P1 AEROSOL CHARACTERIZATION - LC-HRAM-MS

STUDY PLAN / EXPERIMENTAL PLAN & REPORT

PROGRAM NAME	ANALYTICAL CAPABILITY
PROJECT NAME	BRIDGING METHODOLOGY
WORK PACKAGE NAME	P1 AEROSOL CHARACTERIZATION
EXPERIMENTAL WORK TYPE	Exploratory ☒ Developmental ☐ Regulatory submission ☐
OWNER/APPROVER	MARK BENTLEY
AUTHORS	DANIEL ARNDT
DATE OF FINAL APPROVAL	PLEASE REFER TO EDMS

Contents

1 Abstract

This document describes the planning and deliverables for the characterization of the most abundant P1 aerosol constituents using non-targeted screening (NTS) methodologies by liquid chromatography coupled to high resolution accurate mass spectrometry (LC-HRAM-MS). The primary objectives are to determine the major constituents present in P1 NFDPM, in order to provide supporting information for PMI's position regarding the absence of "Tar" in P1 and to deliver product developers with a list of the top 500 most abundant compounds in the aerosol from the THS 2.2 regular (Dorado II 'Ron') as a benchmark for PMI's heat-not-burn portfolio.

2 Introduction

The objective of this study is to identify and semi-quantify heat-not-burn aerosol constituents from the THS 2.2 regular (Dorado II 'Ron') using all available non-targeted methodologies for the analytical platform LC-HRAM-MS.

A set of 4 complementary non-targeted methods using LC-HRAM-MS, designed to cover the broadest possible range of chemical classes present in conventional and MRTP aerosols using liquid chromatography, will be applied. These methods will be used in parallel with their development, in order to characterize the most abundant constituents in the aerosol of the THS 2.2 regular (Dorado II 'Ron') generated using the Health Canada Intense (HC) smoking regime. The methods will not have been formally validated at the point of analysis, however, a work instruction is available.

Identified compounds will be classified in accordance with structure, origin and their potential role within the aerosol.

3 Samples definition

Aerosol will be generated using the THS 2.2 tobacco heating system comprising a commercially produced tobacco heating device (THD 2.4; PDIMS Device Batch B23172; Device Version DV.000174(7)) and the regular version of the THS HeatStick as described in **Table 1**. Mainstream aerosol will be generated and cryogenically trapped according to [1] PMI_RD_WKI_000080 'Trappage des composés aromatiques' using a programmable dual syringe pump (PDSP) and a cryogenic cold trap system. Furthermore the mainstream aerosol will be generated and trapped in accordance to [2] PMI_RD_WKI_000530 'Trappage en phase particulaire pour la détermination des constituants de l'aérosol'.

Table 1 Test Items

Short Name	Description	Product Code	Consumable Batch number	Manufacturing date	Batch size (sticks)
THSR	THS 2.2 Dorado II Ron	ME000004.02	B-25906 / 41-2382704	14.01.2016	(b) (4)
3R4F	Reference	K1908	N/A	N/A	N/A

The commercialized THS 2.2 (THSR) test articles were produced at Intertaba SpA, Via Fratelli Rosselli 4, 40069 Zola Predosa, Italy. The samples will be stored in the climatic chambers in packs. The packs are polypropylene wrapped Mini princess packs consisting of two collations, each collation containing 10 heat sticks.

The Reference Cigarette 3R4F was purchased from the University of Kentucky, Kentucky Tobacco Research and Development Center (for specifications see [3]. (http://www2.ca.uky.edu/refcig/3R4F20Preliminary20Analysis.pdf)).

Mass-produced test items and cigarettes are generally homogeneous. However, most constituents involved in the manufacture are derived from natural products, and therefore result in a final product which is intrinsically variable.

