PHILIP MORRIS INTERNATIONAL

NTDS GC×GC-TOFMS POLAR (RDNEU)

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1 Purpose

This method describes an assay for the detection of significant differences between two samples using comprehensive two-dimensional gas chromatography coupled to a time-of-flight mass spectrometer (GC×GC-TOFMS). The assay is based on a comprehensive chemical characterization of complex mixtures with no predefined target compounds.

To maximize the coverage of the chemical space in terms of polarity and volatility, the non-targeted differential screening (NTDS) GC×GC-TOFMS assay consists of three analytical methods, for nonpolar, polar and volatile compounds, respectively. The analytical methods compare two test items by comprehensive chemical screening and subsequent data evaluation to get summary tables with ranked chemical differences. This work instruction describes the method for polar compounds, which is referred to as “Polar method”.

The high resolution power using comprehensive 2-dimensional gas chromatography, combined with spectral deconvolution, results in high quality electron ionization (EI) mass spectra, improving the search against commercial mass-spectral libraries.

Data acquisition is followed by advanced raw data processing using the ChromaTOF software for automatic peak finding, spectral deconvolution and peak alignment, resulting in an aligned peak table. The software tools CASI Pre-/Post-processor and CASI automate a sequence of important data evaluation steps, e.g. batch processing, data alignment, compound identification, semi-quantification, comparison and ranking.

Ranking of the compounds is done by applying a student’s t-test to filter compounds with a significant difference followed by a ranking procedure that was developed empirically. The ranking procedure considers the relative differences in abundance of each compound as well as the absolute abundance. In addition flexible filtering (e.g. fold change, concentration cut-off) can be applied.

NTDS using GC×GC-TOFMS represents a key methodology to not only comprehensively characterize the chemical composition of aerosols derived from different test items, but also to determine significant differences of these complex matrices. Upon comparison of different reduced risk product (RRP) platforms, the detection of novel compounds or elevated levels of specific constituents provides an important basis for subsequent toxicological assessment.

2 Scope and Applicability

This document applies to the analytical laboratories of the Complex Matrix Analysis team, Testing Labs & Analytical Research (TL&AR), at PMI, RRP in Neuchâtel.

The methods were developed for smoke/aerosol-related samples (e.g. TPM, whole smoke) from conventional cigarettes and prototypes (e.g. RRPs). Nevertheless, the described method can be taken as a universal approach for comparing samples of different matrices in an unbiased way.

3 Responsibilities

Task/Activity	Responsible
Sample collection and sample preparation	Lab technician or equivalent trained
Setting up the instrument and verification of the quality control criteria according to *PMI-RRP-WKI-111668* - Operation of a LECO Pegasus 4D GC×GC-TOFMS system *(RDNEU)*	Lab technician or equivalent trained
Reporting of any procedural deviation to study director	Lab technician or equivalent trained
Selection of the detector voltage	Scientist or equivalent trained
Perform analysis	Lab technician or equivalent trained
Data processing and data export using the LECO ChromaTOF software	Lab technician or equivalent trained
Data evaluation using CASI Pre-processor, CASI and CASI Post-Processor	Lab technician or equivalent trained
Verification and finalization of the dataset	Scientist or equivalent trained
Preparation of a report	Scientist or equivalent trained
Release of results	Study director

Table 1. Tasks and responsible persons for the complete workflow of NTDS GC×GC-TOFMS Polar.

4 Description of the Method

4.1 Principle

This method describes an assay for the detection of the most significant differences between two samples using comprehensive two-dimensional gas chromatography coupled to a time-of-flight mass spectrometer. The assay is based on a comprehensive chemical characterization of complex mixtures with no predefined target compounds.

4.2 Sample Requirements and Workload

An example for the workload is shown in **Table 2**. The time is calculated for one full-time equivalent (FTE) and under the assumption that two test items are compared using three replicates per test item.

Task	Man-hours (1 FTE)
Sample collection	
ARMS request	2
Preparation of solvent and working solutions	16
Setting up the instrument	8
Analytical quality assurance	
Sensitivity test	6
System suitability test	16
Sample preparation	8
Data processing and evaluation	
Raw data processing	32
CASI Pre-Processor, CASI, CASI-Post-Processor	24
Reporting	48
Archiving of study related data	16
TOTAL	176

Table 2. Workload in man-hours, one FTE, comparison of two test items.

4.3 Material, Equipment, Chemicals, Standards and References

4.3.1 Equipment

GC×GC-TOFMS system 1, PMI ID 7764:

Instrument	Instrument ID	PMI ID
Autosampler	Agilent 7683 Series	3484
Injector	Agilent 7683B Series	11650
Gas chromatograph	Agilent 7890A	7765
Mass spectrometer	LECO Pegasus 4D SN 3390	7766
Dewar	Cryotherm Apollo 350	6478

Table 3. GC×GC-TOFMS system 1.

GC×GC-TOFMS system 2, PMI ID 10606:

Instrument	Instrument-ID	PMI ID
Autosampler	Agilent 7683 Series	11651
Injector	Agilent 7683B Series	0896
Gas chromatograph	Agilent 6890N	2938
Mass spectrometer	LECO Pegasus 4D SN 3284	3103
Dewar	Cryotherm Apollo 200	7771

Table 4. GC×GC-TOFMS system 2.

GC×GC-TOFMS system 3, PMI ID 6472:

Instrument	Instrument-ID	PMI ID
Autosampler	Agilent 7683 Series	3494
Injector	Agilent 7683B Series	12457
Gas chromatograph	Agilent 6890N	6474
Mass spectrometer	LECO Pegasus 4D SN 3242	6473
Dewar	Cryotherm Apollo 350	9878

Table 5. GC×GC-TOFMS system 3.

Additional instruments:

Instrument	Instrument-ID (or equivalent)	PMI ID
Analytical balance	Mettler Toledo XP205 Delta Range	3489
Centrifuge	Beckman Coulter Avanti J-E	2132

Table 6. Additional instrumentation.

4.3.2 Chemicals/Reagents

Name	Specification (equivalent or higher)	Supplier (or equivalent)	Product No.
Acetone	Chromasolv plus, p.a. ≥ 99.9%	Sigma-Aldrich	650501
Hexachlorobenzene	Pestanal, analytical standard	Fluka	45522
2,2,4-Trimethylpentane	Chromasolv plus, p.a. ≥ 99.5%	Sigma-Aldrich	650439
Water	Chromasolv	Fluka	39253

Table 7. List of chemicals/solvents used for the Polar method.

4.3.3 Internal Standard Compounds

Name	Specification (equivalent or higher)	Supplier (or equivalent)	Product No.
Furfural-d4	≥ 98.0% purity/atom-%d	CDN	D-2115
4-Hydroxy-4-methyl-2-pentanone-d12	≥ 98.0% purity/atom-%d	CDN	D-6323
5-Hydroxy-2-methyl-d3-pyridine-3,4,6-d3	≥ 98.0% purity/atom-%d	CDN	D-7566
N-Methylnicotinamide-2,4,5,6-d4	≥ 99.0% purity/atom-%d	CDN	D-7642
2-Methylbutyric-d9 acid	≥ 98.0% purity/atom-%d	CDN	D-5267
2-Methyl-2,4-pentane-d12-diol	≥ 98.0% purity/atom-%d	CDN	D-5825
Pentanenitrile-d9	≥ 98.0% purity/atom-%d	CDN	D-5649
Phenol-d6	≥ 98.0% purity/atom-%d	CDN	D-29
N-iso-Propyl-d7-acrylamide	≥ 99.0% purity/atom-%d	CDN	D-6567
Pyridine-d5	≥ 99.0% purity/atom-%d	CDN	D-85

Table 8. List of deuterium labeled internal standard (ISTD) compounds used for the Polar method.

4.3.4 Retention Index Marker Compounds

Name	Specification (equivalent or higher)	Supplier (or equivalent)	Product No.
Methyl hexanoate	≥ 99.0% purity/atom-%d	Sigma-Aldrich	21599
Methyl decanoate-d19	≥ 98.0% purity/atom-%d	CDN	D-5847
Methyl tetradecanoate-d27	≥ 98.0% purity/atom-%d	CDN	D-5854
Methyl hexadecanoate-d31	≥ 98.0% purity/atom-%d	CDN	D-1360
Methyl arachidate	≥ 99.0% purity/atom-%d	Sigma-Aldrich	10941

Table 9. List of retention index marker (RIM) compounds used for the Polar method.

4.4 Procedure

No calibration for quantification is used in this assay. The assay uses a semi-quantification procedure described in **Section 4.5.1**.

4.4.1 Sample Collection

4.4.1.1 Generation of Smoke/aerosol-related Samples

Cigarette smoke/aerosol related samples are generated according to *PMI-RRP-WKI-111729* and *PMI-RRP-WKI-111801*. All the necessary parameters for smoke/aerosol collection are described in the respective ARMS request.

Whole smoke is collected on a Cambridge filter pad with two micro-impingers connected in series. The extraction solution is dichloromethane (DCM)/acetone (80/20, v/v) containing internal standards and retention index markers (ISTDs/RIMs$_{nonpolar}$). After extraction a liquid/liquid extraction with water is performed. A working solution (ISTD_work) of acetone containing internal standards and retention index markers (ISTDs/RIMs$_{polar}$) is added to the aqueous phase. The preparation of stock solutions and working solution is described in *PMI-RRP-FOR-111487* - Chemicals, solvents, solutions and internal standard amount used for NTDS GCxGC-TOF

(RDNEU) and has to be documented in this form according to *PMI-RRP-WKI-113456*. Stock solutions and ISTD_work have to be freshly prepared.

After generation of the smoke/aerosol samples the filter pad is kept in a Pyrex tube. The micro-impingers are sealed and kept in dry ice/isopropanol.

The samples are analyzed as soon as possible after sample generation. In case samples need to be stored storage of the filters/impinger contents/crude extracts or e-liquids/e-liquid extracts has to be documented in *PMI-RRP-FOR-111488* - Storage of samples and study related materials for NTDS *(RDNEU)*.

4.4.1.2 Generation of Other Samples

The described method can be used for comparing samples with different kinds of matrices. The samples will be analyzed as soon as possible after sample generation.

