DataApex
Clarity™
CHROMATOGRAPHY SOFTWARE

DETAILED HYDROCARBON ANALYSIS

Clarity Extension   ENG

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Contents

To facilitate the orientation in the **Detailed Hydrocarbon Analysis** manual and **Clarity** chromatography station, different fonts are used throughout the manual. Meanings of these fonts are:

> *Open File* (italics) describes the commands and names of fields in **Clarity**, parameters that can be entered into them or a window or dialog name.
> WORK1 (capitals) indicates the name of the file and/or directory.
> *ACTIVE* (capital italics) marks the state of the station or its part.
> Chromatogram (blue underlined) marks clickable links referring to related chapters.

The bold text is sometimes also used for important parts of the text and the name of the **Clarity** station. Moreover, some sections are written in format other than normal text. These sections are formatted as follows:

. . . .
Note: Notifies the reader of relevant information.
. . . .
Caution: Warns the user of possibly dangerous or very important information.

. . . .

▌Marks the problem statement or trouble question.
Description: Presents more detailed information on the problem, describes its causes, etc.
Solution: Marks the response to the question, presents a procedure how to remove it.

1 DHA - Detailed Hydrocarbon Analysis

The **DHA Extension** is an optional add-on for the **Clarity Chromatography Software**. It can expand any **Clarity** Instrument as well as **Clarity Offline**. By default, it contains support for the official **ASTM D6730** norm. Additional **ASTM D6730 DANI Optimized Method with/without Cryo** are available exclusively for the DANI company.

The **Clarity DHA Extension** enables data processing and calculations according to a selected norm(s). These methods are often referred to as **PONA**, **PIONA**, **O-PONA**, etc.

2 Specification

The **DHA Extension** enables chromatogram data analysis in compliance with the following norms:

ASTM D6730

- Included in **p/n A33**.
- The standard test method for the determining components in volatile samples of spark- ignition engine fuels and their mixtures containing oxygenate blends using a 100–meter capillary column (with a pre-column) high-resolution gas chromatography. See the documentation of this norm for further details.
- See the details in the chapter **"ASTM D6730"** on pg. **2**.

ASTM D6730 DANI Optimized Method with Cryo

- Not included in **p/n A33**, available exclusively at the DANI company.
- See the details in the chapter **"ASTM D6730 DANI Optimized Method with Cryo"** on pg. **3**.

ASTM D6730 DANI Optimized Method without Cryo

- Not included in **p/n A33**, available exclusively at the DANI company.
- See the details in the chapter **"ASTM D6730 DANI Optimized Method without Cryo"** on pg. **4**.

Custom Method

- Included in **p/n A33**.
- See the details in the chapter **"How to create a custom norm"** on pg. **22**.

2.1 ASTM D6730

Oven

- Linear carrier flow (He) 2.4 ml/min
- Initial temperature 35 °C
- First hold temperature 5 °C
- First hold time 10 min
- First program rate 5 °C/min
- Second temperature ≈ 49
- Second hold time ≈ 55
- Second program rate 1.6 °C/min
- Final temperature 200 °C
- Final hold time 5 min
- Analysis time ≈ 170

Injector
- Temperature 250 °C
- Split ratio 100:1
- Sample size 0.1 - 0.2 uL

Detector
- FID
- Temperature 250 °C
- Hydrogen flow 35 mL/min
- Air flow 350 mL/min
- Make up flow He 20 mL/min

Carrier gas
- Helium
- EPC pressure 301 kPa
- Linear velocity 28 cm/s
- Gas saver flow 25
- Gas saver time 2

2.2 ASTM D6730 DANI Optimized Method with Cryo

Oven
- Initial temperature 5 °C
- Initial time 10 min
- First program rate 5 °C/min
- First hold temperature 50 °C
- First hold time 0 min
- Second program rate 1.5 °C/min
- Final temperature 200 °C
- Final hold time 5 min

Injector
- Temperature 250 °C
- Split ratio 150:1
- Sample size 0.5 uL

Detector
- FID
- Temperature 250 °C
- Hydrogen 30 mL/min
- Air 300 mL/min
- Nitrogen 30 mL/min

Carrier gas
- Helium, pressure constant: 4.16 bar

2.3 ASTM D6730 DANI Optimized Method without Cryo

Oven

- Initial temperature 35 °C
- Initial time 15 min
- First program rate 1 °C/min
- First hold temperature 60 °C
- First hold time 20 min
- Second program rate 2 °C/min
- Final temperature 200 °C
- Final hold time 5 min.