Table 2 Experimental Groups of Study Samples

Description	Smoking Regimen	Method*	Sample Trapping	Type	Short Name
THS regular	HC	RP	Cryogenic Trapping (ColdTrap)	test	THSR
3R4F	HC	RP	Cryogenic Trapping (ColdTrap)	reference	3R4F
Blank	HC	RP	Cryogenic Trapping (ColdTrap)	reference	BRCT
THS regular	HC	HILIC	Cryogenic Trapping (ColdTrap)	test	THSR
3R4F	HC	HILIC	Cryogenic Trapping (ColdTrap)	reference	3R4F
Blank	HC	HILIC	Cryogenic Trapping (ColdTrap)	reference	BHCT
THS regular	HC	RP	Cambridge Pad NFDPM	test	THSR
3R4F	HC	RP	Cambridge Pad NFDPM	reference	3R4F

Description	Smoking Regimen	Method*	Sample Trapping	Type	Short Name
Blank	HC	RP	Cambridge Pad NFDPM	reference	BRNF
THS regular	HC	HILIC	Cambridge Pad NFDPM	test	THSR
3R4F	HC	HILIC	Cambridge Pad NFDPM	reference	3R4F
Blank	HC	HILIC	Cambridge Pad NFDPM	reference	BHNF

* RP – reversed phase chromatography; HILIC – hydrophilic interaction liquid chromatography

The aerosol will be trapped and treated in accordance with the requirements for each of the analytical methods.

4 Research Questions

- What is the identity and the quantity (semi-quantitatively determined) of the most abundant compounds in the THS 2.2 regular (Dorado II 'Ron') amenable to liquid chromatography coupled to high resolution accurate mass spectrometry (LC-HRAM-MS)?

5 Methods

5.1 Design

The latest version of THS 2.2 'Dorado II Ron' (THSR) together with the device THD 2.4 will be used to generate aerosols for the chemical characterization of the most abundant compounds present in NFDPM and cryogenically trapped mainstream aerosol (Cold Trap). The reference cigarette 3R4F will be used to qualify the system and to serve as a comparator for the most abundant chemical constituents found in the aerosol of the THS 2.2 'Dorado II Ron' (THSR). Blank aerosol samples will be used to exclude any potential impact of smoke machines, trapping approaches or analytical methods on the results.

A set of 4 complementary non-targeted methods using LC-HRAM-MS, designed to cover the broadest possible range of chemical classes present in conventional and MRTP aerosols using liquid chromatography, will be applied to analyze the samples using a non-targeted screening approach (NTS).

5.2 Analytical Procedures

5.2.1 Test Item Conditioning

All test articles will be stored in a cooling chamber at 4 ± 3 °C with uncontrolled humidity. Prior to aerosol generation the test articles THSR and 3R4F will be conditioned according to [4] ISO 3402[1] and [5] PMI_RD_WKI_000489[2] for a minimum of 48h and a maximum of 10 days at 22 ± 1 °C and 60 ± 3% relative humidity (RH). The conditioning will be performed in open packages for all test items.

5.2.2 Aerosol Generation

5.2.2.1 Aerosol Generation for Cryogenic Trapped Mainstream Aerosol (Cold Trap)

Mainstream aerosol from THS 2.2 regular and 3R4F will be generated under HC smoking conditions (**Table 3**) and trapped using a cold trap at -200 °C according to [1] PMI_RD_WKI_000080[3]. For the HC smoking protocol (Health Canada, T-115, 1999) [6], the 3R4F cigarettes will be 100% vent-blocked by taping in accordance with [7] PMI_RD_WKI_000399[4]. THSR items will not be taped due to absence of ventilation holes in the filter region. The room conditions for aerosol generation will be 22 ± 2 °C and 60 ± 5% RH.

Table 3 Smoking Regime for Cryogenic Trapped Mainstream Aerosol (Cold Trap)

Short Name	Puff Volume [mL]	Duration [s]	Puff interval [s]	Frequency [min^{-1}]	Puff Count [n]
THSR	55	2	30	2	12
BLANK	55	2	30	2	12
3R4F	55	2	30	2	10 *

* smoked to a fixed butt length of 35mm (normally achieved in approximately 10 puffs)

Each smoking replicate will consist of the accumulated trapped whole aerosol from 2 sticks/cigarettes and afterwards extracted with 2 times 5 mL methanol for reversed phase chromatography and with 2 times 5 mL acetonitrile for hydrophilic interaction chromatography (**Table 4**). The extraction solvents will be provided to the aerosol generation lab on the day of aerosol collection.