4.4.2 Sample Preparation

A step-by-step workflow for sample preparation is described and has to be documented in *PMI-RRP-FOR-111513* - Sample preparation for NTDS GCxGC-TOFMS Polar *(RDNEU)*. The workflow is briefly described here point by point:

- the content of the micro-impingers (twice 10mL DCM/acetone (80/20, v/v)) containing a set of internal standard and retention index marker compounds is added to the Pyrex tube containing the Cambridge filter pad
- the Pyrex tube is shaken by hand until the filter is starting to break
- the extract is centrifuged with 1000 rpm (approximately 233 ×g) for 10 minutes
- a 10 mL aliquot is transferred to a fresh Pyrex tube and 10 mL of water are added to the aliquot
- the sample is vortexed for 20 seconds and centrifuged with 1000 rpm (approximately 233 ×g) for 10 minutes
- while the organic phase (lower) is transferred by means of a pasteur glass pipette into a fresh amber glass vial for the Nonpolar method, the aqueous phase is kept for the Polar method
- 500 µL of ISTD_work are added to the aqueous phase
- the sample is vortexed for 10 seconds
- an aliquot is transferred into an autosampler vial and analyzed by GC×GC-TOFMS in full scan mode
- pool sample(s) is/are created from equal volumes of aerosol/smoke replicates to represent the chemical space of all sample groups

Storage of the aqueous phase, remaining extracts plus filter and processed extracts has to be documented in *PMI-RRP-FOR-111488* - Storage of samples and study related materials for NTDS *(RDNEU)*. Remaining extracts plus filter are kept until the study is closed.

4.4.3 Setting-up of Instruments

Instrument	Parameter	Settings
Injector	injector	cool-on-column, track-oven mode
	injection	on-column
	injection volume	0.1 µL
Gas chromatograph	carrier gas	helium
	flow	1.0 mL/min (constant flow)
	pre-column	2 m SLB-IL60, 0.25 mm ID, 0.20 µm d_f
	column 1 (1st dimension)	30 m DB-FFAP, 0.25 mm ID, 0.25 µm d_f
	column 2 (2nd dimension)	1.9 m VF-624ms, 0.15 mm ID, 0.84 µm d_f
	primary oven temperature program	rate (°C/min) target temp. (°C) duration (min) initial 35.0 2.0 5.0 250.0 23.0
	secondary oven temperature program	rate (°C/min) target temp. (°C) duration (min) Initial 55.0 2.0 4.6 285.0 16.0
Transfer line	temperature	280 °C
Modulator	modulator	enabled
	modulator temperature program	rate (°C/min) target temp. (°C) duration (min) Initial 65.0 2.0 5.0 300.0 19.0
	2-dimension separation time	6 s
	hot pulse time	1.00 s
	cool time between stages	2.00 s
Mass spectrometer	acquisition delay	450 s
	mass range	29-700 u
	data acquisition rate	200 spectra/s
	detector voltage	1450 – 2000 V
	electron energy	-70 V
	temperature ion source	230 °C

Table 10. Setup of the instrument for the Polar method.

Before the main sequence is started, the sensitivity and the chromatographic resolution of the system is tested. In the case of failure of at least one system suitability parameter, a troubleshooting will be initiated (e.g. new analytical column, increase of multiplier voltage, etc.).

Prior to every analysis the instrument has to be checked and the changes documented in *PMI-RRP-FOR-111496* - Preparation of LECO PEGASUS 4D SYSTEM for NTDS GCxGC-TOFMS *(RDNEU)*.

4.4.3.1 Sensitivity Test

The operating procedure of the LECO Pegasus 4D GC×GC-TOFMS system is given in *PMI-RRP-WKI-111668* - Operation of a LECO Pegasus 4D GC×GC-TOFMS system *(RDNEU)*. The preparation of a hexachlorobenzene (HCB) stock solution/sensitivity test solution and the correct procedure to conduct a sensitivity test is described in the WKI and has to be documented in *PMI-RRP-FOR-111514* - Sensitivity test

and system suitability test NTDS GCxGC-TOFMS Polar *(RDNEU)*. The final detector voltage chosen for the subsequent analysis has to be indicated in the form *PMI-RRP-FOR-111514* and a LECO ChromaTOF report (template name "Detector Sensitivity") has to be generated, saved electronically in the study folder and printed, signed and stored in the study binder.

Sensitivity testing of the system is done by measuring the sensitivity test solution prior to the analytical series. A sequence with an increasing multiplier voltage in steps of 50-V-increments is prepared. The maximum detector voltage is given in **Section 4.4.3**. The analysis will be performed in 1-dimensional mode. The main criterion for the sensitivity is the **quant signal to noise ratio (quant S/N)** of the m/z 284 ion of HCB. Additionally the correct name has to be assigned (Benzene, hexachloro-) when matching the EI spectrum to the HCB library. In general an increase of the detector voltage leads to an improvement in S/N, yet at some point the improvements are less pronounced as the noise increases along with the signal. Typically the acceptance criterion is a **quant S/N ≥30**, however the final detector voltage has to be selected by the scientist. The decision has to be commented in the form *PMI-RRP-FOR-111514*.

4.4.3.2 System Suitability Test

The chromatographic system is tested by injection of aliquots of a diluted working solution (ISTD_work). The concentration of the diluted ISTD_work has to be in accordance to the concentration of the ISTDs and RIMs in the measured samples. The samples are called SST (system suitability test) and are distributed equally across the analytical series (SST_1, SST_2, …). The sample preparation procedures are described in *PMI-RRP-FOR-111487* - Chemicals, solvents, solutions and internal standard amount used for NTDS GCxGC-TOF *(RDNEU)* and briefly in **Section 4.4.4**.

Test parameters are:

- **retention time** of selected compounds to test the flow and temperature programs of the analytical system
  → stability of the retention times is monitored
- **peak shape** to test the inertness of the analytical system, especially of the analytical columns
  → tailing factor is monitored

Compound	1st Dimension RT (s)		2nd Dimension RT (s)		Tailing Factor 2nd Dimension
Pentanenitrile-d9	708	±80s	4.425	±0.6s	<5
2-Methyl-2,4-pentane-d12-diol	1368	±80s	3.245	±0.5s	<5
N-Methylnicotinamide-2,4,5,6-d4	2646	±90s	3.315	±0.5s	<5

Table 11. Acceptance criteria for the system suitability test using the Polar method.

ChromaTOF processing and data evaluation of the system suitability test is described in *PMI-RRP-FOR-111514* - Sensitivity test and system suitability test NTDS GCxGC-TOFMS Polar *(RDNEU)*. A LECO ChromaTOF (template name "System Suitability Test") report has to be generated for each SST sample, saved electronically in the study folder and printed, signed and stored in the study binder. The results of the SST have to be documented in *PMI-RRP-FOR-111514*, saved electronically in the study folder and printed and stored in the study binder.

4.4.4 Preparation of Solutions and Media

Preparation of stock solutions, working and extraction solution is described and has to be documented in *PMI-RRP-FOR-111487* - Chemicals, solvents, solutions and internal standard amount used for NTDS GCxGC-TOF *(RDNEU)*. All solutions are labeled with solution name, concentration, type of solvent, preparation date, storage condition and the initials of the person who prepared the solution (in accordance with *PMI-RRP-WKI-111814*). The solutions are stored at -10 to -80 °C. The stock solutions are prepared by adding the required volume of solvent using volumetric pipettes of accuracy degree AS with quality certificate to the weighed standard compound. The working solution is only prepared by using measuring flasks of accuracy degree A with quality certificate and volumetric pipettes of accuracy degree AS with quality certificate or piston pipettes according to work instruction *PMI-RRP-WKI-111700* or microliter syringes, respectively. For weighing an analytical balance with an accuracy of at least 0.01 mg has to be used according to *PMI-RRP-WKI-111726*. Exact weights have to be printed and printouts added to the form *PMI-RRP-FOR-111487*.

Calculation of concentrations:

In case of weighing solid compounds:

$$\text{Concentration (mg/mL)} = \frac{\text{Compound weight (mg)} \bullet (\text{purity atom-\%d (≥\%)} / 100) \bullet (\text{purity content (≥\%)} / 100)}{\text{Total volume (mL)}}$$

In case of adding liquid compounds:

$$\text{Concentration (mg/mL)} = \frac{\text{Compound volume (mL)} \bullet \text{density (g/mL)} \bullet (\text{purity atom-\%d (≥\%)} / 100) \bullet (\text{purity content (≥\%)} / 100)}{\text{Total volume (mL)}}$$

4.4.4.1 Solvents

Name	Storage/Stability
Water/acetone (4:1) v/v	1 year at ambient temperature

Table 12. Preparation of the solvent.

4.4.4.2 Stock Solutions of Internal Standards and Retention Index Markers

The appropriate volumes that need to be weighed and pipetted according to work instructions *PMI-RRP-WKI-111726* and *PMI-RRP-WKI-111700*, respectively, for the ISTD and RIM stock solutions are described in *PMI-RRP-FOR-111487* - Chemicals, solvents, solutions and internal standard amount used for NTDS GCxGC-TOF *(RDNEU)*.

Concentrations depend on the exact weight and the purity (content and atom-%d) of the used compounds. The exact values are documented in *PMI-RRP-FOR-111487*.

Solution Name	Weight (mg)	Volume (µL)	Density (g/mL)	Volume (mL)	Solvent
Furfural-d4	-	10.0	1.156	10	Acetone
4-Hydroxy-4-methyl-2-pentanone-d12	-	12.5	0.931	10	Acetone
5-Hydroxy-2-methyl-d3-pyridine-3,4,6-d3	10 ±3	-	-	10	Acetone
N-Methylnicotinamide-2,4,5,6-d4	10 ±3	-	-	10	Acetone
2-Methylbutyric-d9 acid	-	12.5	0.936	10	Acetone
2-Methyl-2,4-pentane-d12-diol	-	12.5	0.925	10	Acetone
Pentanenitrile-d9	-	12.5	0.795	10	Acetone
Phenol-d6	10 ±3	-	-	10	Acetone
N-iso-Propyl-d7-acrylamide	10 ±3	-	-	10	Acetone
Pyridine-d5	-	10	0.980	10	Acetone
Methyl hexanoate	-	12.5	0.884	1	Acetone
Methyl decanoate-d19	-	12.5	0.873	1	Acetone
Methyl tetradecanoate-d27	-	25.0	0.855	2	Acetone
Methyl hexadecanoate-d31	30 ±3	-	-	3	Acetone
Methyl arachidate	30 ±3	-	-	3	Acetone

Table 13. Target weights and volumes for the ISTD and RIM stock solutions.