Injector

- Temperature 250 °C
- Split ratio 150:1
- sample size 0.5 uL

Detector

- FID
- Temperature 250 °C
- Hydrogen 30 mL/min
- Air 300 mL/min
- Nitrogen 30 mL/min

Carrier gas

- Helium, pressure constant: 4.16 bar

2.4 Custom DHA Method

In the **DHA Extension**, it is possible to set up a custom method, which can be used to calculate the DHA parameters of your chromatograms. For a description on how to create a custom norm, see the chapter **"How to create a custom norm"** on pg. **22**.

3 Installation

The DHA Extension is activated by entering an appropriate user code during the installation of **Clarity** or later using the *Help - User Code…* command from the *Clarity* main window.

To enable the DHA Extension on an *Instrument*, you have to set the corresponding instrument type. To set the instrument type, click the ⊡ button in the *System Configuration* dialog.

In the invoked *Instrument Type Setting* dialog, select the GC-DHA option.

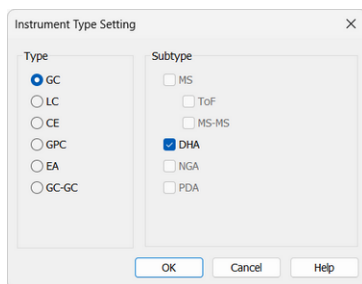*Caution:*   The DHA Extension is available only on **GC** instrument type.

Fig. 1: Enabling the DHA Extension on an Instrument

4 DHA Extension Description

This chapter lists and describes the features changed in or added into **Clarity** by the **DHA Extension**.

4.1 Chromatogram window

A new DHA tab is available in the *Chromatogram* window. Furthermore, there is a new *DHA* menu at the top of the window.

4.1.1 DHA

The *DHA* tab is divided into two panes:

- Left pane includes the DHA Results and the DHA Group Results tabs.
- Right pane includes the DHA Settings.

4.1.2 DHA Settings

In this pane, you can set up the *DHA Method* - choose a norm and edit its parameters.
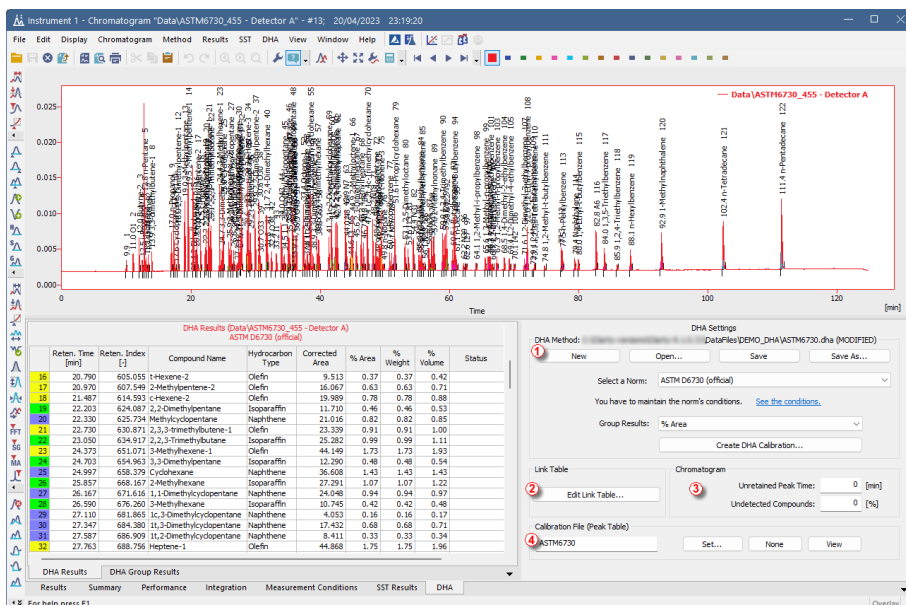

Fig. 2: Chromatogram window - DHA - DHA Settings

DHA Method ①

Displays the currently opened *DHA Method*. All settings in the *DHA Method* are saved in the *.DHA file, which is independent on the method opened from the *Instrument* window. The last opened *DHA Method* used in the *Chromatogram* window is saved in the project file (for more details, see also the chapter Project).