[1] ISO 3402 'Tobacco and tobacco products -- Atmosphere for conditioning and testing 4th edition'

[2] PMI_RD_WKI_000489 'Preparation of items'

[3] PMI_RD_WKI_000080 'Trappage des composés aromatiques'

[4] PMI_RD_WKI_000399 'Blocage de la ventilation du papier de bout des cigarettes'

Table 4 Experimental Details for the Preparation of Cryogenic Trapped Aerosol

Short Name	Number of Replicates [n]	Items per Replicate [accumulation]	Extraction Solvent	Extraction Volume [mL]
THSR_RP	3	2	Methanol	2 x 5[5]
3R4F_RP	3	2	Methanol	2 x 5[4]
Blank_RP	3	/	Methanol	2 x 5[4]
THSR_HILIC	3	2	Acetonitrile	2 x 5[4]
3R4F_HILIC	3	2	Acetonitrile	2 x 5[4]
Blank_HILIC	3	/	Acetonitrile	2 x 5[4]

5.2.2.2 Aerosol Generation for NFDPM

Mainstream aerosol from THS 2.2 regular and 3R4F will be generated under HC smoking conditions (**Table 5**) and trapped using Cambridge filter pad to [2] PMI_RD_WKI_000530[6]. For the HC smoking protocol (Health Canada, T-115, 1999) [6], the 3R4F cigarettes will be 100% vent-blocked by taping in accordance with [7] PMI_RD_WKI_000399[7]. THSR items will not be taped due to absence of ventilation holes in the filter region. The room conditions for aerosol generation will be 22 ± 2 °C and 60 ± 5% RH.

Table 5 Smoking Regime for NFDPM

Short Name	Puff Volume [mL]	Duration [s]	Puff interval [s]	Frequency [min^{-1}]	Puff Count [n]
THSR	55	2	30	2	12
BLANK	55	2	30	2	12
3R4F	55	2	30	2	10 *

* smoked to a fixed butt length of 35mm (normally achieved in approximately 10 puffs)

[5] The 2 times 5 mL extraction volume can be combined into a single vessel after extraction

[6] PMI_RD_WKI_000530 'Trappage en phase particulaire pour la détermination des constituants de l'aérosol'

[7] PMI_RD_WKI_000399 'Blocage de la ventilation du papier de bout des cigarettes'

The Nicotine free dry particulate matter (NFDPM amount: approximately 100 - 150 mg) from each aerosol collection (**Table 6**) will be collected on a 44 mm Cambridge glass fiber filter pad (GF) according to the procedure described in [2] PMI_RD_WKI_000530[8]. Each sample replicate will consist of the accumulated crude condensate from 2 sticks/cigarettes. After sample generation, the GF is stored in a cleaned (3 times methanol rinsed and dried) Pyrex® tube. The tubes will be provided to to the aerosol generation lab on the day of sample collection.

Table 6 Experimental Details for the Preparation of NFDPM

Short Name	Number of Replicates [n]	Items per Replicate [accumulation]	Comment
THSR_RP	3	2	Pad Only
3R4F_RP	3	2	Pad Only
Blank_RP	3	/	Pad Only
THSR_HILIC	3	2	Pad Only
3R4F_HILIC	3	2	Pad Only
Blank_HILIC	3	/	Pad Only

[8] [2] PMI_RD_WKI_000530 'Trappage en phase particulaire pour la détermination des constituants de l'aerosol'

5.2.3 Sample Preparation

5.2.3.1 Sample Preparation for Cryogenic Trapped Mainstream Aerosol (Cold Trap)

Aliquots (200µL) of the cold trap extracts derived from **Table 4** will be diluted with methanol (700µL) for RP analysis or acetonitrile (700µL) for HILIC analysis. 100µL of internal standard solution (WS IS_1) will be added to each sample and, after vial closure, mixed for 5 minutes using an Eppendorf ThermoMixer (5°C; 2000 rpm). Aliquots (1.5 µL) of the diluted extracts will be injected and analyzed by LC-HRAM-MS in full scan mode and in data-dependent fragmentation mode for compound identification (**Table 7** and **Table 8**). Each diluted extract will be analyzed 5 times (analytical replicates) in full scan mode and data-dependent fragmentation mode.