4.4.4.3 Working Solution of ISTDs and RIMs

The ISTD and RIM working solution (ISTD_work) is prepared in a 10 mL measuring flask which is filled up to the final volume with acetone.

Concentrations depend on the exact weight and the purity of the used compounds. The exact values are documented in *PMI-RRP-FOR-111487*.

Used Stock Solution	Volume Stock Solution (µL)	Target Concentration (µg/mL)
Furfural-d4	90	10
4-Hydroxy-4-methyl-2-pentanone-d12	220	25
5-Hydroxy-2-methyl-d3-pyridine-3,4,6-d3	300	30
N-Methylnicotinamide-2,4,5,6-d4	200	20
2-Methylbutyric-d9 acid	440	50
2-Methyl-2,4-pentane-d12-diol	350	40
Pentanenitrile-d9	160	15
Phenol-d6	200	20
N-iso-Propyl-d7-acrylamide	100	10
Pyridine-d5	100	10
Methyl hexanoate	140	150
Methyl decanoate-d19	380	400
Methyl tetradecanoate-d27	720	750
Methyl hexadecanoate-d31	1600	1600
Methyl arachidate	2000	2100

Table 14. Target volumes for the working solution of ISTDs and RIMs.

4.4.5 Number of Determinations

Each sample will be generated minimum in triplicate and analyzed. Blanks, SST samples, pool sample(s) and one technical replicate of each matrix will be evenly distributed across the sequence. The term technical replicate describes the process of injecting the same sample extract multiple times across a sequence to assess the instrumental variability.

4.4.6 Daily Verification or According to Use

Prior to each study the LECO Pegasus 4D system is verified according to *PMI-RRP-FOR-111496* - Preparation of LECO PEGASUS 4D SYSTEM for NTDS GCxGC-TOFMS *(RDNEU)* and tested in terms of sensitivity and system suitability according to *PMI-RRP-FOR-111514* - Sensitivity test and system suitability test NTDS GCxGC-TOFMS Polar *(RDNEU)*.

4.4.7 Testing Procedure

This method describes an assay for detection of significant differences between two samples by GC×GC-TOFMS. The composition of different complex mixtures, like cigarette smoke or RRP aerosol, is compared in a hypothesis-free unbiased way (non-targeted). Compounds found to be different between samples are ranked according to relevance considering the relative difference in abundance of each compound as well as the absolute abundance. Focus of the approach is the comprehensive chemical characterization of a complex mixture using three GC×GC-TOFMS methods, which are allocated to different polarities and volatilities of the constituents. The methods are not intended to assess absolute quantitative amounts of the detected compounds, the concept is rather based on a semi-quantitative assessment.

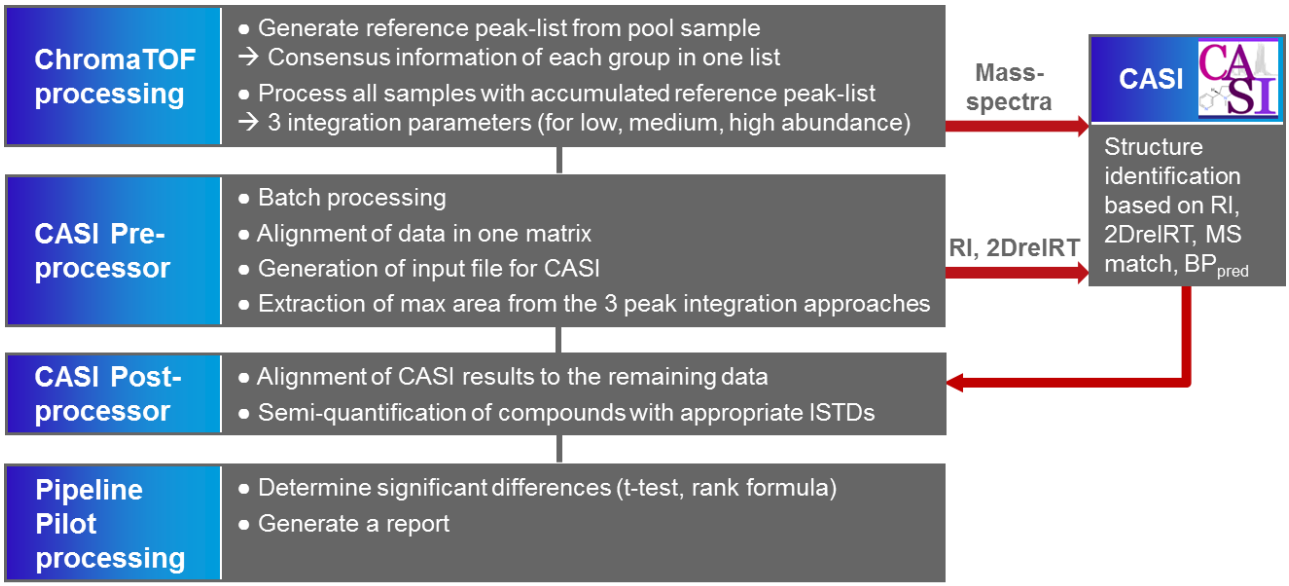


Figure 1. Overview of the data processing steps.

Data processing is divided into multiple steps, (1) ChromaTOF processing, (2) CASI Pre-processor, (3) CASI, (4) CASI Post-processor, and (5) Pipeline Pilot processing (see **Figure 1**).

First the raw data has to be processed by the LECO ChromaTOF software. After assembling all the relevant information each sample is processed with three different parameter settings to optimally assess the peak area of minor, medium and major peaks. Files are exported in .csv format.

The data is further processed using CASI Pre-processor, which processes the batches and aligns the data in one matrix. The maximum area of each peak is determined and an input file for CASI is created. The CASI input file contains the mean of retention indices (RI) and 2nd dimension retention times (2DRT) for all compounds. Together with the casi_input file the associated EI mass spectral library (converted with lib2nist) is submitted to CASI.

The CASI platform increases the accuracy for analytical identification of compound structures and accelerates and standardizes the identification process. It assures reproducibility and enables scientists to have higher confidence in the correct assignment of mass spectra to the right compounds. CASI automatically identifies, on-the-fly and with highest confidence, possible relevant structures from mass spectra associated with chromatographic values, including models for retention index, 2-dimensional relative retention time and boiling point.

CASI Post-Processor combines the high confidence identifications of CASI to the existing data matrix. Subsequently, semi-quantification is performed according to predefined rules.

In the final step, a Pipeline Pilot script is used to determine the significant differences and to transform the data to a suitable reporting format.

Alternatively, a manual Microsoft Excel process can be used instead of CASI Pre- and Post-processor. This procedure was employed before the automated CASI Pre- and Post-processor procedure, but became obsolete due the enormous time-consumption and the error-proneness. However it will be briefly described in **Section 4.4.7.2.3**.

4.4.7.1 Data Processing using the LECO ChromaTOF Software

A step-by-step description for the ChromaTOF processing is given in *PMI-RRP-FOR-111512* - Data processing in ChromaTOF NTDS GCxGC-TOFMS Polar *(RDNEU)*. All the steps have to be documented. Different type of sheets can be selected according to the number of items that need to be compared. In the following an example for two items (e.g. 3R4F versus P1) is shown.

4.4.7.1.1 Generation of the Reference Peak Matrix from the Pool Sample

- **Processing of the pool sample**
  - computing of the baseline
  - finding peaks above the baseline
  - identifying peaks by library search (select only “ISTDs_RIMs” library)
  - integration of the peaks (area, height)

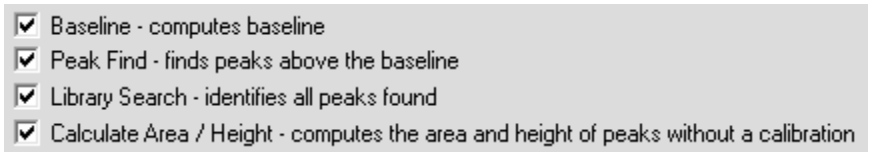


Figure 2. Processing steps that need to be activated in the Data Processing Method (DPM).

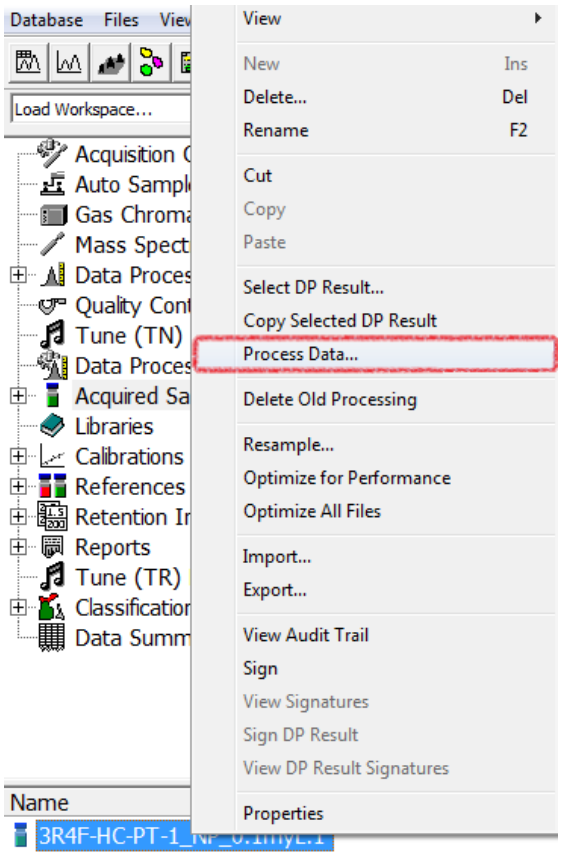


Figure 3. Processing of the pool sample.

