated with the analytes of interest or for unexpected shifts in retention times.

The selected ions served as the ions employed in the SIM mode. The response from these selected ions was used to calculate component concentrations. A list of compounds, their selected ions, and retention times (RT) can be found in Tables 2 and 3. One GC-MS system was used for the analytes in Table 2 while a second GC-MS system was used for the analytes in Table 3. Calibration standards were analyzed periodically throughout the entire experiment to guard against instrument drift, sample evaporation and partial or incomplete injections.

Multiple injections, 6, of the standard solutions yielded a %RSD variation of approximately 2% for each component. Linear equations were derived from the plots of concentration vs. selected ion abundance, having forced the line through a zero intercept. In all cases the R^2 values for the linear curves were greater than 0.9998 with all points falling either on or close to the calibration line. Calculations of the yield per cigarette were made by multiplying the methanol concentration determined from the calibration

Figure 1. SIM chromatogram of mainstream smoke from Product E.

Figure 2. SIM chromatogram of megastigmatrienone section of Product E

Figure 3. SIM chromatogram of acid/β-damascenone section of Product E

curve by the extraction volume of 20 mL followed by dividing this total yield by the number of cigarettes, 40. The %RSD for the three injections was found to be compound specific, but was consistently between 5% and 10%. Calculations of the yield on a ng/mg WTPM basis were made by dividing the yield per cigarette by the mg WTPM value. This value was then adjusted from a $\mu\text{g}/\text{mg}$ WTPM value to a ng/mg WTPM by multiplying by 1000.

For the purposes of these analyses, the detection limit for each analyte was set to the lowest calibration standard, approximately 1 ng/ μL . This value also served as the limit of quantitation for all analytes.

RESULTS AND DISCUSSION

Tobacco smoke is a very complex matrix containing at least 4000 compounds (21). Among those classes of compounds having significant sensory attribute contributions to tobacco smoke are pyridines, pyrazines, carboxylic acids, furfurals, and carotenoid degradation products such as megastigmatrienones, β -damascenone, and sclareolide. Concentrations for these classes of compounds have been available for some time but yet no single analytical method has been developed capable of providing quantitative data on the concentrations of a wide array of these components in one procedure (10). The concentration ranges obtained for these compound classes vary from less than 1 to more than 1000 $\mu\text{g}/\text{cig}$ depending on the type and design of the cigarette rod.

The compounds targeted for these analyses were selected based in part on their documented sensory characteristics as well as their olfactory detection thresholds. For example, pyrazines have been shown to possess powerful sensory characteristics such as toasted, roasted, nutty, sweet, bread-like and peanut-like. In addition, their threshold levels span a range in concentration from multiple ppm to unit ppb levels as a function of structure (15,23,24). The selected carboxylic acids, 2-methylpropionic, 3-methylbutyric and 3-methylpentanoic acid, are noted for their sweaty sock and cheese-like notes at ppb levels (10,25). Likewise, pyridines have been described as having green, sweet, and tobacco-like notes at ppm levels (10). The carotenoid degradation compounds such as the megastigmatrienones, β -damascenone and sclareolide have powerful cedar, woody notes at the ppb level (10). The furfurals have been described as having sweet-spicy to caramellic notes in the ppm range (11).

Thus, the first stage or challenge in the development of this target compound approach involved the development of an adequate separation of all of the compounds of interest, preferably employing one column type and one separation set of conditions. After experiments with selected column types including, FFAP, DB-5, DB-1701, coupled with column oven variations, a DBWAXetr column was selected which provided baseline separation for the ingredients listed in Tables 2 and 3. The DBWax style column had previously been effectively used for the separation of tobacco materials (22). The SIM chromatogram, employing the DBWaxetr column, from the mainstream smoke from Product E is shown in Figure 1. The SIM chromatograms from the other products were very similar qualitatively. Portions of the SIM chromatogram have been expanded in Figures 2 to 4 to indicate the types of separation obtained and to provide some information on the chromatographic signal to noise conditions obtained for the analyses. Baseline separation of all selected components with acceptable signal to noise performance was possible with an overall injection to injection turnaround time of approximately one hour.

Figure 4. SIM chromatogram of a portion of the pyridine section of Product E

Figure 5. Concentration, µg/cig, of targeted pyrazines in the mainstream smoke of selected products

Figure 6. Concentration, µg/cig, of targeted carboxylic acids in the mainstream smoke of selected products

Smoke samples from the six products were generated as described above. One injection of each sample from three smokings was made and %RSD calculations were performed on each component of interest. The values for each component, µg/cig, within each sample and %RSD values are listed in Table 4.