New

Creates a new *DHA Method* with default settings.

Open

Opens the *Open DHA Method* dialog where you can select the *DHA Method* you want to use.

Save

Saves the settings of the current *DHA Method*.

Save As…

Opens the *Save DHA Method As* dialog for saving the *DHA Method* into a new file.

Select a Norm

Select the norm you want to use for your calculations.

- -

Caution: Based on the purchased DHA Extension type, you may be able to select only some of the norms from the drop-down menu. For more information, see the chapter the chapter **"Specification"** on pg. **2**.

In the case you select a norm you have not purchased, an error dialog is displayed:
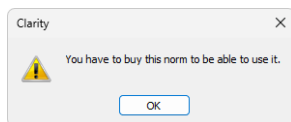

Fig. 3: Error when selecting a non-available norm

See the conditions

By clicking on the link, you can display the help containing the conditions valid to each norm.

Group Results

Selects the units used in the DHA Group Results.

Create DHA Calibration

By clicking this button, the Create DHA Calibration dialog is displayed. It allows you to create a *DHA Calibration* and display it in the *Calibration* window.

In the *Calibration*, the peak detection must be set to one signal only; otherwise there will be warnings in DHA Results.

Link Table ②

In situations, when the *Compound Names* in norms are different from names set in the calibration, you can use the *Link Table* to link those compound names to ensure that all compounds are included in the calculations. For more details see the chapter **Link Table** on pg. **8**.

Edit Link Table…

Opens the Link Table dialog, where you can create and edit links between compound names in calibrations and norms.

Chromatogram ③

Unretained Peak Time

This parameter defines the column dead time in minutes. This field corresponds to the same field in the Performance tab.

When creating a *DHA Calibration* from a DHA standard, it is necessary to fill in here the *Unretained Peak Time* (which is usually the retention time of methane).

....

Caution: *Retention Indexes* can be calculated either with or without the *Unretained Peak Time*. This depends on the selection of the *Retention Indexes use Log. Interpolation with Unretained Peak* option in the **Calibration Options**. For DHA calculations, this option should be checked (default setting), so that *logarithmic interpolation with unretained peak time* is used for calculation of *Retention Indexes*. When this option is not checked, the indexes are calculated using *linear interpolation*.

Undetected Compounds

This parameter defines the value in % of compounds which are undetected in the DHA Results - column *% Weight* (*% Volume* is calculated from the *% Weight* column). The total weight of the detected compounds will be calculated as: 100-(*Undetected Compounds*) [%]. The weights of the individual detected compounds will be normalized to this total weight.

Calibration File (Peak Table) ④

This field and group of buttons work identically as the buttons in the *Results* tab.

Set…

Opens the *Select Calibration* dialog for linking a **Calibration**.

None

No calibration will be used.

View…

Displays the linked calibration.

4.1.2.1 Link Table

This dialog can be used for editing the *Link Table* in the DHA Extension. The *Link Table* is used in those cases when you have unresolved compound names in the Result Table. For example, you are using compound names specific to your language, but in the selected norm, there is a different name for the same compound. The *Link Table* allows you to link the different compound names so you can use your custom compound names in calibrations and chromatograms as needed.
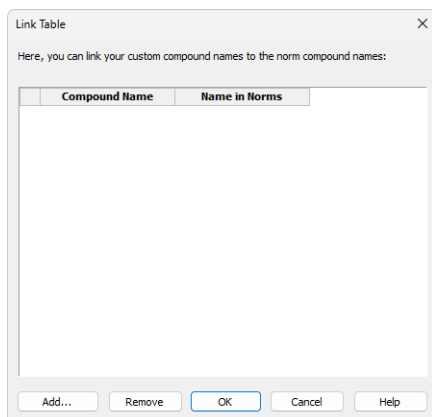
Fig. 4: Link Table dialog

1st column in the table
Number of the *Link Table* row.