- **Repeated processing of the pool sample with classification and RI method**
  - computing of the baseline
  - finding peaks above the baseline
  - identifying peaks by library search (select only “ISTDs_RIMs” library)
  - integration of the peaks (area, height)
  - calculation of retention index
  - classification (exclusion of, e.g. bleed, high abundant compounds like nicotine)

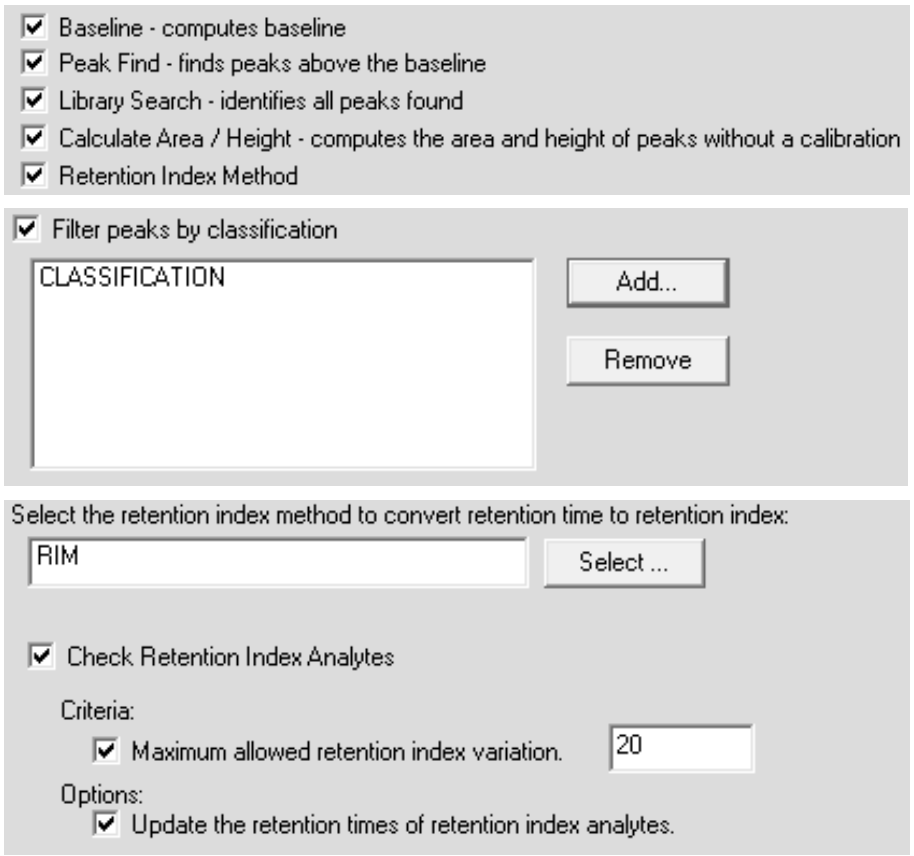


Figure 4. Processing steps that need to be activated in the DPM of the repeated processing.

- **Evaluate the data and create a new calibration of the pool sample**
  - evaluate correct finding of ISTD and RI compounds
  - sort according to Quant S/N, delete peaks with S/N <50
  - flag false/noise peaks
  - import processed and flagged data into new calibration
  - select quantitation parameters in the calibration

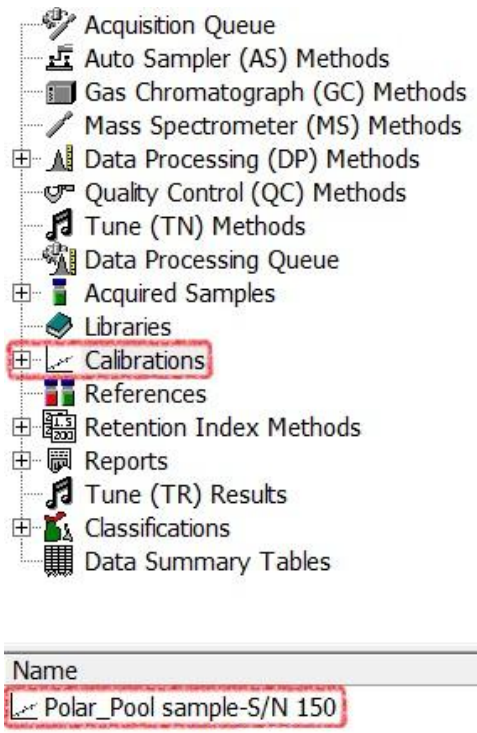


Figure 5. Creating a “New” calibration under tab “Calibrations”.

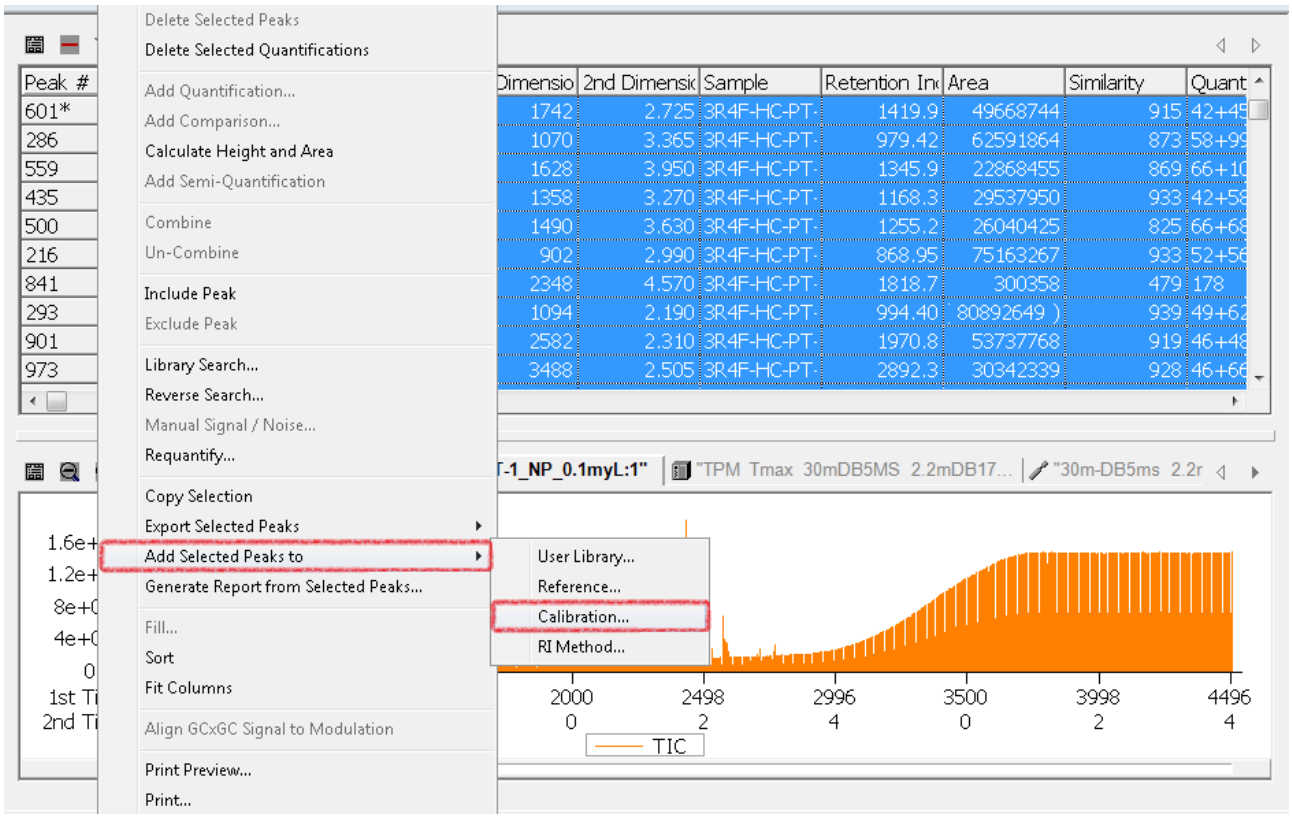
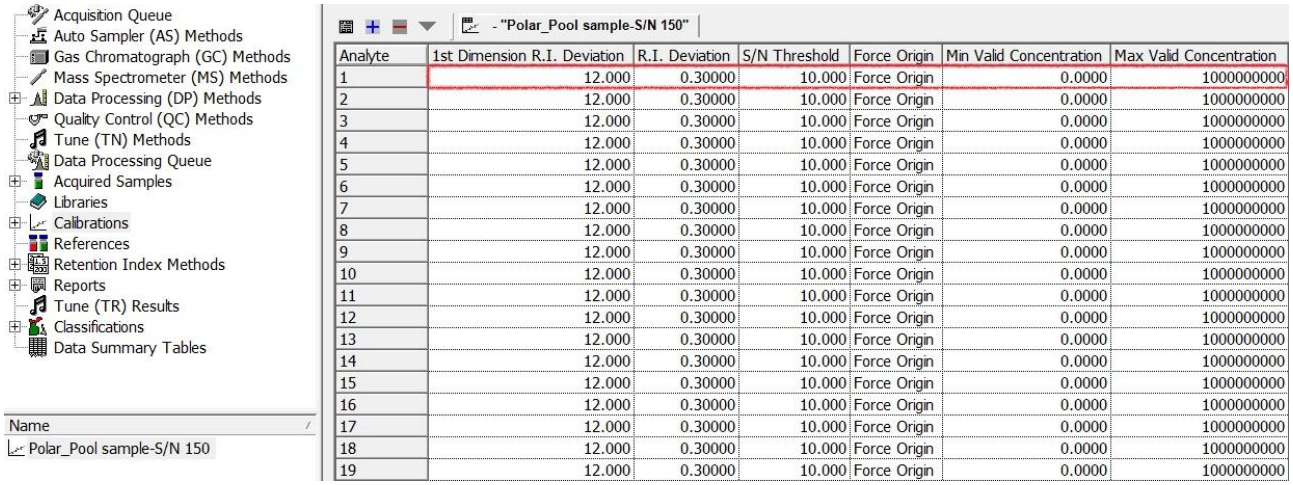


Figure 6. Select the processed pool sample, select all peaks and add selected peaks to calibration.



