APPLICATION AND USE OF E-CIGARETTES HPHC METHODS FOR THE ANALYSIS OF HEATED TOBACCO PRODUCTS

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ENTHALPY ANALYTICAL

INTRODUCTION

The interest in e-cigarette research has resulted in the development of methods for the quantitative analysis of chemical constituents present in their aerosols. The emissions of e-cigarettes contain not only nicotine, propylene glycol, glycerin, water and flavors, but also nicotine related impurities and thermal degradation products. We have developed and validated analytical methods for the analysis of e-cigarette aerosols including the compounds listed in the US FDA Draft PMTA guidance document. These include primary constituents, aldehydes, VOCs, organic acids, metals, aromatic amines, nicotine related impurities and benzo[a]pyrene. An emerging tobacco product; Heated Tobacco Products (HTPs) produce an aerosol by heating of tobacco. Since tobacco is not combusted in these products, the majority of the resulting aerosol is comprised of water, humectants and volatile compounds. Given the similarities between e-cigarettes and HTPs, we hypothesized that e-cigarettes methods would also be suitable for analysis of HTPs emissions.

SAMPLE ANALYSIS

In this study, we have applied our validated e-cigarette methods to the analysis of the iQOS™ HEETS ™ HTPs. The compounds analysed included the FDA's PMTA Guidance list of 59 compounds plus selected metals, organic acids and additional nicotine degradants. These are methods on our ISO 17025 scope of accreditation.

The iQOS™ HTP aerosols were collected under intense smoking conditions (55ml, 2 sec puffs with 30 sec interval), using a filter holder optimized for collection of e-cigarette aerosols. HEETS ™ sticks (Amber) and the iQOS™ devices were purchased commercially. All iQOS™ samples were generated using a 20 port linear smoking machine under Canadian Intense (3 cigarettes or sticks /sample) conditions. A total of 54 constituents and HPHCs were determined for this study. A brief over view of the methods used is given below.

- **Collection of glycols**, **nicotine and water** was performed using filter pads extracted in isopropanol. Water was analyzed using GC-TCD; other constituents were analyzed using GC-FID.
- **Collection of Carbonyl Compounds** was performed using impingers containing DNPH trapping solution. Detection was accomplished by HPLC-UV/Vis.
- **Collection of Volatile Organic Compounds** was performed in methanol containing impingers fitted with fritted stems and placed in a dry ice/isopropanol bath. Samples were analyzed by GC-MS.
- **Collection of Tobacco Specific Nitrosamines** (TSNAs) was performed using filter pads extracted in water, then solvent-exchanged into dichloromethane. Detection was accomplished by GC-MS/MS.
- **Collection of Metals** was performed using 47 mm quartz filter pads, extracted and digested with 10% [v/v] nitric acid. Detection was accomplished by ICP-MS.
- **Collection of Primary Aromatic Amines** (PAAs) was performed using filter pads extracted with hexanes and water. The hexanes layer was then derivatized with pentafluoropropionic anhydride. Extracts were analyzed by GC-MS.
- **Collection of Nicotine degradants** was performed using filter pads extracted in a mix of methanol and water. Detection was accomplished by LC-MS/MS.
- **Collection of Benzo[a]pyrene** was performed using filter pads extracted in methanol and then diluted with water. The extract is further prepared using solid phase extraction and detection was accomplished by GC-MS.
- **Collection of Ammonia** was performed using a filter pad and impinger containing 40 mN aqueous sulfuric acid. The filter pads were extracted with the impinger contents. Detection was accomplished by an ion chromatograph equipped with a conductivity conductor.
- **Collection of Organic Acids was** performed using two water containing impingers in series. Detection was accomplished by an ion chromatograph equipped with a conductivity conductor.

For comparison purposes, HPHC data was collected from the 3R4F reference cigarette. Cigarettes were purchased from the University of Kentucky. Metals smoke samples were collected on a 20 port rotary smoking machine, all other smoke samples were collected on a 20 port linear smoking machine. Samples were collected under Intense smoking conditions. A total of 25 constituents and HPHCs were determined for this study. Common smoke HPHCs not included on the FDA Draft PMTA guidance were omitted from this study. Sample generation and analysis was conducted under applicable ISO standards, CORESTA, or Health Canada Methods.

RESULTS: ENDS COMPOUNDS

The iQOS™ HTP products were analyzed for the presence of compounds that may potentially be present in ENDS aerosol. Several classes of compounds were evaluated in the trapped HTP aerosol including glycols, metals, nicotine degradants and organic acids. **Glycols** (propylene glycol, glycerin, ethylene glycol, and diethylene glycol) are shown in Table 1. **Metals** (copper, iron, silver and tin) are shown in Table 2. **Nicotine degradants** (Myosmine, Nornicotine, ß-nicotyrine, Anatabine, Anabasine, Cotinine and Nicotine-N-oxide) are shown in Table 3. **Organic Acids** (acetic acid, formic acid, oxalic acid, benzoic acid, glycolic acid, and lactic acid) are shown in Table 4.