5.2.3.2 Sample Preparation for NFDPM

10 mL of extraction solvent (methanol for RP, acetonitrile for HILIC) will be added to each Pyrex® tube containing the GF derived from **Table 6**. The filter is extracted by thoroughly shaking the Pyrex® tube (disintegrating the GF), vortexing for 5 min and finally centrifuging (4500 g, 5 min, 10 °C). The extract will then filtered using a glass fiber frit to avoid any transfer of glass fiber particles. Aliquots (200µL) of the NFDPM extracts will be transferred into a chromatographic vial and diluted with methanol (700µL) for RP analysis or acetonitrile (700µL) for HILIC analysis. Internal standard solution (100 µL, WS IS_1) will also be added to each sample. The vial will be sealed and then mixed using an Eppendorf ThermoMixer (5 min, 5 °C, 2000 rpm). An aliquot (1.5 µL) of the diluted extracts will be injected and analyzed by LC-HRAM-MS in full scan mode and in data-dependent fragmentation mode for compound identification (**Table 7** and **Table 8**). Each diluted extract will be analyzed 5 times (analytical replicates) in full scan mode and data-dependent fragmentation mode.

5.2.3.3 Pool Sample Generation

To enable advanced data processing, a pool sample will be created and used as a reference point for data processing (Section 3.5) in order to have a single sample representing the entire trapped chemical space. Aliquots (100µL) of each aerosol sample generated will be combined and mixed well. Aliquots (200µL) of this pooled aerosol sample will be prepared for chromatographic analysis as detailed above.

5.3 Analytical Methods

The NTS assay using LC-HRAM-MS will be performed to evaluate the most abundant compounds in the cryogenic trapped aerosol and NFDPM aerosol fraction of the THS 2.2 regular (Dorado II 'Ron').

The samples will be analyzed by LC-HRAM-MS using a Thermo QExactive™ high resolution mass spectrometer in both full scan mode and data dependent mode in accordance with [8] PMI_RD_WKI_001225[9]. In total, 4 different methods will be applied in order to cover a wide range of substances with different ionization properties and compound classes. Samples will be analyzed using RP chromatography with electrospray ionization (ESI) in both positive and negative mode and with atmospheric pressure chemical ionization (APCI) in positive mode, and using HILIC chromatography in ESI positive ionization mode. For RP chromatography mode, samples extracted with methanol will be used. For HILIC chromatography mode, samples extracted with acetonitrile will be used.

For the subsequent identification of constituents, samples will be measured using a data dependent fragmentation method, complementary to the full scan analysis. Instrumental parameters are presented in **Table 7** and **Table 8**.

The methods will be conducted according to the following WKI, which contain all necessary information related to required instrumentation and materials:

- PMI_RD_WKI_001225 NTDS NTDS LC-HR-MS PROGENESIS QI

The non-targeted screening methodology represents a subset of the non-targeted differential comparison, described in the WKIs. The comparative evaluation is not in scope for this study.

Table 7 Instrument Scan Events

Scan Event	Scan Event Details	Detection	Fragmentation	Resolution
(1)	Full Scan	FTMS	-	70000
(2)	MS/MS Top 3 most intense from (1)	FTMS	HCD	17500

[9] PMI_RD_WKI_001225 'NTDS LC-HR-MS PROGENESIS QI'

Table 8 Instrument Parameters

Parameter	
General Parameters	
In-source CID [eV]	Off (0.0 eV)
Default Charge State	1
Full MS	
Microscans	1
Resolution	70000
AGC Target	3e6
Maximum IT [ms]	100
Scan Range [Da]	80 - 800
Spectrum Data Type	Profile
dd-MS^2	
Microscans	1
Resolution	17500
AGC Target	1e5
Maximum IT [ms]	150
Loop Count	3
TopN	3
Isolation Window [m/z]	4
Scan Range [Da]	80 - 800
Stepped NCE [eV]	25, 50, 75
dd Settings	
Underfill Ratio [%]	1.00
Intensity Threshold	6.7e3
Apex Trigger	Off
Dynamic Exclusion [s]	10

The accurate mass measurements allow the determination of elemental composition (proposed sum formula) for precursor ions derived from the full scan analyses, and the elemental composition of the fragments using data dependent fragmentation experiments. Combining these information results in a high certainty for the proposed elemental composition of a compound and additionally identified structural features. Semi quantification will be carried out by means of derived consecutive replicates per sample.