Analyte	1st Dimension R.I. Deviation	R.I. Deviation	S/N Threshold	Force Origin	Min Valid Concentration	Max Valid Concentration
1	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
2	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
3	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
4	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
5	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
6	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
7	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
8	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
9	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
10	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
11	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
12	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
13	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
14	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
15	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
16	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
17	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
18	12.000	0.30000	10.000	Force Origin	0.0000	1000000000
19	12.000	0.30000	10.000	Force Origin	0.0000	1000000000

Figure 7. Selection of the quantitation parameters. Values have to be filled down.

4.4.7.1.2 Comparison of the Whole Sample Set against the Reference Peak List and Export Data

- **Prepare a report-file folder structure for the export of the .csv files**

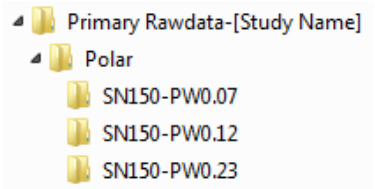


Figure 8. Folder structure for the export of the .csv files.

- **Prepare three quantitation methods with alternating peak widths in the 2nd dimension**
  - computing of the baseline
  - finding peaks above the baseline
  - disable library search
  - calculation of retention index
  - apply calibration
  - enable export of the peak information in ASCII CSV format

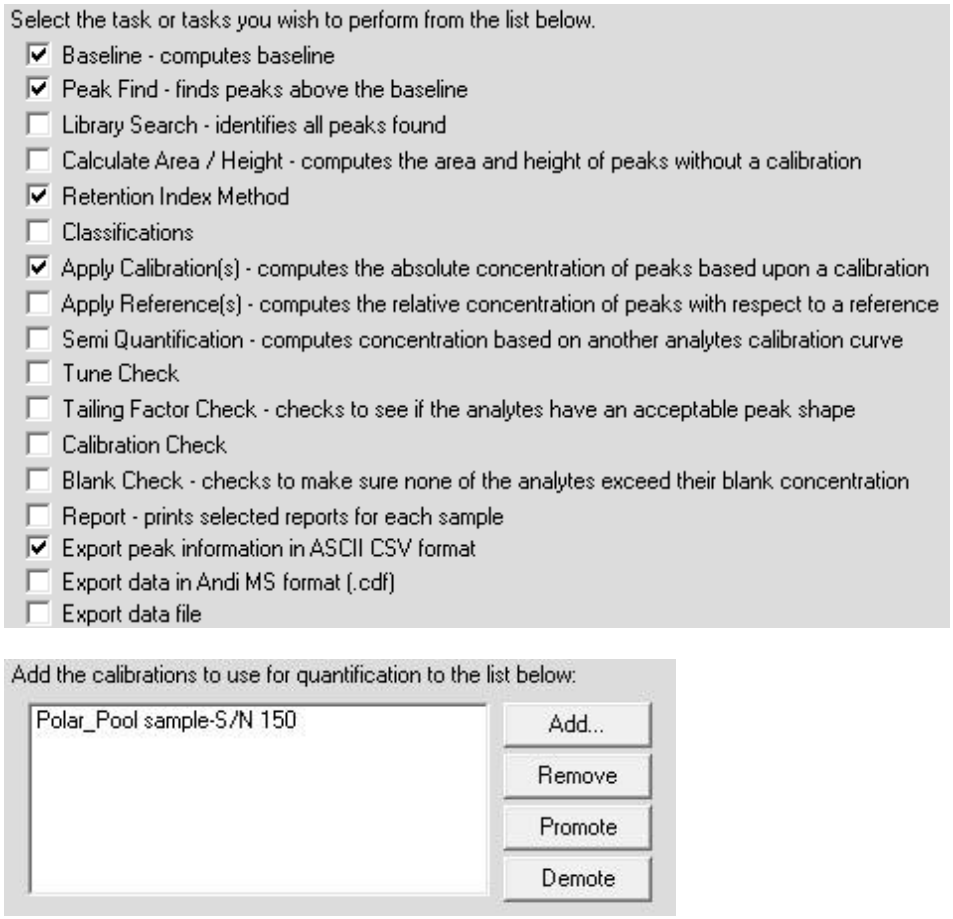


Figure 9. Processing steps that need to be activated in the DPM of the final three methods.

- method 1: peak width 0.07 sec
- method 2: peak width 0.12 sec
- method 3: peak width 0.23 sec
- keep remaining parameters consistent

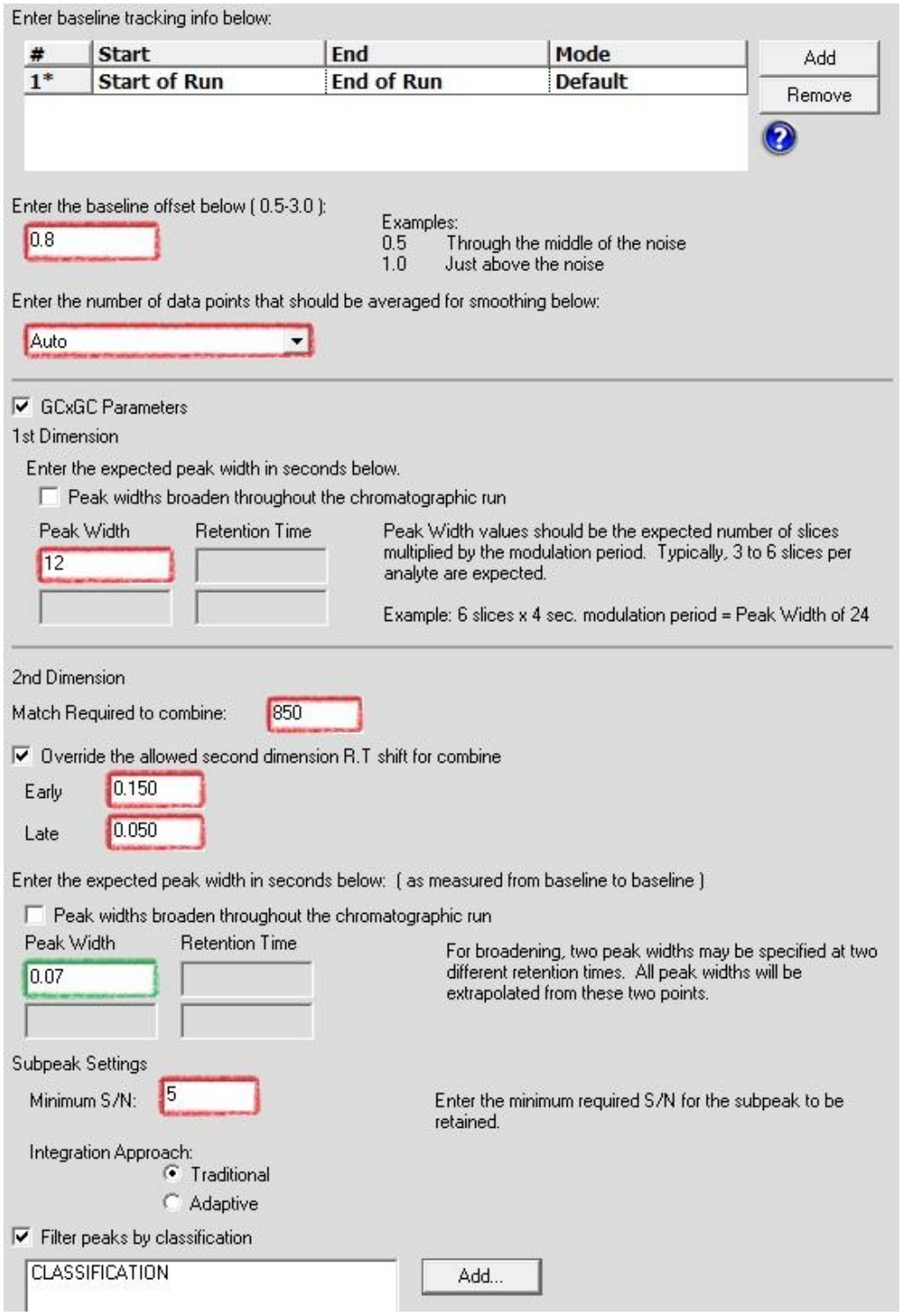


Figure 10. Alternate peak width in 2D (green square); keep remaining parameters constant (red squares).

- choose header “@SampleName[]”
- export all peaks , except “Contaminants / Unknowns”
- ensure export of peak information **in the right order**