Compound Name
The compound name in the *Calibration* that you want to link with the name in norms, and thus to include in the DHA Results and DHA Group Results.

Name in Norms
The compound name as given by the norm (if the name is in multiple norms, only one occurrence is displayed).

Add…
Displays the Add Link dialog for adding a new record to the link table.

Remove
Removes the selected record from the *Link Table*.

OK
Saves the settings to the *Link Table* and exits the dialog.

Cancel
Exits the dialog without saving any changes to the *Link Table*.

4.1.2.1.1 Add Link

The *Add Link* dialog is used for adding a new item to the Link Table. It is useful in the case you want to link a compound name from the DHA Results to a compound name in a norm. Linking a compound name to the name in norms includes such compound in the DHA calculations.
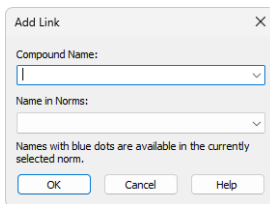
Fig. 5: Add Link dialog

Compound Name

Enter the compound name you want to link to a name in norms. Unresolved compounds in the chromatogram are suggested in the drop-down menu.

Name in Norms

Select the appropriate compound name from norms. Names from all supported norms are available in the drop-down menu. For better orientation, blue dots are displayed on the compounds applicable to the norm selected in the DHA Settings.
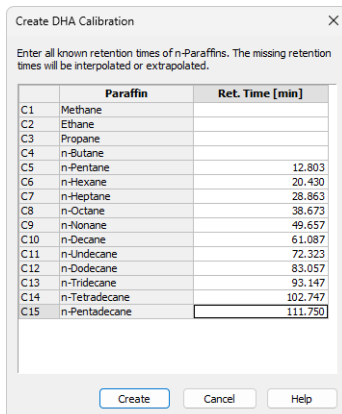
4.1.2.2 Create DHA Calibration

This dialog is displayed when you click the *Create DHA Calibration…* button in the DHA Settings. The present table contains a list of n-paraffins and their *Retention Times* as detected from the measured standard. The dialog allows you to manually adjust the *Retention Times* of the particular compounds. The opened standard chromatogram must contain from 6 to 11 calibrated peaks of the standard n-paraffins.

Note: The standard based on which a DHA calibration can be created **must** be calibrated. Because of this, it is necessary to have a separate non-DHA calibration for the standard n-paraffins samples. The DHA demo data also contain two demo calibrations. For more details see the chapter **How to try the DHA Extension** on pg. **19**..

After clicking the *Create* button, a *DHA Calibration* is created and displayed in the *Calibration* window. **Clarity** will perform an interpolation based on the *Retention Times* of the n-paraffins standard and automatically calculate *Retention Times* of all other peaks according to the *Retention Indexes* in the DHA norm.

The main purpose of this dialog is to enter the retention times of light hydrocarbons - methane, ethane, propane, and butane.

Fig. 6: Create DHA Calibration dialog

The minimum number of n-paraffins in your standard is 6 and the maximum is 11 (the more compounds, the more precise the interpolation). If this condition is not met, the following error message is displayed:
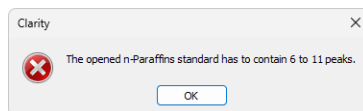

Fig. 7: The opened n-Paraffins standard has to contain 6 to 11 peaks.

