Enthalpy Analytical, Inc.
Statement of Qualifications
Testing of Electronic Cigarettes and e-liquids

Introduction:

Enthalpy Analytical, Inc., established in 1993, is a leading independent tobacco testing laboratory offering regulatory and research testing for tobacco products. Enthalpy offers analytical services for electronic cigarettes and e-liquids, in addition to traditional tobacco products including cigarettes, whole tobacco and smokeless tobacco. Enthalpy is accredited by A2LA and the ISO 17025 standard. Enthalpy occupies two facilities with over 60,000 ft² of laboratory and office space located in Durham, NC and Richmond VA. Our nationally accredited laboratory is equipped with modern linear and rotary smoking machines and an array of analytical systems designed for the collection, analysis and reporting of electronic cigarettes and e-liquids constituents. Client projects are managed in a secure network environment following strict quality guidelines. Enthalpy Analytical, Inc. is committed to providing our customers with the most accurate results available, robust testing solutions, and unmatched customer service.

Electronic Cigarettes and e-Liquid Services:

Enthalpy offers analysis and testing of electronic cigarettes and e-liquids for the presence of nicotine, propylene glycol, glycerin, carbonyls (i.e. diacetyl, formaldehyde, etc.), and the FDA's abbreviated list of harmful and potentially harmful constituents (HPHC), including tobacco specific nitrosamines (TSNA). Enthalpy has analytical methods in place to meet requirements of the 2016 Tobacco Product Directive (TPD). Our scientists utilize national and internationally accepted test methods, enhanced and adapted for our laboratory, to deliver accurate, reliable, consistent, and high quality data. All e-cigarette and aerosol methods have been validated for quality assurance including evaluation for linearity, recovery from the matrix, and verification of limits of detection. All analytical equipment is qualified and calibrated with full documentation. Our laboratories offer testing under ISO 17025 or GLP guidelines.

Smoke Laboratory:

Enthalpy operates three smoke labs at its Durham, NC and Richmond VA facilities. Environmental conditions in the lab are maintained to exacting specifications for humidity and temperature control using custom configured control systems. Equipment within the lab is supported by a backup electric generator and uninterruptable power system (UPS). Enthalpy houses quality controlled facilities for conditioned storage and sample preparation. Dedicated freezers and refrigerators are available for storage of samples and analytical standards. All laboratory data collected from smoking machine runs and analytical system outputs are stored on a secure, access-limited network with firewall and intrusion protection systems.

Key Personnel:

Enthalpy expert staff is composed of professionals and scientists who are experts in providing analytical services. Our technical staff employs experts in tobacco, air, and biomedical testing. Our combined technical staff offer decades of experience in analytical testing services**.** Our clients' work is performed by seasoned professionals expert in the techniques and equipment required to effectively complete client projects.

Steve Eckard, PE–Vice President founded Enthalpy Analytical, Inc. in 1993 and has grown the organization into one of the premier laboratories in the U.S. As a project designer and manager Steve applies his wide ranging testing and engineering expertise to ensure that Enthalpy's activities meet the quality standards required by our clients and regulatory agencies. Mr. Eckard is a NC registered Professional Engineer and has authored numerous papers on GC and regulatory issues in the air-testing field. Our clients value Steve's assistance in designing testing programs, training and regulatory consultation. (B.S. Chemical Engineering, Carnegie-Mellon)

Dr. Gene Gillman –Site Manager has considerable experience investigating the constituents and chemistry of tobacco smoke and more recently in electronic cigarettes and e-liquids. He has many years of experience in developing and validating relevant LC, LC-MS, GC-MS and biological based test methods. He brings expertise from both a cigarette manufacturing and a tobacco testing contract research organization (CRO) perspective. In his most recent position, he was the laboratory manager of an ISO 17025 accredited laboratory specializing in qualitative and quantitative analysis of tobacco smoke. Dr. Gillman has worked with domestic and international study groups and trade organizations. (Ph.D. Chemistry, Wake Forest University; B.S. Chemistry, Appalachian State University)