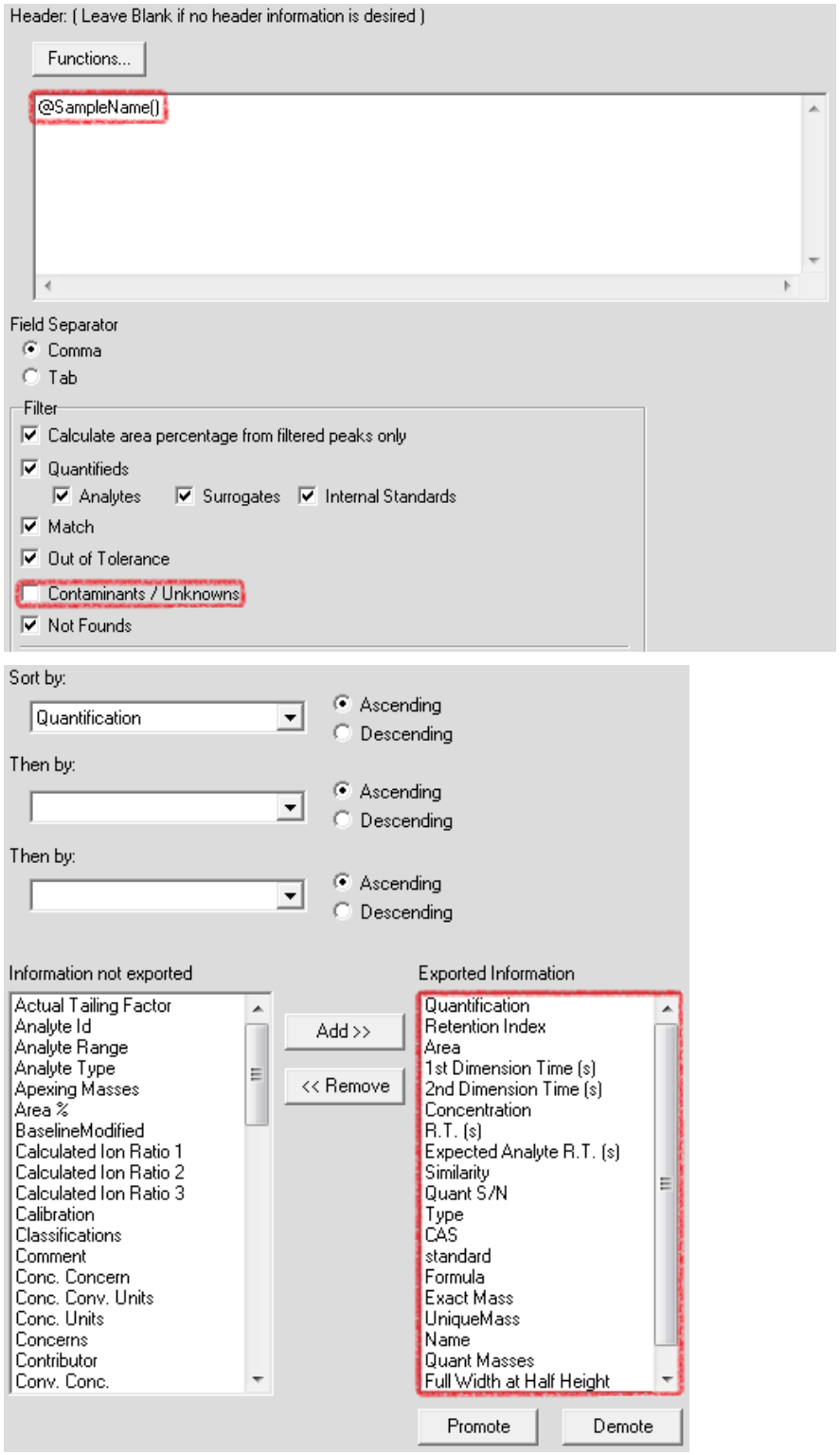


Figure 11. Critical parameters that have to be inserted, deactivated or given in the right order.

- **Process all samples using the final quantitation methods 1, 2 and 3**

4.4.7.1.3 Transfer of the Final Calibration into a Library

- Create a new user library in ChromaTOF and name it according to the study
- Select all entries of the final ChromaTOF calibration and add them to the newly created user library
- Ensure to deactivate the box “Enter additional user information for each spectrum”

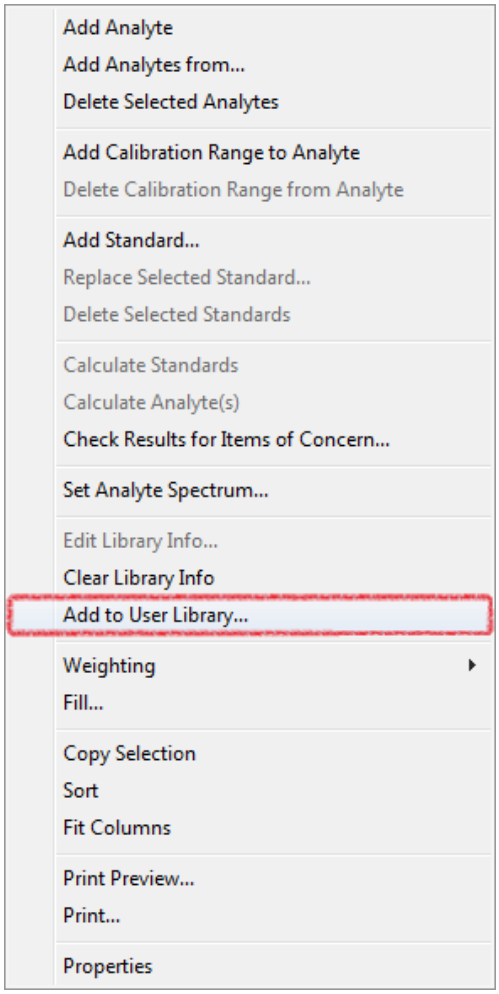


Figure 12. Adding information from a calibration to a user library.

4.4.7.2 Submitting Metadata to CASI Pre-processor, CASI and CASI Post-processor

A step-by-step description is presented in *PMI-RRP-FOR-111491* - Submitting metadata for CASI pre-processor, CASI, CASI post-processor *(RDNEU)*. All the steps have to be documented.

Two files are needed as input in order to run through all the CASI processes. Both files have to be saved in the study folder "…\Primary Raw data-[Study name]\Polar\".

The file "Concentration_ISTDs.txt" is generated from the respective polar sheet in *PMI-RRP-FOR-111487* - Chemicals, solvents, solutions and internal standard amount used for NTDS GCxGC-TOF *(RDNEU)*. The file has to be saved with the exact name "Concentration_ISTDs.txt". The group names (column → test item) in "Concentration_ISTDs.txt" have to be in accordance with the groups defined in the exported file names.

The JCAMP file "Library.HPJ" is generated by converting the library, which was created in **Section 4.4.7.1.3**, with lib2nist converter. Here, no naming convention is necessary.

4.4.7.2.1 CASI Pre-processor

CASI Pre-processor performs a fully automated batch processing, which includes replacement of saturated signals by the approximate value, alignment of the data, calculation of RI and 2DRT means, and determination of the maximum peak area for each signal within the three quantitation methods. In case the max area of an ISTD is not between 50 and 200 % of the mean of max area values across all samples the value is replaced by the mean of the max area.

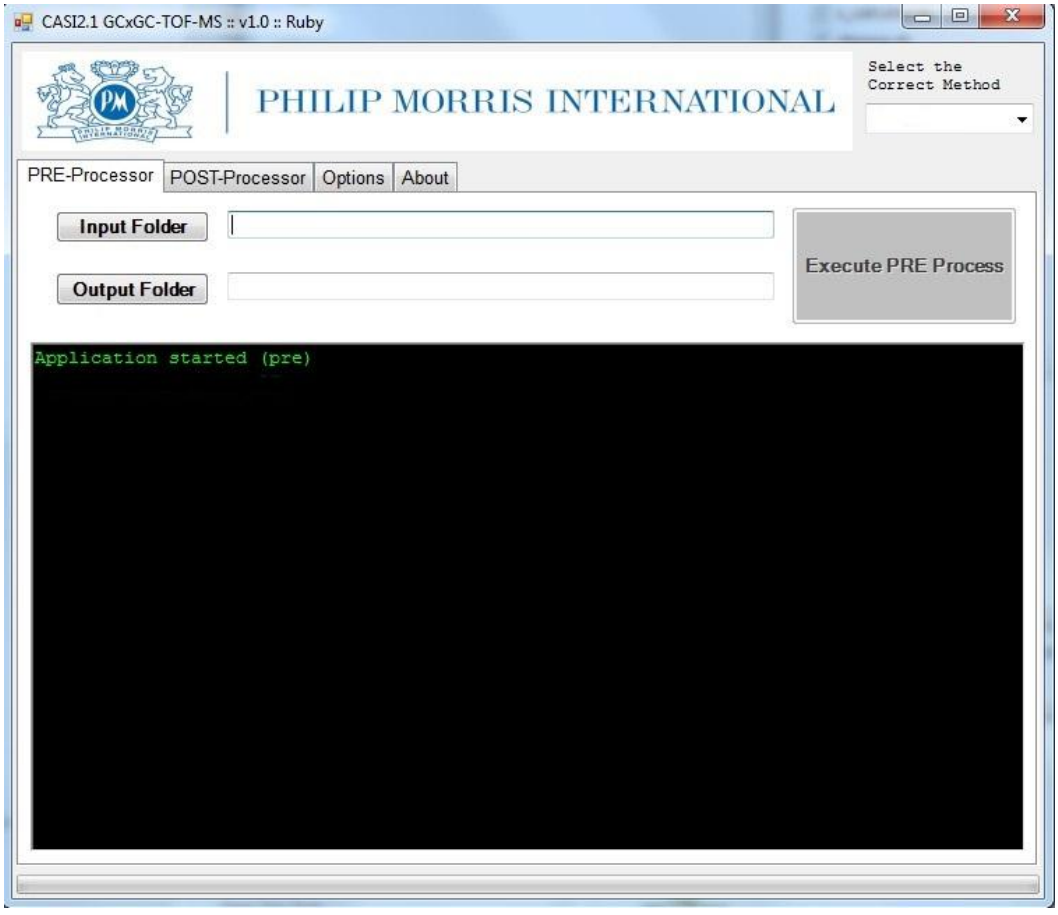


Figure 13. User interface of CASI Pre- and Post-processor.

CASI Pre-processor outputs in total four files, two warning files and two files that are needed for further processes.

The file “warning.txt” lists all signals, which (A) were saturated and therefore replaced or (B) could not be found in respective samples.

The file “warning-area-dcompounds.txt” lists the max areas of the ISTDs that were out of the defined range and thus replaced (original max area values are shown).

The file “input-postprocess.txt” contains the accumulated information and is required as an input file for CASI Post-processor.

The file “casi_input.txt” comprises average RI and 2DRT values, which are needed for CASI.

4.4.7.2.1 CASI

Computer Assisted Structure Identification is a powerful platform that enhances the accuracy of compound structure identification and accelerates and standardizes the identification process. CASI’s automatic identification process operates on-the-fly and facilitates a higher confidence in the correct assignment of mass spectra to the right compounds as relevant structures are associated with chromatographic values, including models for retention index, 2nd dimension retention time and boiling point.