4.1.3 DHA Results

The *DHA Results* section on the *DHA* tab contains information about the detected compounds and calculated results. The calculation details can be found the chapter **"Appendix - Mathematical Formulas"** on pg. **25**..
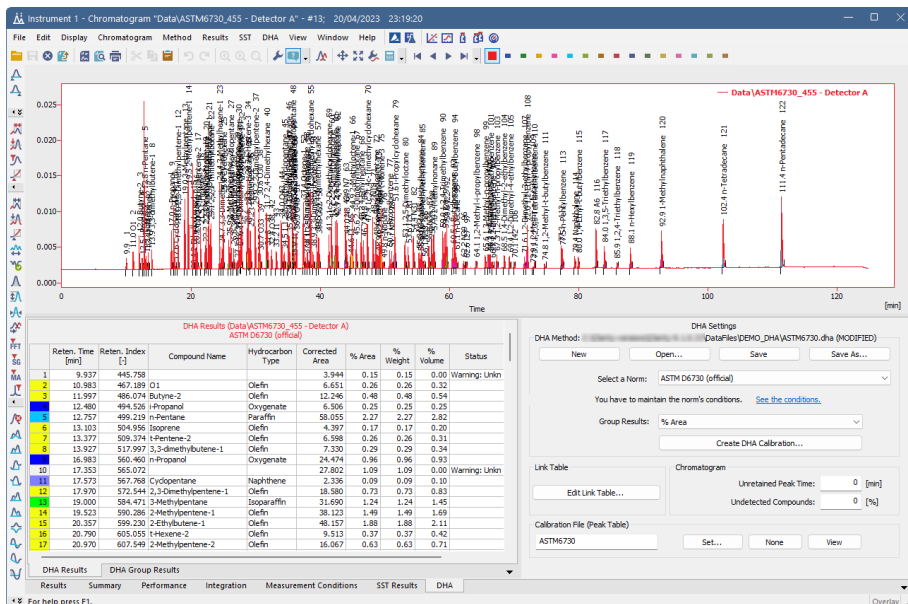
Fig. 8: Chromatogram window - DHA - DHA Results

DHA Results

Above the table, you can see information about the signal from which the results were calculated, and also the selected norm. To obtain valid data, you must create a calibration first using *Create DHA Calibration*. The calibration must use the same norm as selected for displaying the Results. Otherwise, you will see one of the following warnings:

Warning	Description
Linked calibration was not generated by DHA	The *Calibration* you have linked to the chromatogram was not created using the DHA Extension. Create it using the n-paraffins known sample via the *Create DHA Calibration…* button.
Norm selected for DHA Method \<norm name\> and DHA Calibration \<norm name\> do not match	Different norms are selected in *DHA Results* and *DHA Calibration*.
Unretained Peak Time has to be enabled in Calibration	The *Retention Indexes use Log. Interpolation with Unretained Peak* option in the *Calibration Options* dialog in the linked *Calibration* is not enabled.

Warning	Description
Unretained Peak Time in Chromatogram <number> and DHA Calibration <number> do not match	Your linked *DHA Calibration* was generated with a different *Unretained Peak Time* than the one set in the current *DHA Method*. Correct this in the DHA Settings or create a *DHA Calibration* again with the correct *Unretained Peak Time*.
Unretained Peak Time is non-zero	This is an informational message that appears when the *Unretained Peak Time* is set to a non-zero value.
DHA Calibration is not linked	Calibration is not linked. You will not see any results until you link a *DHA Calibration*.

Reten. Index
Retention Index of a compound. Calculated as described in the chapter **"Appendix - Mathematical Formulas"** on pg. **25**.

Compound Name
If the chromatogram contains calibrated peaks or groups, their names are shown in this column. The compound names are retrieved from the norm table (the DHA.CSV file located in the CLARITY\DATFILES\COMMON directory) according to the selected norm, corresponding to the calculated retention indexes based on the calibration.

Hydrocarbon Type
The hydrocarbon type of the compound retrieved from the selected norm.

Corrected Area
Corrected Area of a specific compound in the measured chromatogram, is calculated from the chromatogram peak area multiplied by the relative response factor (more details in the chapter **"Appendix - Mathematical Formulas"** on pg. **25**.).

% Area
% Area of the a specific compound in the measured chromatogram. The value is based on the corrected area value (this means that it is in fact based on the compound amount - more details in the chapter **"Appendix - Mathematical Formulas"** on pg. **25**.).