Kathy Humphries–TSC Group Manager is directly responsible for the delivery of Enthalpy's TSC Durham services and is an experienced bench chemist. She has over 25 years of laboratory and management experience and has served in a variety of roles over the span of her career including delivery of quality assured delivered work supporting pesticide residue analysis. She is proficient in the laboratory analytical techniques including gas chromatography, high performance liquid chromatography supporting organic and inorganic assays. (B.A. Chemistry, Salem College)

Melissa Johnson-Lab Manager is directly responsible for the delivery of Enthalpy's TSC Richmond services

Bryan Tyler – Sales Manager is responsible for providing customer service and managing client project arrangements. Bryan's knowledge of our analytical services, method performance and regulatory requirements makes him an asset to client projects. Mr. Tyler serves as a single point of client contact throughout each project. (B.A. Chemistry, B.S. Animal Science, MBA, North Carolina State University)

Dr. Karen Carter - Business Development Director (Ph.D. Chemistry, University of Virginia; M.Sc. Medicinal Chemistry, Medical College of Virginia; B.S. Chemistry, Old Dominion University)

e-Aerosol & e-liquid Test Methods and Analytes

- Nicotine, Propylene Glycol (PG), Vegetative Glycerin (VG), Diethylene Glycol (DEG), Water, Menthol
- Volatile Organic Compounds (VOC) and Semi-Volatile Organic Compounds (SVOC)
- Diacetyl, 2,3-Pentanedione (Acetyl Propionyl), Acetion
- Aldehydes & Ketones (Carbonyls): i.e. formaldehyde, acetaldehyde, acrolein, and crotonaldehyde
- Minor Alkaloids, Nitrosamines (TSNA), and Nicotine Degradants including Nicotine-N-Oxide (NNO)
- Polycyclic Aromatic Hydrocarbons (PAH) and Aromatic Amines
- Metals: arsenic, cadmium, chromium, copper, nickel, lead, tin, zinc
- Aerosol Particle Size and Stability Studies

e-Cigarette Device Test Methods:		
Matrix	**Analysis Method(s)**	**Target Compounds**
Vapor Phase Profile	GC-MS Scan Method	Identification of unknown peaks formed during vaporization
Device Testing	Automatic and manual devices (with buttons). Testing of variable wattage devices. Temperature controlled devices and measurement of coil temperature during puffing.	Nicotine, Propylene Glycol and Glycerin or thermal degradation products such as formaldehyde, acetaldehyde and acrolein.
Particle Transfer	Polymer characterization Contaminant testing Materials characterization High resolution SEM & TEM imaging	Unknown particles

Quality Assurance Program:

Enthalpy's nationally accredited laboratory operates under a comprehensive quality assurance (QA) system designed to ensure the validity, reproducibility, and accuracy of our measurements. ISO17025:2005 quality procedures serve as the basis for our primary TSC accrediting body, A2LA as well as the traditional environmental laboratory accrediting body NELAC (National Environmental Laboratory Accreditation Conference). Our laboratory is also certified to offer testing under GLP guidelines. Our quality assurance program includes Enthalpy's Quality Assurance Plan, Enthalpy's Handbook of Standard Operating Procedures (SOPs), and the standard methodologies published by international, federal and industry organizations. Our QA unit operates independently of technical staff and is directly managed by Enthalpy's Vice President.

Each project is delivered to the client according to customer specifications and Enthalpy's QA guidelines. Upon completion, each project receives a thorough review by Enthalpy's Quality Assurance staff prior to submission to our client. As a demonstration to our commitment to QA, we maintain a low ratio (1:4) of QA reviewer staff to analytical chemists. This ratio provides the resources for independent review of each data set while providing timely release of data in adherence with the overall project schedule.

Sample Analysis:

ISO Guide 34 Standards
Analytical standards used for the quantitation of HPHCs in tobacco and tobacco smoke shall meet the requirements of ISO guide 34 (if commercially available).

Laboratory Blanks
Laboratory blanks are performed on reagents used for each sample batch. This step confirms the cleanliness of the materials and systems used to collect client samples and ensures that detected analytes are a result of the samples being collected and not contaminants in the collection matrix. Analysis of method blanks confirms the cleanliness of the entire collection and analysis process, including smoking machines, impingers and sample recovery accessories.