The next step in the evaluation centered on an examination of the data in Table 4 for the presence of any significant trends in the concentration of the selected analytes as a function of product type. A portion of the data can be more

Figure 7. Concentration, µg/cig, of targeted carotenoid degradation compounds in the mainstream smoke of selected products

Figure 8. Concentration, µg/cig, of targeted pyridines in the mainstream smoke of selected products

conveniently examined when plotted as in Figures 5 to 8. From an evaluation of the data in Table 4, coupled with the visual cues available in Figures 5 to 8, several conclusions can be drawn from the data: 1) Product C had consistently the highest levels of pyrazines while Product A was consistently the lowest; 2) methylpyrazine and 2,6-dimethylpyrazine were the dominant pyrazines detected, however, from a sensory/threshold perspective, pyrazines with four carbons attached, i.e., dimethylethylpyrazines possess much lower threshold values than the pyrazines with 1 or 2 carbons attached, i.e., methyl and dimethylpyrazines; 3) Product E had the highest levels of the targeted carboxylic acids; 4) pyridine, 3-methylpyridine and 3-ethylpyridine were the dominant pyridines detected; 5) Product A was consistently the lowest in pyridines; 6) Product D was consistently the lowest in carotenoid degradation compounds, while Product E was consistently the highest; 7) Product E had the highest levels of furfurals and cyclotene.

To this point in the discussion of the results, the data have focused on the yield of targeted components on a per cigarette basis. While comparisons on a per cigarette basis are suitable in their own right and acceptable for comparisons, an assessment of the yields of these components as a function of cigarette yield, i.e., WTPM, is also applicable. The average WTPM values (mg/cig) for the three smokings of Product A, Product B, Product C, Product D, Product E, and 2R4F, were 9.1, 12.1, 12.5, 10.4, 9.5, and 11.0, respectively.

The data clearly indicate a considerable range in WTPM, thus the comparative yields of the selected analytes as a

Table 4. Mainstream smoke electrostatic tube targeted analyte concentrations for selected products, µg/cig^a