% Weight
% Weight of a specific compound in the measured chromatogram. The value is based on the entire sample amount. It may be corrected by the undetected compounds percentage (like water etc., determined by external methods) entered in the *Undetected Compounds* field. When this field is non-zero, the *% Area* and *% Weight* values are equal. Upon entering an *Undetected Compounds* content, the *% Weight* values will be recalculated accordingly (more details in the chapter **"Appendix - Mathematical Formulas"** on pg. **25**.).

% Volume
% Volume of a specific compound in the measured chromatogram. It is calculated as the *(% Weight)/(Relative Density)* ratio normalized to the sum of 100% (more details in the chapter **"Appendix - Mathematical Formulas"** on pg. **25**.).

Note: In case any of the mentioned properties (*Corrected Area*, *% Area*, *% Weight*, *% Volume*) are not calculated for a specific compound, however there is no error, the necessary calculation parameters are probably not defined in the selected norm.

Status

A Warning may appear if the compound name is not found in the selected norm. You can match the present compound name with a compound name in the norm by clicking the Edit Link Table… button. In the displayed *Link Table* dialog, you can link compounds from the chromatogram with the compound names in the norm.

4.1.4 DHA Group Results

On the *DHA Group Results* tab, you can find results calculated according to hydrocarbon types. The results are automatically recalculated each time you open or change the chromatogram or select different norm or modify its parameters.
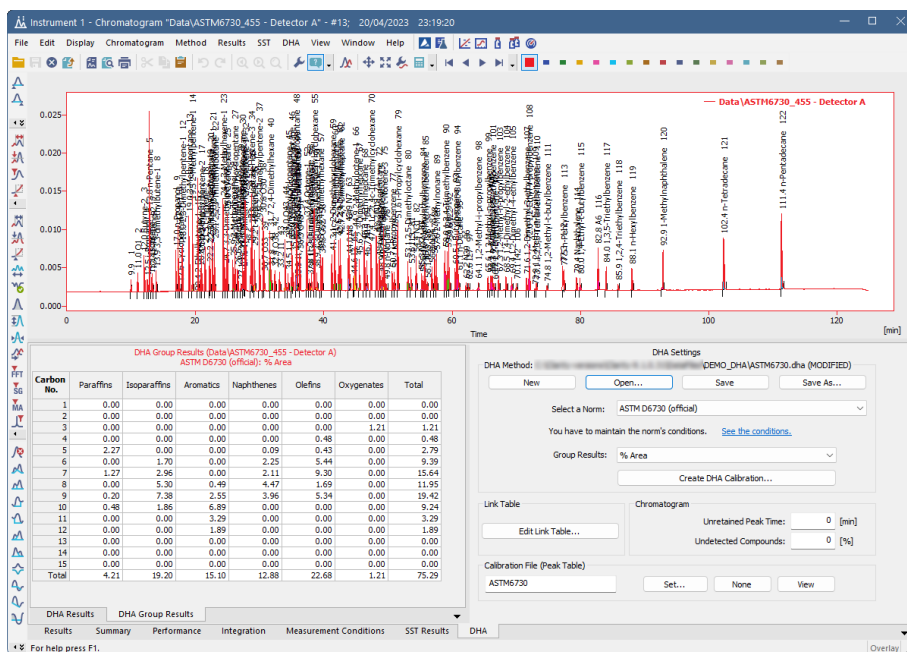
Fig. 9: Chromatogram window - DHA Group Results

DHA Group Result Table

This table displays the results in a special pivot table. The units of the results in the table depend on the units you have selected in the *DHA Settings - Norm - Group Results* combo-box. You can see the summary results per carbon number or per a group of paraffins. Warnings may appear in the table header (see the chapter **"DHA Results"** on pg. **11**. for more details).

Note: Group results will be calculated only for compounds with a carbon number defined in the selected norm. Compounds without a definite carbon number are omitted from the *DHA Group Results* (thus the *Total* values do not have to add up to *100*).