Method Validation:

Methods performed in our laboratory are validated routinely and before use with client samples. However, not all methods have been validated for all possible matrixes. When a method is used outside of the validation scope, a validation study will be conducted with the client samples to ensure system suitability. This study typically includes analysis of laboratory generated matrix spikes at three concentrations and a short-term sample stability study. All employees must complete a demonstration of competency (DOC) of validated methods before being allowed to perform analysis and report data. Employees also receive ongoing training via continued demonstration of capabilities, which is documented in their individual training record which must remain up to date. Enthalpy does not employ subcontractors or temporary staff to perform in-house analytical work.

Select Test Methods

E-Liquid Test methods:

AM-200 Nicotine, Menthol, and humectants in e-liquid by GC-FID

This analytical method describes the procedure for the determination of compounds that may be found in e-liquid used in e-cigarettes. The selected compounds include nicotine, menthol, two humectants (propylene glycol and glycerol) and two humectants that are potential contaminants/adulterants (diethylene glycol and ethylene glycol). Internal standard is added to a weighed amount of e-liquid, which is subsequently diluted with isopropanol and vortexed briefly. The resulting extract is analyzed by gas chromatography (Hewlett Packard Model 6890) coupled to a flame-ionization detector (FID). Separation of the analytes is achieved using an RTX-Wax analytical column. Quantitation is by internal standard technique.

AM-232 pH in e-Liquid

E-Liquid is diluted with water and vortexed until a homogeneous solution is obtained. The pH of the aqueous e-liquid extract is then measured at 15 minute intervals until the pH reading (of the last two readings) are within 0.2 pH units of one another.

AM-225 Determination of Nitrosamines in e-liquids

This analytical method describes the procedure to quantitatively measure the following four tobacco-specific N-nitrosamines (TSNAs) in e-liquid (used in e-cigarettes) by LC-MS/MS using electrospray ionization (ESI) in the positive mode: N-Nitrosoanabasine (NAB), N-Nitrosoanatabine (NAT, 4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), and N-Nitrosonornicotine (NNN). An aliquot of e-liquid is diluted with water and the internal standard is added. The solution is vortexed for approximately 10 seconds. Samples are then analyzed by LCMS/MS using electrospray ionization in the positive mode. Separation is achieved using a Waters Xterra C18 50 x 2.1 mm; 2.5 µm column and gradient elution. Sample results are quantitated by internal standard using deuterated analogs of the analytes.

ENT270 Minor Alkaloids and Nicotine Degradants in E-Liquid

The e-Liquid samples were analyzed for nicotine n-oxide, nornicotine, myosmine, anabasine, cotinine, and antabine. E-Liquid aliquots are weighed and extracted with a 70:30 methanol/water solution. Sample extracts are vortexed until thoroughly mixed. When mixed, the samples are placed into autosampler vials to be analyzed via Waters Acquity LC/MS/MS. LC/MS/MS equipped with a Waters TQ Detector.

ENT301 Carbonyls in E-liquid and E-liquid Flavorings (HPLC UV-Vis)

The e-Liquid samples were analyzed for acetaldehyde, acetoin, diacetyl, formaldehyde, and 2,3-pentanedione (aka acetyl propionyl) following the analytical procedures in Enthalpy SOP ENT301. An aliquot of e-Liquid and/or e-Liquid flavor is derivatized with 2,4-dinitrophenylhydrazine (DNPH). After the derivatization reaction is complete, samples are neutralized with pyridine and analyzed via HPLC/UV.

AM-235 Selected Metals in e-liquid

E-liquid is digested in nitric acid at room temperature and brought to volume with type 1 water. Analysis is performed on an Agilent 770x ICP-MS.