5.4 Data Processing

The data evaluation process consists of several steps:

- Data import into metabolomics data mining software Nonlinear Dynamics Progenesis® QI
- Alignment
- Experimental design setup (defining one or more groups for aligned runs)
- Peak picking
- Normalization using internal standards
- Deconvolution
- Compound identification (accurate mass and adduct search against database)
- Compound review (managing compound identities and exploring identities and expression between conditions)
- Processing of aligned and normalized (csv-)dataset with EXCEL
- Semi-quantification of compounds
- Extraction of obviously different compounds (compounds of interest)
- Sorting of information according to RANK parameters
- Manual verification of results

5.4.1 Semi-Quantification of Compounds

Although normalization of the peak abundance against an internal standard will be performed within Progenesis® QI, semi-quantification of the compounds will be calculated using EXCEL. Semi-quantification will be performed on the basis of the peak area ratio between the analytes and the appropriate internal standard, which is chosen depending upon the ionization mode used. Since no calibration is performed, the results will be semi-quantitative. The ability for compounds to ionize varies strongly in APCI positive, since it is a soft ionization technique, therefore values derived should only be used as a rough estimation of abundance. If absolute quantitative data are needed, the respective analytes will be calibrated using a certified reference substance.

5.5 Data Evaluation

Data Fusion and Sorting for Abundance

A meta-result list will be prepared separately for Cold Trap and for NFDPM analysis, either using the software tools Excel or Pipeline Pilot using the fused dataset of the 4 available methods (RP ESI positive, RP ESI negative, RP APCI positive and HILIC ESI positive) and sorted for abundance.

Cleaning for Systemic Compounds (Blank)

The meta-result list will be cleaned for compounds present in blank samples with a concentration more than 50% of the aerosol result.

Retrieving additional Structural Information

The cleaned meta-result list will be complemented by PhysChem properties and other relevant information on a proposed chemical structure that is available in UCSD (Unique Compound and Spectral Database) with the help of chemoinformatic tools.

Defining Compound Scope

The results of the meta-analysis will be considered for defining the number of compounds to be identified using reference standards.

Current planning:

- Confirmed identification using reference standards (if practicable) for the top 500 constituents of the fused dataset of LC-HRAM-MS, GC-HR-TOF-MS and GCxGC-TOF-MS platforms, semi-quantified to be above 100 ng/stick
- Tentative identification for constituents semi-quantified to be above 10 ng/stick (per method)

Alternative planning:

- Confirmed identification using reference standards (if practicable) for all constituents semi-quantified to be above 100 ng/stick (per method)
- Tentative identification for constituents semi-quantified to be above 10 ng/stick (per method)

5.6 Compound Identification

Compounds will be identified based on the accurate mass, isotopic similarity and detected adducts, retention time compared to reference database, MS^2 fragments compared to theoretical fragmentation and MS^2 compared to reference database. The compound identification comprises the following 5 steps.

- **Compound Identification using UCSD Database Fragmentation (Step I)**
  The first identification step comprises the accurate mass comparison of the acquired data against the in-house UCSD database via [9] MetaScope algorithm (Precursor tolerance: 5ppm). In addition the compound retention times are compared with the in-house retention time database (Retention within 0.5 minutes). All acquired MS^2 spectra are compared against in-house MS^2 library (Fragment tolerance: 10 ppm).

- **Compound Identification using UCSD Theoretical Fragmentation (Step II)**
  The second identification step comprises the theoretical fragmentation of the in-house UCSD database via MetaScope algorithm and MetFrag algorithm [10]. The actual acquired MS^2 spectra are matched against in-silico (theoretical) fragmented spectra of the UCSD candidate compounds (Fragment tolerance: 10 ppm). In addition the compound retention times are compared with the in-house retention time database (Retention within 0.5 minutes).

- **Compound Identification using HMDB Theoretical Fragmentation (Step III)**
  The third identification step comprises the theoretical fragmentation of the HMDB database [11] via MetaScope algorithm and MetFrag algorithm. The actual acquired MS^2 spectra are matched against in-silico (theoretical) fragmented spectra of the HMDB candidate compounds (Fragment tolerance: 10 ppm).

- **Compound Identification using NIST MS/MS Library (Step IV)**
  The fourth identification step comprises the accurate mass comparison (neutral mass) and the match of actual acquired MS^2 spectra against MS^2 fragment spectra library of NIST [12] candidate compounds (Precursor tolerance: 5 ppm, Fragment tolerance: 5 ppm).