Compound	2R4F µg/cig	%RSD	Product A µg/cig	%RSD	Product B µg/cig	%RSD	Product C µg/cig	%RSD	Product D µg/cig	%RSD	Product E µg/cig	%RSD
Pyridine	2.23	1.58	1.45	2.39	2.83	5.04	2.78	1.83	2.05	0.86	2.84	1.57
2-Methylpyridine	0.76	1.41	0.50	2.19	1.08	5.44	1.14	2.59	0.67	1.26	0.92	2.18
2,6-Dimethylpyridine	0.28	2.70	0.20	6.73	0.36	4.80	0.37	6.15	0.24	5.92	0.33	3.37
2-Ethylpyridine	0.41	1.91	0.20	4.58	0.48	5.14	0.42	4.77	0.28	1.99	0.45	9.36
3-Methylpyridine	1.73	2.59	1.17	1.17	2.26	5.11	2.05	0.79	1.16	1.67	2.56	3.19
4-Methylpyridine	0.49	2.75	0.25	11.27	0.60	3.76	0.39	9.59	0.21	5.45	0.38	4.02
2,5-Dimethylpyridine	0.35	3.24	0.18	10.06	0.44	5.59	0.32	2.89	0.16	9.61	0.32	6.87
2,4-Dimethylpyridine	0.37	2.60	0.23	6.69	0.51	4.60	0.40	3.44	0.19	6.97	0.38	7.71
2,3-Dimethylpyridine	0.21	1.13	0.12	22.18	0.26	4.03	0.16	11.96	0.06	3.87	0.17	15.21
2,4,6-Trimethylpyridine	0.18	2.10	0.06	1.11	0.21	3.45	0.09	6.88	0.04	4.69	0.08	7.10
3-Ethylpyridine	0.51	3.42	0.37	8.64	0.60	3.94	0.59	8.36	0.36	8.56	0.71	4.83
4-Ethylpyridine	0.28	3.10	0.21	12.43	0.33	3.47	0.36	11.62	0.12	1.86	0.33	10.00
5-Ethyl-2-methylpyridine	0.22	3.86	0.17	8.19	0.25	3.66	0.26	9.04	0.17	8.34	0.27	6.19
2,3,5-Trimethylpyridine	0.05	4.11	0.05	2.30	0.07	5.75	0.07	3.17	0.03	4.83	0.07	8.00
3-Butylpyridine	0.03	2.53	0.00		0.00		0.00		0.00		0.03	4.45
Cyclotene	5.10	5.16	4.98	4.76	5.55	3.39	6.02	2.72	3.67	2.97	7.54	3.85
Pyrazine	0.12	5.44	0.07	2.46	0.19	3.68	0.22	0.64	0.12	3.52	0.16	6.11
Methylpyrazine	0.61	2.91	0.47	1.56	0.94	3.57	1.16	0.64	0.67	2.12	0.69	4.16
2,6-Dimethylpyrazine	0.52	1.75	0.38	1.64	0.84	2.49	1.04	0.53	0.51	0.82	0.51	1.97
Ethylpyrazine	0.21	1.77	0.17	2.29	0.32	3.38	0.35	0.96	0.17	1.75	0.31	5.51
2,3-Dimethylpyrazine	0.15	7.66	0.10	7.41	0.21	6.85	0.25	4.01	0.12	2.16	0.17	9.44
2-Ethyl-6-methylpyrazine	0.24	3.03	0.18	1.89	0.35	2.47	0.42	0.45	0.20	5.65	0.27	5.94
2-Ethyl-5-methylpyrazine	0.08	3.09	0.06	1.45	0.12	3.53	0.14	0.88	0.07	10.48	0.10	3.06
Trimethylpyrazine	0.29	1.71	0.21	0.88	0.47	4.84	0.61	5.02	0.30	0.81	0.31	10.07
2,5-Dimethyl-3-ethylpyrazine	0.05	22.90	0.04	14.43	0.07	11.92	0.07	24.93	0.03	21.56	0.05	6.22
2,6-Dimethyl-3-ethylpyrazine	0.17	7.07	0.08	3.42	0.15	2.80	0.25	14.46	0.09	20.51	0.21	14.79
Furfural	0.76	2.73	0.71	4.36	0.91	5.70	1.11	5.32	0.62	8.95	1.60	7.13
Tetramethylpyrazine	0.07	15.57	0.04	11.46	0.08	8.06	0.12	31.54	0.04	17.17	0.11	25.29
2-Methylpropionic acid	1.50	3.77	1.32	2.38	1.55	3.46	1.60	9.10	1.20	2.94	1.79	2.17
5-Methylfurfural	0.62	3.02	0.69	3.02	0.86	4.61	0.92	1.41	0.52	3.09	1.25	2.13
3-Methylbutyric acid	4.94	3.31	4.60	1.18	5.62	1.70	5.65	0.78	4.31	1.00	6.48	2.05
3-Methylpentanoic acid	7.11	2.00	8.20	2.06	8.71	2.85	10.73	1.53	8.72	1.54	12.84	4.17
β-Damascenone	0.46	4.21	0.49	4.10	0.55	0.72	0.60	1.24	0.33	4.57	0.71	1.05
Megastigmatrienone I	2.72	3.60	2.36	1.47	2.47	0.79	2.56	1.68	1.76	0.72	3.07	2.96
Megastigmatrienone II	6.91	2.67	5.60	1.24	6.47	1.44	6.65	2.06	4.42	0.74	7.33	2.65
Megastigmatrienone III	2.53	2.35	2.29	1.94	2.44	2.35	2.58	1.81	1.75	0.51	2.90	2.59
Megastigmatrienone IV	8.42	4.37	7.22	1.36	7.90	0.85	8.36	0.19	5.97	6.94	9.37	1.85
Sciaroleide	0.65	2.92	0.66	23.31	0.74	13.21	0.69	7.55	0.62	5.06	0.89	3.78

^a A value of 0.00 indicates that the analyte was below limit of quantitation.

Table 5. Mainstream smoke electrostatic tube targeted analyte concentrations for selected products, ng/mg WTPM ^a