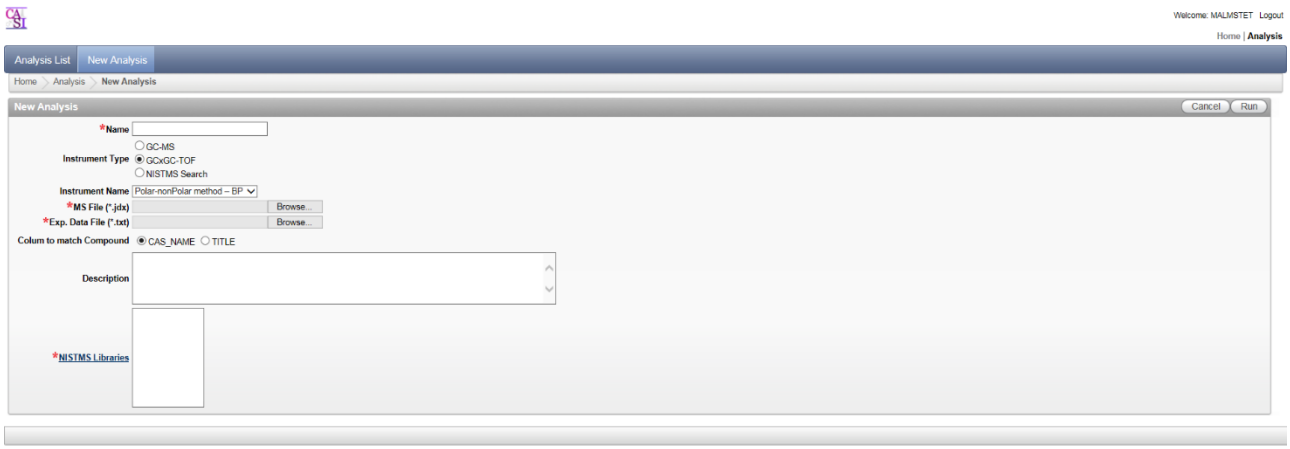


Figure 14. User interface of CASI.

A user manual for CASI is available in EDMS (*PMI-RRP-WKI-111624*).

CASI requires two files, the “Library.HPJ”, which was converted from the original library with lib2nist converter, and the “casi_input.txt“ generated by the CASI Pre-processor.

When the CASI process is finished the “casi_report_ntds.txt” and the “casi_report.sdf” are exported. Both files are uploaded to the Pipeline Pilot script “IS_determination_polar_GC×GC”. The script performs group-type classification, assigns each compound to a specific internal standard (according to rules defined in **Section 4.5.1**) and merges the data into one file. The merged file is saved as “casi_report_ntds.txt” (ensure the correct name). The file is further required to run CASI Post-processor.

4.4.7.2.2 CASI Post-processor

CASI Post-processor aligns the data comprised in "casi_report_ntds.txt" to the data of the file "input-postprocess.txt". Semi-quantification is performed according to specific rules, which are defined in **Section 4.5.1**. The output file of CASI Post-processor is the file "concentration.txt", which contains the final processed data.

4.4.7.2.3 Manual Excel Processing

Before the CASI processors were developed the metadata was processed manually in a predefined MS Excel workflow. The individual steps are briefly described point by point in the following:

- open .csv file and delete saturation comments in field "area"
- sort table: field "standard" and then "quantification" ascending
- sort standards for "standard" descending
- verify ISTD and RT-INDEX alignment => correction if necessary
- rename .csv data into [SNx]-[Pwy]-[sample name]-[repetition number]-[method], e.g. SN150-PW0.12-3R4F_HC1-P.xls
- repeat first 5 steps for all .csv files
- generate EXCEL reference calculation file
- import compound name, area data and information on sample, measurement-no., S/N and PW into EXCEL
- verify correct data-table alignment (conditional format, compare names)
- correction of misalignments in data
- get maximum area of values from the different integration parameters
- prepare NIST format library from final calibration file, prepare chromatography file for CASI, data have to be averaged
- align CASI results with chromatography data
- perform analysis on elemental composition
- copy worksheet and transform formulas to values
- check ISTD areas according to criteria:
  - area must be within 50% and 200% of mean area
  - non-accepted areas are replaced by mean area of valid dataset
- semiquantify all samples according to rules described in **Section 4.5.1**
- copy worksheet e.g. 3 times (depending on the number of comparisons) and transform formulas to values
- rename as table P1reg vs P2reg / P1reg vs 3R4F / P2reg vs 3R4F
- perform t-test on the data sets
- sort tables for p-values, separate data with p-values >0.05 as "not significantly different"
- calculate EFFECT, INDEX and RANK

- sort for RANK, split tables in positive and negative list, sort for absolute values within categories
- verify results (manually)

The time-consuming manual processing procedure has been replaced and each step verified by CASI Pre- and Post-processor.

4.4.7.3 Pipeline Pilot Processing

The Pipeline Pilot script “NTDS_Comparison and Report” is specifically dedicated to perform t-tests and ranking on the dataset. In addition the script transforms all of the information into a suitable reporting format. Therefore the “concentration.txt” has to be uploaded to the webport, the study name/comparisons entered and the process executed. The output file is a final report file in Excel format.

The user guide “User Guide Pipeline Pilot Web Port Protocols”, Elyette Martin, version 1.0 describes all the available Pipeline Pilot webport protocols (available on DISCO).

4.5 Calculation and Records

4.5.1 Semi-quantification of Compounds

The calculation of peak areas (integration) for a high number of diverse compounds is a critical step due to the different chromatographic behavior of individual compounds. In order to enhance the quality of the integration process, the samples are processed three times using different peak integration parameters as described in **Section 4.4.7.1.2**. Then, the maximum value of the integration results for each component will be used for further calculation.

For semiquantification, each compound will be referred to one of the internal standards. Every internal standard is allocated to a certain compound class. If a compound cannot be classified by a corresponding internal standard or is unknown a secondary classification according to 2DRT applies. A detailed description of the rules is given in **Table 15**.

Compound	Case of Known Compounds	Case of Unknown Compounds
Furfural-d4	internal standard for • compounds with at least one ring (without nitrogen) and one carbonyl function • (thio)ethers (without N-containing rings)	internal standard for compounds with 2DRT > 2.5 and ≤ 4.25 sec
4-Hydroxy-4-methyl-2-pentanone-d12	internal standard for carbonyls (without a ring)	-
5-Hydroxy-2-methyl-d3-pyridine-3,4,6-d3	internal standard for compounds with at least one N-containing ring and one oxygen-containing functional group (except amide)	-
N-Methylnicotinamide-2,4,5,6-d4	internal standard for compounds with at least one ring and one amide function	-
2-Methylbutyric-d9 acid	internal standard for acids, thioacids (without N-containing rings)	-
2-Methyl-2,4-pentane-d12-diol	internal standard for alcohols (without a ring)	-
Pentanenitrile-d9	internal standard for nitriles	internal standard for compounds with 2DRT > 4.25 sec
Phenol-d6	internal standard for compounds with at least one ring and one alcohol (without N-containing rings)	internal standard for compounds with 2DRT ≤ 2.5 sec
N-iso-Propyl-d7-acrylamide	internal standard for compounds without a ring and at least one amide function	-
Pyridine-d5	internal standard for N-containing rings without oxygen-containing functional groups	-

Table 15. Assignment of compound classes to specific ISTDs.

4.5.2 Extraction of Significant Differences

The extraction of significantly different compounds between the different test items is done by applying a two-tailed two-sample t-test with unequal variances (heteroscedastic) on the data set (2 groups, 3--5 replicates). The results provide the probability (p) of significant differences between two samples and/or test items. Comparisons with $p \leq 0.05$ will be considered to be significantly different. On the contrary, compounds with $p > 0.05$ will be excluded from further calculations/processing.

TTEST(Dataset Lx, Dataset Ly, tails, type)

Lx: measured values of test item 1 to be compared with Ly

Ly: measured values of test item 2 to be compared with Lx

tails = 2; two-tailed distribution

type = 3; heteroscedastic

4.5.3 Ranking of Detected Compounds

The sorting of significantly different compounds by their relevance is done by applying an empirically developed ("RANK") formula on the t-test filtered data set.

This "RANK" formula mathematically combines two criteria:

- difference of the variable ("Effect" (%))
- abundance of the variable ("Average Concentration" (e.g., µg/cig. or µg/article, or µg/mg TPM)).

$$RANK = \frac{Effect^3}{1000} \times Average\ Concentration$$

$$Effect = \frac{(Ly-Lx)}{(Ly+Lx)} \times 100$$

$$Average\ Concentration = \frac{Lx+Ly}{2}$$

The data set is divided into positive (Lx > Ly) and negative (Lx < Ly) rank values and sorted by increasing absolute rank values for the positive as well as the negative effect. A lower rank value shows more significant differences than a higher rank value.

4.5.4 Flexible Filtering

The final processed data can be filtered according to e.g. fold change or a concentration cut-off can be applied depending on the request or individual requirement.

4.5.5 Results Recording/Data Transfer/Documentation

A detailed description for data management is given in *PMI-RRP-FOR-111519* - NTDS GCxGC-TOF-Data Management *(RDNEU)*. The form summarizes all the administrative data, certificates, paper-rawdata, method specific forms and sample naming information that are needed for the study, and the GC×GC-TOFMS rawdata, processed data, CASI Processor data and result tables that are generated during the study with the respective location of storage. To conclude the study all steps have to be documented in *PMI-RRP-FOR-111519*, saved electronically in the study folder and printed and stored in the study binder.

Initial raw data are generated on the instrument acquisition computer, which are then copied including all study relevant instrument methods using the ChromaTOF inbuilt archiving functionality to a central data repository (currently HPC data share \\rd-hpc-samba.app.pmi). Autosampler sequences are copied to the same repository.

The initial raw data and all related data are restored on a local data evaluation computer using the ChromaTOF inbuilt restore from archive function. The data are deleted from the acquisition computer when the ChromaTOF data processing is finished successfully, provided that no file was corrupted during transfer.

Intermediate results (.csv files) of the data processing are stored in the primary raw data folder of the study directory in the \\rd-hpc-samba.app.pmi data share. Processed data files including all study relevant files are backed up to the same data repository using the ChromaTOF inbuilt archiving function.

After successful data processing using CASI Pre-processor, CASI and CASI Post-processor and subsequent confirmation of the results the processed data are deleted from the data evaluation workstation. Ultimately the entire study including a complete set of raw data and processed data must be maintained on the Long Term Repository (LTR, currently PMRDLabData_NeuchatelData \\cifs.arch10.store.pmi) environment.

Study related documents	Type
Certificates of ISTDs, syringe and analytical columns	Original/printout
Smoke/aerosol generation request and protocol	Printout/signed printout

Table 16. Study related documents.

Study relevant raw data and instrument related data for backup	File-type
Instrument raw data of samples, SSTs and sensitivity test	ChromaTOF database
Acquisition methods: GC and MS methods	ChromaTOF database
Autosampler methods	ChromaTOF database

Table 17. Study relevant raw data and instrument related data for backup.