Table 1: Propylene glycol, glycerin, ethylene glycol, and diethylene glycol in the iQOS™ HTP

Compound Units	Diethylene glycol mg/stick	Ethanol mg/stick	Ethylene glycol mg/stick	Glycerin mg/stick	Propylene glycol mg/stick
Average	ND (<0.05)	ND (<0.06)	ND (<0.06)	4.78	0.12
Std Dev	NA	NA	NA	0.21	0.00
RSD	NA	NA	NA	4.4%	2.6%

Table 2: Copper, iron, silver and tin in the iQOS™ HTP

Compound Units	Copper ng/stick	Iron ng/stick	Silver ng/stick	Tin ng/stick
Average	ND (<0.333)	<LOQ (<16.6)	ND (<0.1)	<LOQ (<8.32)
Std Dev	NA	NA	NA	NA
RSD	NA	NA	NA	NA

Table 3: Myosmine, Nornicotine, ß-nicotyrine, Anatabine, Anabasine, Cotinine and Nicotine-N-oxide in the iQOS™ HTP

Compound Units	Anabasine µg/stick	Anatabine µg/stick	b-Nicotyrine µg/stick	Cotinine µg/stick	Myosmine µg/stick	Nicotine-N-Oxide µg/stick	Nornicotine µg/stick
Average	1.07	3.40	<LOQ (<0.34)	0.92	0.87	<LOQ (<0.33)	0.46
Std Dev	0.13	0.28	NA	0.09	0.06	NA	0.04
RSD	11.9%	8.4%	NA	10.2%	7.1%	NA	9.4%

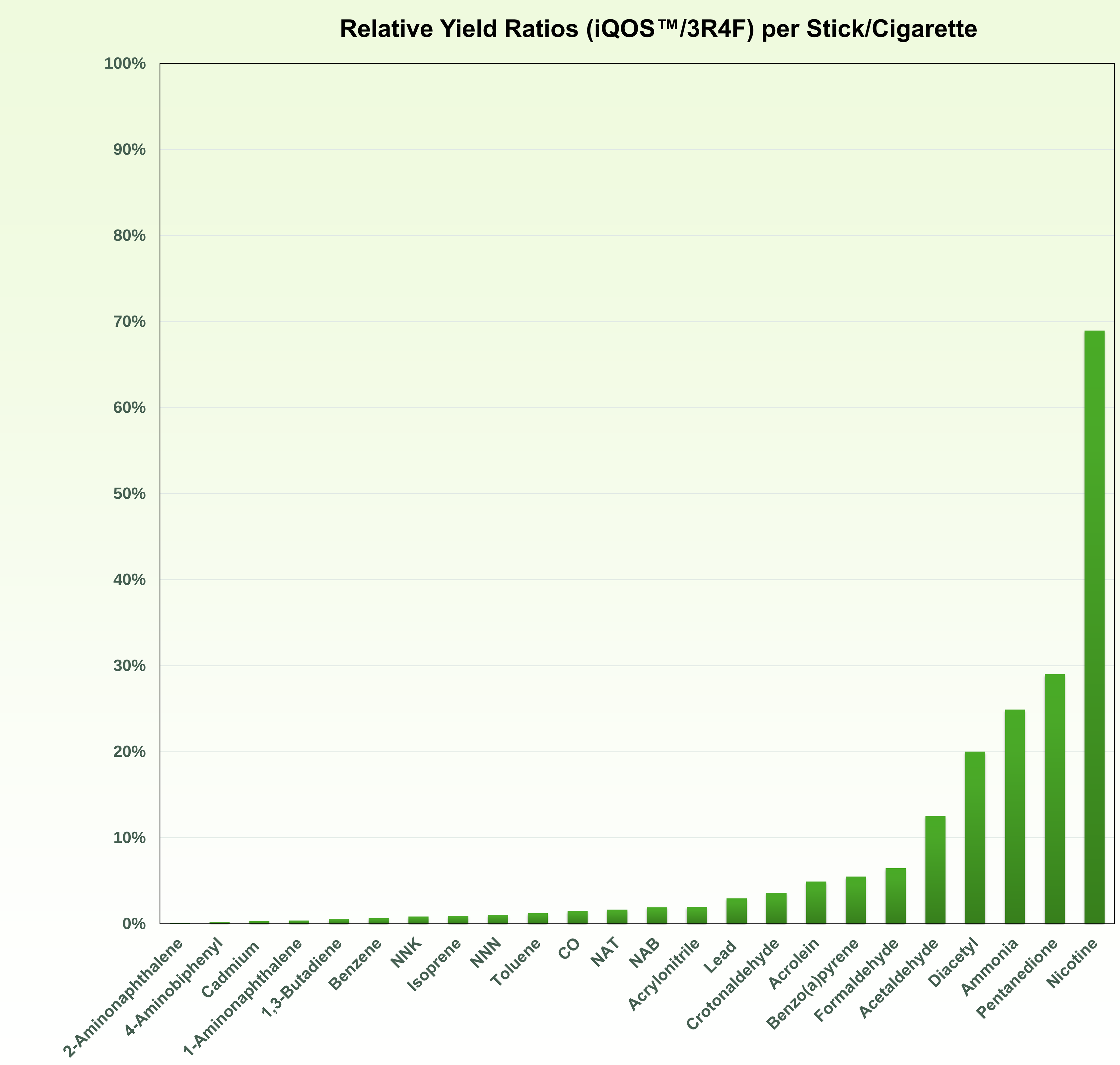
Table 4: Acetic acid, formic acid, oxalic acid, benzoic acid, glycolic acid, and lactic acid in the iQOS™ HTP