Compound	2R4F	Product A	Product B	Product C	Product D	Product E
Pyridine	202.80	160.38	233.79	221.98	196.08	300.57
2-Methylpyridine	68.90	55.30	89.23	90.66	64.47	96.90
2,6-Dimethylpyridine	25.69	22.37	29.45	29.20	23.18	35.32
2-Ethylpyridine	37.67	22.42	39.76	33.21	26.56	47.47
3-Methylpyridine	156.97	128.57	187.23	163.93	110.89	270.98
4-Methylpyridine	44.28	27.14	49.34	31.26	19.71	40.02
2,5-Dimethylpyridine	31.69	19.37	36.05	25.23	15.48	33.70
2,4-Dimethylpyridine	33.32	24.95	42.13	31.81	18.38	39.76
2,3-Dimethylpyridine	19.42	13.33	21.51	13.05	6.06	17.82
2,4,6-Trimethylpyridine	16.14	6.26	17.33	7.47	4.11	8.60
3-Ethylpyridine	46.03	41.10	50.04	47.13	34.64	74.61
4-Ethylpyridine	25.43	22.92	27.66	28.57	11.09	34.84
5-Ethyl-2-methylpyridine	20.25	19.26	20.90	21.08	16.65	28.26
2,3,5-Trimethylpyridine	4.81	5.04	5.59	5.44	3.22	7.34
3-Butylpyridine	3.15	0.00	0.00	0.00	0.00	3.12
Cyclotene	463.68	549.57	458.91	481.08	351.32	797.63
Pyrazine	10.79	8.14	15.42	17.71	11.59	16.49
Methylpyrazine	55.13	51.51	78.13	92.36	63.91	72.54
2,6-Dimethylpyrazine	47.06	42.47	69.75	82.76	48.79	53.82
Ethylpyrazine	19.02	19.11	26.45	27.77	16.31	32.92
2,3-Dimethylpyrazine	13.45	10.90	17.27	20.15	11.94	17.57
2-Ethyl-6-methylpyrazine	21.56	20.16	28.78	33.21	19.37	28.43
2-Ethyl-5-methylpyrazine	7.39	6.72	9.77	11.52	6.45	10.28
Trimethylpyrazine	26.68	23.64	39.28	48.50	28.28	32.63
2,5-Dimethyl-3-ethylpyrazine	4.44	4.03	6.09	5.83	3.18	5.16
2,6-Dimethyl-3-ethylpyrazine	15.59	9.15	12.55	20.07	9.02	22.19
Furfural	68.78	78.10	74.92	88.37	59.23	169.14
Tetramethylpyrazine	6.55	4.73	6.39	9.25	3.37	12.00
2-Methylpropionic acid	136.48	145.89	127.99	127.93	115.45	188.90
5-Methylfurfural	56.64	76.38	70.96	73.62	49.54	132.48
3-Methylbutyric acid	448.75	507.22	465.13	451.45	413.30	685.52
3-Methylpentanoic acid	646.24	904.65	720.57	857.35	836.10	1357.86
β-Damascenone	42.26	54.00	45.11	47.91	31.76	75.10
Megastigmatrienone I	247.12	259.85	204.03	204.77	168.73	325.02
Megastigmatrienone II	628.20	617.87	535.02	530.77	423.23	775.44
Megastigmatrienone III	230.05	252.21	201.49	205.75	168.10	306.82
Megastigmatrienone IV	764.98	796.59	653.05	667.59	572.34	991.31
Sclareolide	59.00	73.25	61.19	54.74	59.63	94.47

^a A value of 0.0 indicates that the analyte was below the limit of quantiation.

function of WTPM could possibly be different from the comparisons observed on a per cigarette basis. Thus, all of the concentration data presented previously was normalized to a per mg of WTPM. These values are located in Table 5. As with the previous data a more convenient way to view the data can be found in Figures 9 to 12.

When normalized to WTPM yields, the trend in compound concentrations established on a per cigarette basis was re-established on a ng/mg WTPM basis. That is, 1) Product C had consistently the highest levels of pyrazines while Product A was consistently the lowest; 2) methylpyrazine and 2,6-dimethylpyrazine were the dominant pyrazines detected from all products; 3) Product E had the highest levels of the targeted carboxylic acids; 4) pyridine, 3-methylpyridine and 3-ethylpyridine were the dominant pyridines detected for all products; 5) Product A was consistently the lowest in pyridines; 6) Product D was consistently the lowest in carotenoid degradation compounds, while Product E was consistently the highest; 7) Product E had the highest levels of furfurals and cyclotene.

CONCLUSION

A new method has been described as a relatively simple means for providing accurate and precise data on flavorful and important sensory compounds in mainstream tobacco smoke. When applied to the analysis of the electrostatic tube precipitated (ESP) mainstream smoke from five commercially available cigarettes and a University of Kentucky Reference Cigarette, 2R4F, the method was able to detect significant quantitative differences in the mainstream particulate phase amounts of pyrazines, pyridines, furfurals, carboxylic acids, β-damascenone, sclareolide, and megastigmatrienones. This new quantitative method is based on the gas chromatographic-selected ion monitoring-mass spectrometric (GC-SIM-MS) determination of the selected smoke constituents. The accuracy and precision of the approach were well within acceptable parameters having in the majority of cases relative standard deviation (RSD) values consistently around 5%. Sample preparation was simple requiring only the dissolution of the trapped particulate material in a known volume of methanol

Figure 9. Concentration, ng/mg WTPM, of targeted pyrazines in the mainstream smoke of selected products

Figure 10. Concentration, ng/mg WTPM, of targeted carboxylic acids in the mainstream smoke of selected products

Figure 11. Concentration, ng/mg WTPM, of targeted pyridines in the mainstream smoke of selected products

followed by injection of this clear dark colored solution into the gas chromatograph.

After simple sample collection and preparation, the method provides for the quantitative and qualitative evaluation of cigarette products from the perspective of the amount and type of flavor components yielded during burning.

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Figure 12. Concentration, ng/mg WTPM, of targeted carotenoid degradation compounds in the mainstream smoke of selected products

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