Aerosol Test methods:

AM-201 Nicotine, Menthol Humectants and Contaminants in E-Cigarette Aerosol by GC-FID

This analytical method describes the procedure for the determination of compounds that may be found in the aerosol produced from e-cigarettes. The selected compounds include nicotine, menthol, two humectants (propylene glycol and glycerol) and two potential contaminants/adulterants (diethylene glycol and ethylene glycol). Analysis is accomplished by gas chromatography with flame ionization detection (GC/FID).Sample e-cigarette aerosol is collected on a 44 mm Cambridge filter pad (CFP). Ethyl acetate and internal standard solution are added to the CFP. The sample is extracted for 30 minutes on an orbital shaker. The resulting extract is analyzed by GC/FID. Separation of the analytes is achieved using an RTX-Wax analytical column. Quantitation is by internal standard technique.

AM-220Selected Tobacco-Specific N-Nitrosamines In E-Cigarette Vapor by LC-MS/MS

This analytical method describes the procedure to quantitatively measure the following four tobacco-specific N-nitrosamines (TSNAs) in e-cigarette vapor by LC-MS/MS: N-Nitrosoanabasine (NAB), N-Nitrosoanatabine (NAT), 4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), and N-Nitrosonornicotine (NNN). This analytical method is applicable to the quantitation of TSNAs in vapor collected from the puffing of e-cigarettes (mainstream smoke, MS) and vapor collected from the environment where e-cigarette "smoking" has occurred (environmental tobacco smoke, ETS) using Cambridge filter pads. TSNAs can be collected from MS or ETS using a 44-mm CFP. Once collection is complete, the CFP is transferred to an amber vial and extracted with methanol-water (1:1 v/v). The extract is mechanically shaken for 30 minutes. Extracts are subsequently filtered (PTFE; 0.2 µm), and then analyzed by LCMSMS using electrospray ionization in the positive mode. Separation is achieved using a Waters BEH UPLC C18 column and gradient elution. Sample results are quantitated by internal standard using deuterated analogs of the analytes.

AM-221 Selected Primary Aromatic Amines (PAAs) In E-Cigarette Vapor By GC-MS

This method describes the procedure to quantitatively measure the following primary aromatic amines (PAAs) in e-cigarette vapor by gas chromatography mass spectrometry (GC-MS) using negative chemical ionization (NCI): 1-Aminonaphthalene; 2-Aminonaphthalene; 3-Aminobiphenyl; 4-Aminobiphenyl. PAAs can be collected from MS or ETS using a 44-mm CFP. Once collection is complete, the CFP is transferred to an amber vial and extracted with20 mL dichloromethane for approximately 30 minutes. An aliquot of the sample extract is concentrated, dried and derivatized with pentafluoropropionic acid anhydride (PFPA) followed by analysis by GC-MS using NCI.

ENT305 Carbonyls in E-Cigarette Samples (HPLC UV-Vis)

The e-cigarette samples were analyzed for acetaldehyde, acrolein, acetone, crotonaldehyde, and formaldehyde following the analytical procedures in Enthalpy SOP ENT305. E-cigarettes are smoked using a smoking machine. The smoke is passed through a single impinger on ice containing a derivatization solution. The derivatization solution generates a colored adduct that can be visualized on an HPLC with a UV-Vis Diode

Array Detector or Variable Wavelength Detector. Samples are collected using a standard puff profile or using a client-requested puff profile.

Total Particulate Matter of Trapped Aerosol

The total particulate matter (TPM) collected for each puff block during smoking for the evaluation of propylene glycol (PG), glycerin (VG), and nicotine samples is determined by weighing the trapping system (filter pad) before and after each collection event. The total amount of aerosol is determined by the difference in mass.

Particle Size Distribution of E-cigarette Aerosol

The particle size distribution of e-cigarette aerosol is measured across the lifetime of the device. This is done via cascade impaction using a Marple personal sampler, MSP135-8. This impactor is designed for a flow rate of 2 liters per minute, (33.3 ml/sec) which can be used to activate e-cigarettes. E-cigarettes can be activated for 3 or 4 seconds. This experiment allows for the determination of the particle size distribution using 35 to 60 ml puff volumes. The total mass collected on each impactor stage is compared to the amount of mass lost from the e-cigarettes and used to calculate average particle size in the aerosol.

E-Cigarette Device Test Methods

Sample Stability

Enthalpy offers stability testing encompassing various sample storage conditions for the evaluation of e-liquid and e-cigarette product lifetimes. Our extensive program provides fully validated and continuously monitored storage environments for all ICH and WHO test conditions. We can also support study design and management of forced degradation studies to assess the "stability-indicating" power of the analytical methods under development for stability testing.