- **Compound Identification using ChemSpider Theoretical Fragmentation (Step V)**
  The fifth identification step comprises the theoretical fragmentation of ChemSpider with ChemIDplus and FDA as connected data sources via MetFrag algorithm. The actual acquired MS^2 spectra are matched against in-silico (theoretical) fragmented spectra of the ChemIDplus [13] and FDA [14] candidate compounds. The elemental composition comprises H: 0-200, C: 0-100, N: 0-10, O: 0-30, P: 0-2 and S: 0-2 is considered as filter.

Table 9 Number of Compounds per Database

Database / Data Source	Number of Compounds / Datasets
UCSD	11,252
UCSD in-house MS/MS library	201
HMDB	37,619
NIST 14 MS/MS library	8,351
ChemIDplus	322,916
FDA	1,529

5.7 Statistical Analysis

Mean and standard deviation values only will be calculated.

6 Administrative Aspects

6.1 Study Timelines

No.	Tasks / **Deliverables**	Description	Responsible	Delivery date
1.	**Work package in place**	**Work package description P1 aerosol characterization – LC-HRAM-MS approved**	**D. Arndt**	**Q2, 2015**
2.	**Study Plan in place**	**Study Plan P1 aerosol characterization – LC-HRAM-MS approved**	**D. Arndt**	**Q1, 2016**
3.	Start Date Experimental	Start aerosol sample generation	D. Arndt	Q1, 2016 (Mar)
4.	End Date Experimental	Sample generation, preparation, acquisition and data processing finished	D. Arndt	Q2, 2016 (Apr)
5.	Data Evaluation finished	Data evaluation finished (generation of data table)	D. Arndt	Q3, 2016 (Jul)
6.	**Data Summary I (Generic Identification RP ESI pos)**	**Data summary for RP ESI positive compounds**	**D. Arndt**	**Q3, 2016 (Aug)**
7.	**Data Summary II (Generic Identification RP ESI neg)**	**Data summary for RP ESI negative compounds**	**D. Arndt**	**Q3, 2016 (Sep)**
8.	**Data Summary III (Generic Identification RP APCI pos)**	**Data summary for RP APCI positive compounds**	**D. Arndt**	**Q3, 2016 (Oct)**
9.	**Data Summary IV (Generic Identification HILICI ESI pos)**	**Data summary for HILIC ESI positive compounds**	**D. Arndt**	**Q3, 2016 (Oct)**
10.	Confirmed Identification finished	Reference standards measured and proposed structures (generic identification) confirmed	D. Arndt	Q4, 2016 (Dec)
11.	**Data Summary Final (Confirmed Identifications all Methods)**	**Final data summary**	**D. Arndt**	**Q4, 2016 (Dec)**

6.2 Roles and Responsibilities

The internal key contributors are as follows:

Name	Function in the project
Mark Bentley	Summary WP Owner/Manager
Daniel Arndt	WP Manager – P1 Aerosol Characterization - LC-HRAM-MS
Stefania Della Gatta	Scientist - SME
Christoph Buchholz	Scientist - SME
Arno Knorr	WP Manager – P1 Aerosol Characterization - GCxGC-TOF-MS
Philippe Guy	WP Manager – P1 Aerosol Characterization - GC-HR-TOF-MS
Pavel Pospisil	Manager Computational Chemistry – Data Management
Elyette Martin	Scientist Computational Chemistry
Antonio Castellon	Scientist Computational Chemistry
Deborah Forte	Test Item Management

7 References

[1] PMI_RD_WKI_000080 'Trappage des composés aromatiques'

[2] PMI_RD_WKI_000530 'Trappage en phase particulaire pour la détermination des constituants de l'aérosol'

[3] University of Kentucky (http://www2.ca.uky.edu/refcig/3R4F20Preliminary20Analysis.pdf)

[4] ISO 3402 'Tobacco and tobacco products -- Atmosphere for conditioning and testing 4th edition'

[5] PMI_RD_WKI_000489 Reception and preparation of test and reference item

[6] Health Canada, T-115, Official Method T-115, Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, prepared by the Department of Health dated December 31, 1999

[7] PMI_RD_WKI_000399 'Blocage de la ventilation du papier de bout des cigarettes'

[8] PMI_RD_WKI_001225 'NTDS LC-HR-MS PROGENESIS QI'