Study relevant processed data and intermediate results for backup	File-type
Processed raw data of samples, system suitability tests and sensitivity test	ChromaTOF database
Data Processing methods: Data Processing methods, calibrations, retention index methods, classifications	ChromaTOF database
Results sensitivity test + report	Textfile (.pdf)
Results system suitability tests + report	Textfile (.csv and .pdf), Excel-form
Results sample data processing	Textfiles (.csv)
CASI Pre-processor, CASI, and CASI Post-processor input/output files	Textfiles (.txt)
Converted library	JCAMP (.HPJ)
Further Data Evaluation and Reporting files	Excel-files

Table 18. Study relevant raw data and instrument related data for backup.

Study relevant forms for backup	File-type
PMI-RRP-FOR-111487 - Chemicals, solvents, solutions and internal standard amount used for NTDS GCxGC-TOF *(RDNEU)*	Excel-file, signed printout
PMI-RRP-FOR-111488 - Storage of samples and study related materials for NTDS *(RDNEU)*	Excel-file, signed printout
PMI-RRP-FOR-111491 - Submitting metadata for CASI pre-processor, CASI, CASI post-processor *(RDNEU)*	Excel-file, signed printout
PMI-RRP-FOR-111496 - Preparation of LECO PEGASUS 4D SYSTEM for NTDS GCxGC-TOFMS *(RDNEU)*	Excel-file, signed printout
PMI-RRP-FOR-111512 - Data processing in ChromaTOF NTDS GCxGC-TOFMS Polar *(RDNEU)*	Excel-file, signed printout
PMI-RRP-FOR-111513 - Sample preparation for NTDS GCxGC-TOFMS Polar *(RDNEU)*	Excel-file, signed printout
PMI-RRP-FOR-111514 - Sensitivity test and system suitability test NTDS GCxGC-TOFMS Polar *(RDNEU)*	Excel-file, signed printout
PMI-RRP-FOR-111519 - NTDS GCxGC-TOF-Data Management *(RDNEU)*	Excel-file, signed printout

Table 19. Study relevant forms for backup.

4.6 Testing Scope, Repeatability, Reproducibility

Type	Name	Title	Author	Version
Assay Lifecycle Management Process Validation Report	Rep_AEC_003	Validation Report (Fast Validation) Non-Targeted Differential Screening (NTDS) Assay Using Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry (GC×GC-TOF) September 2008 (available on DISCO)	Arno Wittig	1.0

Table 20. Documentation.

4.6.1 Testing Scope

NTDS GC×GC-TOFMS Polar describes an assay for the detection of the most significant differences between two complex samples. The assay is non-targeted, i.e. it is based on a comprehensive chemical characterization of complex mixtures with no predefined target compounds. Depending on the matrix the testing scope can vary and thus cannot be defined specifically.

4.6.2 Repeatability, Reproducibility

Each sample will be generated minimum in triplicate and analyzed. Blanks, SST samples, pool sample(s) and one technical replicate of each matrix will be evenly distributed across the sequence. The repeatability of the SST and technical replicate samples are assessed by means of the area values of the ISTDs.

4.6.3 Acceptability Limits

The acceptability limits of the method are described in **Section 4.4.3.1** (sensitivity test) and **4.4.3.2** (system suitability test).

In case the criteria of the sensitivity test are not met, the multiplier voltage will be increased in steps of 50-V-increments until the acceptance criteria are fulfilled (further described in Section **4.4.3.1**). If the detector voltage exceeds 1900 V a replacement has to be considered.

If the acceptance criteria of the system suitability test are not met, the deviation needs to be commented in *PMI-RRP-FOR-111514* - Sensitivity test and system suitability test NTDS GCxGC-TOFMS Polar *(RDNEU)*. In case of multiple deviations the samples following the respective SST sample in the sequence are rendered invalid.

4.7 Safety

- The usual good practices of a laboratory are required, information is available in the “Laboratory EHS Handbook”
- Be aware of the risk assessment of the labs
- Work in an exhaust hood and wear safety glasses
- Take care of the relevant MSDS (material safety data sheets, storage location: T0.182 - 186)
- Store the inflammable products away from a heat source or a flame

4.8 Calibration and Maintenance of Instruments

Name	Title
PMI-RRP-WKI-111668	Operation of a LECO Pegasus 4D GC×GC-TOFMS system *(RDNEU)*

Table 21. Documentation for calibration and maintenance of the instruments.

5 Reference Documents

Knorr, A., Monge, A., Stueber, M., Stratmann, A., Arndt, D., Martin, E., Pospisil, P., Computer-assisted structure identification (CASI)--an automated platform for high-throughput identification of small molecules by two-dimensional gas chromatography coupled to mass spectrometry, Analytical Chemistry, 85(23) (2013) 11216-24

Shellie, R. A., Welthagen, W., Spranger, J., Ristow, M., Zrostlikova, J., Fiehn, O., Zimmermann, R., Statistical methods for comparing comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry results: Metabolomic analysis of mouse tissue extracts, Journal of Chromatography A, 1086 (2005) 83-90

Zhu, S., Lu. X., Dong, L., Xing, J., Su, X., Kong, H., Xu, G., Wu, C., Quantitative determination of compounds in tobacco essential oils by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry, Journal of Chromatography A, 1086 (2005) 107-114

International Organization for Standardization: International Standard ISO 3308, Routine analytical cigarette-smoking machine - Definitions and standard conditions, 4th ed., 2000

International Organization for Standardization: International Standard ISO 3402, Tobacco and tobacco products – Atmosphere for conditioning and testing, 4th ed., 1999

University of Kentucky, Kentucky Tobacco Research and Development Center: The reference cigarette, Lexington: The University of Kentucky Printing Services, 2003

6 Related Documents

Type	Name	Title
SOP	*PMI-RRP-SOP-111561*	Change control for CASI *(RDNEU)*
SOP	*PMI-RRP-SOP-111562*	Change control for CASI pre&post-processors *(RDNEU)*
SOP	*PMI-RRP-SOP-111686*	Perform Analysis *(RDNEU)*
User Guide	User Guide Pipeline Pilot.docx	User Guide Pipeline Pilot webport protocols, Elyette Martin, version 1.0 (available in DISCO)
Validation Report	rep_AEC_033	Validation Report (Fast Validation) Non-Targeted Differential Screening (NTDS) Assay Using Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry (GC×GC-TOF), September 2008, Arno Wittig, version 1.0 (available on DISCO)
WKI	*PMI-RRP-WKI-111700*	Management of pipettes *(RDNEU)*
WKI	*PMI-RRP-WKI-111768*	Management of laboratory fridges and freezers *(RDNEU)*
WKI	*PMI-RRP-WKI-111726*	Management of Balances *(RDNEU)*
WKI	*PMI-RRP-WKI-111784*	Equipment Logbook creation and content *(RDNEU)*
WKI	*PMI-RRP-WKI-111729*	Trappage des volatiles et semi-volatiles *(RDNEU)*
WKI	*PMI-RRP-WKI-111801*	Trappage en phase particulaire pour la détermination des constituants de l'aérosol *(RDNEU)*
WKI	*PMI-RRP-WKI-111814*	Management and labeling of chemicals *(RDNEU)*
WKI	*PMI-RRP-WKI-113456*	Rules for Handwritten Documented Information *(RRPCE)*
WKI	*PMI-RRP-WKI-111668*	Operation of a LECO Pegasus 4D GC×GC-TOFMS system *(RDNEU)*
WKI	*PMI-RRP-WKI-111624*	User guide for CASI *(RDNEU)*
WKI	*PMI-RRP-WKI-111625*	User guide for CASI Pre&post-processors *(RDNEU)*

Table 22. Related documents.

7 Records

Type	Name	Title
FOR	*PMI-RRP-FOR-111487*	Chemicals, solvents, solutions and internal standard amount used for NTDS GCxGC-TOF *(RDNEU)*
FOR	*PMI-RRP-FOR-111488*	Storage of samples and study related materials for NTDS *(RDNEU)*
FOR	*PMI-RRP-FOR-111491*	Submitting metadata for CASI pre-processor, CASI, CASI post-processor *(RDNEU)*
FOR	*PMI-RRP-FOR-111496*	Preparation of LECO PEGASUS 4D SYSTEM for NTDS GCxGC-TOFMS *(RDNEU)*
FOR	*PMI-RRP-FOR-111512*	Data processing in ChromaTOF NTDS GCxGC-TOFMS Polar *(RDNEU)*
FOR	*PMI-RRP-FOR-111513*	Sample preparation for NTDS GCxGC-TOFMS Polar *(RDNEU)*
FOR	*PMI-RRP-FOR-111514*	Sensitivity test and system suitability test NTDS GCxGC-TOFMS Polar *(RDNEU)*
FOR	*PMI-RRP-FOR-111519*	NTDS GCxGC-TOF-Data Management *(RDNEU)*

Table 23. Records.

8 Revision History

Version No.	Description of change (including reason for change)	Type of change
1.0	New version	1
2.0	Addition of another instrument, minor changes in the process (improvement of the overall performance)	2
3.0	Improvements of the process by implementing the use of a pool sample, changes in data storage location (LTR)	2
4.0.0	*Content transferred in the new OMSP template. Change document names into document OMSP IDs*	*2*

(1. Major change/new version; 2. Minor change; 3. Review without change); at least the last three major versions (i.e. 1.0, 2.0. etc.) are listed in the Revision History.

9 Abbreviations

Abbreviation/Definition	
2DRT	2nd dimension retention time
ARMS	advanced request management system
CASI	computer-assisted structure identification
DCM	dichloromethane
DISCO	document improvement system customer oriented
DPM	data processing method
EDMS	electronic document management system
EI	electron ionization
FTE	full-time equivalent
GC	gas chromatography, gas chromatograph
GC×GC	comprehensive two-dimensional gas chromatography
HPC	high performance computer
ISTD	internal standard
JCAMP	joint committee on atomic and molecular physical data
LTR	long term repository
MS	Microsoft, mass spectrometer, mass spectrometry
NTDS	non-targeted differential screening
PQ	performance qualification
RIM	retention index marker
RRP	reduced risk product
S/N	signal-to-noise ratio
SOP	standard operating procedure
SST	system suitability test
TOFMS	time-of-flight mass spectrometer
TPM	total particulate matter
WKI	work instruction

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