Conditions offered:
25 °C ± 2 °C/60% RH ± 5% RH (Standard)
40 °C ± 2 °C/75% RH ± 5% RH (Accelerated)
-20 °C ± 5 °C (Control)

Weight Loss for the E-cigarette Device

The amount of material lost from each device for each puff block is determined by weighing the e-cigarette before and after each collection event. The total weight loss is determined by the difference in mass.

Device Pressure Drop

Pressure drop for each device is measured before and after aerosol collection. Each device is uniquely identified to allow for comparison of pressure drop changes. Pressure drop is measured with a fully discharged battery attached to each device and the rate is read once the reading has stabilized.

Evaluation of Liquid Remaining After Normal Use of Electronic Cigarette

The liquid is extracted from a used device and is analyzed in duplicate by GC/MS. These samples will be compared to the liquid extracted from unused devices filled with the same liquid. Constituents other than the primary (propylene glycol, vegetable glycerin, nicotine) will be identified on a qualitative basis. In addition we can generate a full mass spectra which will have compounds identified qualitatively with an objective of

identifying >80% of the peaks in each sample using the NIST 2014 library. Qualitative analyte identification and instrument mass spectra are presented in a format which will allow the systematic comparison of used and unused devices to be tested.

Other analyses of the liquid such as Water by Karl Fisher Titration (KFT), as well as Aldehydes (Formaldehyde, Acrolein, Acetaldehyde, and Acetone) by HPLC/UV may be performed.

Evaluation of changes in vapor composition resulting from e-liquid vaporization

Typically, ten e-cigarettes are smoked on a rotary smoking machine using either a 35 or 55 ml puff volume. Aerosol is collected first onto 92mm pads and followed by a chilled impinger containing 30mL of methanol. The pads are extracted in methanol.

E-liquid samples are analyzed by GC/FID for nicotine content and are then adjusted to a known nicotine concentration and n-heptadecane is added as an internal standard. Starting e-liquids stock and vaporized samples are then compared by GC/FID. Any differences in the samples are reported. Additional qualitative analysis by GC/MS can be performed to help identify any differences between e-liquid stock and resulting vaporized samples.

The samples are then analyzed and compared by GC/MS. All samples are diluted to equivalent concentrations, with 100µg/mL of quinoline internal standard added to each extract. The samples are analyzed by GC/MS in scan mode using several columns with different stationary phases. Suggested phases includes a low polarity phase, Crossbond® 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane) column, and a polar phase, Crossbond® polyethylene glycol column. Compounds will be identified based on library matching and estimated concentrations will be reported based on relative response to the quinoline internal standard.

Selected Analytical Projects

Major Manufacturer of Disposable E-Cigarette	**FDA abbreviated HPHC list measurement** -Enthalpy Analytical, Inc. provided analytical services to amajor manufacturer of disposable e-cigarettes in the winter of 2012. Data was reported according to the client's project schedule and was in an electronic format.
Voluntary Industry Standards Group	**Enthalpy performed analysis of e-liquids and USP grade Nicotine** for a confidential client.

Other Capabilities:

Enthalpy partners with MVA Scientific Consultants (www.mvc-inc.com), located in Duluth, Georgia, for other identification and characterization of particle characterization and unknown material present in in-cigarettes and e-cigarette aerosol. MVA is accredited to ISO/IEC 17025, by A2LA, and is cGMP compliant.

Technical Presentations and Papers:

Gillman, I.G.; Kistler, K.A.; Stewart, E. W.; Paolantonio, A.R., "Effect of variable power levels on the yieldof total aero-sol mass and formation of aldehydes in e-cigarette aerosols" Reg. Tox. Pharm. **2016**;75:58-65.

2015 CORESTA Joint Study Group Meeting, October 4-8, **2015,** , Effect of power level on the yield of total aerosol mass and formation of aldehydes in e-cigarette aerosols, Gillman I.G.; Stewart E.W.; Paolantonio A.R.