[9] MetaScope, Algorithm of the Systems Biology Research Group at the University of California, San Diego, USA

[10] Wolf S., Schmidt S., Müller-Hannemann M., Neumann S.; '*In silico fragmentation for computer assisted identification of metabolite mass spectra*' , 11:48, BMC Bioinformatics 2010

[11] Wishart DS, Jewison T., Guo AC, Wilson M., Knox C., et al., '*HMDB 3.0 — The Human Metabolome Database in 2013*'. Nucleic Acids Res. 2013. Jan 1;41(D1):D801-7

[12] National Institute of Standards and Technology NIST, 100 Bureau Drive, Stop 1070, Gaithersburg, MD 20899-1070, USA (http://www.nist.gov/srd/nist1a.cfm)

[13] ChemIDplus database, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894, USA (http://www.chemspider.com/DatasourceDetails.aspx?id=34)

[14] FDA database, U.S. Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA (http://www.chemspider.com/DatasourceDetails.aspx?id=75)

8 Related Documents

- WPD_P1 Characterization_GCxGC-TOF.doc,
  (b) (4)
- WPD_P1 Characterization_GC-HR-MS.doc,
  (b) (4)
- WPD_P1 Characterization_LC-HRAM-MS.docx,
  (b) (4)
- Rep_AEC_034 Validation Report (Fast Validation) Non-Targeted Differential Screening (NTDS) Assay Using Liquid Chromatography-High Resolution-Mass Spectrometry (LC-HR-MS), September 2008 (b) (4)

9 Abbreviations

Abbreviation/Term	Explanation
AGC	Advanced Gain Control
APCI	Atmospheric Pressure Chemical Ionization
CASI	Computer-Assisted Structure Identification
CID	Collision Induced Dissociation
dd	Data Depended
EDMS	Electronic Document Management System
ESI	Electrospray Ionization
FDA	U.S. Food and Drug Administration
FOR	Form
GCxGC-TOF-MS	2-Dimensional Gas Chromatography coupled to time-of-flight Mass Spectrometry
GC-HR-TOF-MS	Gas Chromatography coupled to High Resolution time-of-flight Mass Spectrometry
GF	Cambridge Glass Fiber Pad
HC	Health Canada
HCD	Higher Energy Collisional Dissociation
HILIC	Hydrophilic Interaction Chromatography
HMDB	Human Metabolome Database
ISO	International Standard Organization
IS	Internal standard
IT	Ion Inject Time
LC-HRAM-MS	Liquid Chromatography High Resolution Accurate Mass Spectrometry
LIMS	Laboratory Information Management System
MS^2	First Order Fragmentation

Abbreviation/Term	Explanation
MRTP	Modified Risk Tobacco Product
NCE	Normalized Collision Energy
neg	negative
NFDPM	Nicotine free dry particulate matter
NIST	National Institute of Standards and Technology (USA)
NTDS	Non-targeted differential screening
NTS	Non-targeted screening
P1	Platform 1
PDIMS	Product Development Information Management System
PDSP	Programmable Dual Syringe Pump
pos	positive
ppm	Parts per Million (here: mass error)
PT	Product Testing
RH	Relative Humidity
RP	Reversed Phase Chromatography
SME	Subject Matter Expert
THD	Tobacco Heating Device
THS	Tobacco Heating System
THSR	Tobacco Heating System Regular
UCSD	Unique Compounds & Spectra Database
WKI	Work Instruction
WS	Working Solution

For complete definition, refer to PMI OPS Glossary and PMI RD Glossary.

10 Review and Approval

This document has been approved by:

Name	Function	Date / Signature
Mark Bentley Manager Complex Matrix Analysis	Summary Work Package Owner/Manager	P.P. S. D. L 24. Mar. 2016
Daniel Arndt Supervisor Complex Matrix Characterization	Associated WP Owner / Study Director	23 MARS 2016
Stefania Della Gatta Scientist Complex Matrix Characterization	SME	P.P. A. Mey 24. Mar. 2016
Christoph Buchholz Associate Scientist Complex Matrix Characterization	SME	23 MARS 2016
Deborah Forte Supervisor Test Item Management	SME	24 Mar 2016

Electronic signatures, please refers to EDMS

11 Quality Assurance Documentation

This study will not be conducted in accordance with Good Laboratory Practice (GLP).