Compound Units	Acetic Acid µg/stick	Benzoic Acid µg/stick	Formic Acid µg/stick	Glycolic Acid µg/stick	Lactic Acid µg/stick	Oxalic Acid µg/stick
Average	284.5	ND (<2.74)	124.0	ND (<3.15)	41.7	ND (<2.64)
Std Dev	24.4	NA	9.6	NA	2.6	NA
RSD	8.6%	NA	7.8%	NA	6.3%	NA

RESULTS: 3R4F COMPARISON

The iQOS™ HTP was analyzed for the presence of compounds included in the FDA PMTA Guidance that overlapped with known combustion productions in cigarette smoke. The data for iQOS™ HTP product was generated using methods developed and validated for e-cigarette aerosols. The data for the 3R4F reference product was generated using methods developed and validated for combustible cigarette smoke. The compounds determined included carbonyl compounds (Diacetyl, 2,3-Petandione, Formaldehyde, Acetaldehyde, Acrolein, Crotonaldehyde), metals (Cadmium and Lead), Tobacco Specific Nitrosamines (NNK,NNN, NAT and NAB), aromatic amines (1-Aminonaphthalene, 2-Aminonaphthalene and 4-Aminobiphenyl), volatile organic compounds (1,3-butadiene, Acrylonitrile, Benzene, Isoprene, and Toluene), Benzo[a]pyrene, Ammonia, Carbon Monoxide and Nicotine. Data is presented in relative yield ratios by compounds: analyte yield of each compound in the iQOS™ divided by the analyte yield in the 3R4F. Data is presented on a per stick or cigarette basis.

Fig 1: Relative Analyte Yield of the iQOS™



DISCUSSION

The iQOS™ HTP product was analyzed for 54 compounds routinely measured in our laboratory in e-cigarettes emissions. The aerosol methods used in this study were developed specifically for the less complex e-cigarette aerosol produced by the heating of glycerin and propylene glycol instead of the complex aerosol produced by the combustion of tobacco. During the course of this study, we found that our e-cigarette methods were fit for the purpose of analyzing HTP products. Data was compared to published analytical results on the iQOS™ HTPs and all target analyte results were found to be in excellent agreement [1,2] with two exceptions. Our results for TSNAs were lower (Total TSNAs = 10.5 ng/stick) than reference results.[3] This value was confirmed with our tobacco smoke LC-MS/MS method. No assignable cause was found. The other compound that did not match the previously reported value was water. Our e-cigarette method for water yielded 38.6 mg/stick. While this value was higher than measured with the standard ISO method, it was lower than the value reported for the *in situ* filter extraction method.[4]

CONCLUSION

We found that methods developed and validated for e-cigarettes could be successfully applied to the analysis of HTPs emissions. With the exception of nicotine and water, the emissions from HTPs were found to be more similar in nature to e-cigarette aerosol than cigarette smoke. We found that e-cigarette methods were well suited for the analysis of HTPs due to the lower calibration ranges and selected compound list as compared to methods developed for conventional cigarettes. The iQOS™ HEETS ™ HTPs were compared against the 3R4F reference cigarette and we found that the yields of VOCs, aromatic amines, benzo[a]pyrene and TSNAs were reduced by 99% and carbonyl compound yields were reduced by 87% when testing under intense smoking conditions. These results were consistent with previously published studies.[3,4]

REFERENCES

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3) Addendum to FDA Briefing Document, January 24-25, 2018, Meeting of the Tobacco Products Scientific Advisory Committee; sata source: section 3.3.2 and section 6.1.1.3.4 of the Modified Risk Tobacco Product application (MRTPAs) and appendix A of an amendment to the MRTPAs submitted on 8 December 2017.

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