Konstantinos E Farsalinos · Gene Gillman · Konstantinos Poulas · Vassilis Voudris. Tobacco-Specific Nitrosamines in Electronic Cigarettes: Comparison between Liquid and Aerosol Levels·International Journal of Environmental Research and Public Health 07/**2015**; 12(8):9046-9053

Konstantinos E Farsalinos · Gene Gillman · Matt S Melvin · Amelia R Paolantonio · Wendy J Gardow · Kathy E Humphries · Sherri E Brown · Konstantinos Poulas · Vassilis Voudris, Nicotine Levels and Presence of Selected Tobacco-Derived Toxins in Tobacco Flavoured Electronic Cigarette Refill Liquids,International Journal of Public Health 03/**2015**; 12(4):3439-3452.

Konstantinos E. Farsalinos; Kurt A. Kistler; Gene Gillman; Vassilis Voudris, Evaluation of electronic cigarette liquids and aerosol for the presence of selected inhalation toxins, Nicotine & Tobacco Research, **2014**,17(2).

Tobacco Merchants Association Deeming Regulations Conference, June 16, **2014**, Leesburg, Virginia, Invited Speaker, I. Gene Gillman.

Tobacco Merchants Association E-cigarette Conference, October 30, **2013**, Leesburg, Virginia, Invited Speaker "eCig Vapor Analysis" I. Gene Gillman

67thTobacco Science Research Conference, September 15-18, **2013**, Williamsburg, Virginia, Invited Symposium Speaker "The changing role of the contract research laboratory in the tobacco industry" I. Gene Gillman

2012 CORESTA CONGRESS, September 23-27, **2012**, Sapporo, Japan "Determination of volatile organic compounds from US FDA list of Harmful or Potentially Harmful Compounds in mainstream cigarette smoke by GC-MS " I. Gene Gillman and Katherine E. Humphries

66th Tobacco Science Research Conference, September 11-14, **2012**, Concord, North Carolina " Determination of aromatic amines through the use of tandem mass spectrometry coupled to gas phase chromatography" Sherri S. Brown and I. Gene Gillman

66th Tobacco Science Research Conference, September 11-14, **2012**, Concord, North Carolina "Applications of GC-tandem mass spectrometry to the analysis of chemical constitutes in mainstream cigarette smoke" I. Gene Gillman, Sherri S. Brown and Katherine E. Humphries

2011 CORESTA Joint Study Groups Meeting, October 9-13, **2011**, Graz, Austria "Determination of a wide range of N-nitroso-compounds in smokeless tobacco through the use of tandem mass spectrometry coupled to gas phase chromatography" I. G. Gillman, T. D. Daniels, K.A. Wilkinson, K.E. Humphries, and S.S. Brown

65th Tobacco Science Research Conference, September 18-21, **2011**, Lexington, Kentucky "Determination of a wide range of N-nitroso-compounds in both tobacco smoke and in smokeless tobacco through the use of

tandem mass spectrometry coupled to gas phase chromatography ” I. G. Gillman, T. D. Daniels, K.A. Wilkinson, K.E. Humphries, and S.S. Brown

Enthalpy Analytical TSC Group – Major Analytical Equipment:

Sample Measurement and Smoking Equipment

Cerulean Quantum Plus	Physical properties
Cerulean SM 450	(15) 20 port linear smoking machines
Cerulean CR20	20 port rotary smoking machine.
Borgwaldt RM1	2, Single port smoking machine
Borgwaldt RM20	20 port rotary smoking machine

Analytical Instruments

Instrument	Inventory	Target Analytes
LC/MS Triple Quad Waters	6	Minor Alkaloids
GC/MS Triple Quad Agilent Model 7000QQQ	2	TSNA
GC/MS Agilent Model 5975	14	VOC, SVOC,PAH
HPLC/IC Dionex Model 3000 Agilent Model 1100	4	Ammonia, Nitrate
ICP-MS Agilent 7500	1	Metals analysis
HPLC/UV various	16	Carbonyls
GC/FID Agilent 5890/6890	16	Nicotine
Gravimetric Balance Sartorius /Mettler	10	TPM