4.2 Report Setup

In the *Report Setup* dialog, tab *DHA*, you can set the printing options for the data in the DHA Extension.
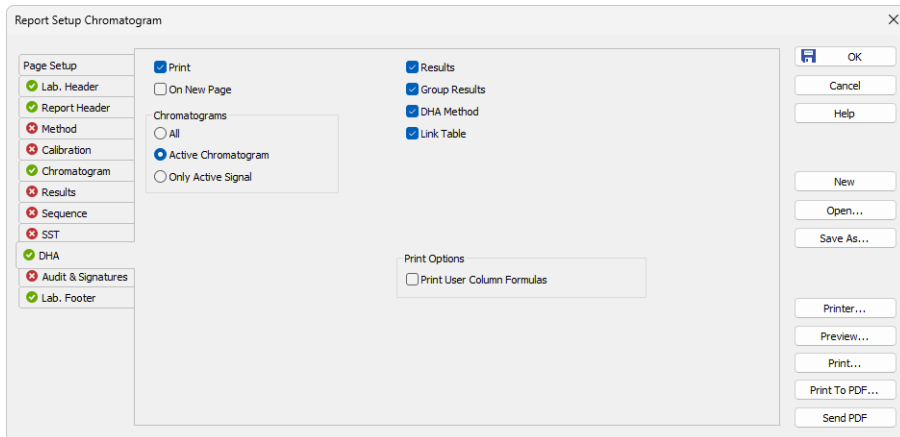

Fig. 10: Report Setup DHA dialog

Print

Enable/disable the printing of the DHA details. If checked, the symbol ✔ will be shown before the tab name, and other fields on the tab will become available.

On New Page
If checked, the **DHA Section** is printed on a new page.

Chromatograms

> **All**
> Prints data for all chromatograms opened in the *OVERLAY MODE*.

> **Active Chromatogram**
> Prints data for the *ACTIVE Chromatogram* only.

> **Only Active Signal**
> Prints data for the *ACTIVE Signal* only.

Results
If checked, prints the *DHA - Results*.

Group Results
If checked, prints the *DHA - Group Results*.

DHA Method
If checked, prints the information about the *DHA Method*.

Link Table
If checked, prints the Link Table.

Print Options

Defines additional options of the printed report.

Print User Column Formulas

Adds calculation formulas used in the User Columns including *User Variables* at the end of the appropriate table to be printed.

Note: In the GLP mode, formulas are printed automatically, even if this option is unchecked.

4.3 Export Data

In the *Export Data* dialog, you can set whether the *DHA - Result Table* and the *DHA - Group Result Table* will be included in the exported data. For more details, see the chapter Export Data in the **Clarity Reference Guide**.
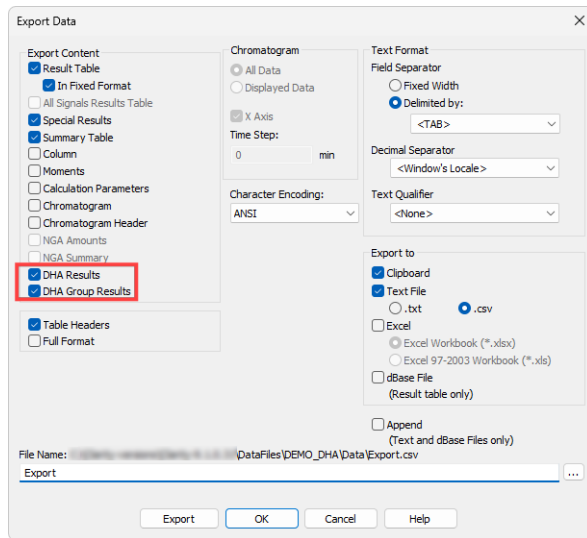

Fig. 11: Export Data dialog

5 How to try the DHA Extension

5.1 How to try the DHA demo project

In this chapter, you can find instructions on how to try the DHA Extension using the DHA demo project.

Step 1

In the *System Configuration* window (menu *Clarity - Configuration…*), open the *Instrument Type Setting* dialog via the ⌷ button, and check the *DHA* option on *Instrument 1*.

For more details, see also the chapter Installation.

Step 2

After closing the *System Configuration* window, open *Instrument 1*. Now, open the DHA demo project: in the *Login Dialog*, select **DEMO_DHA** in the *Select Project* drop-down list, and click the *OK* button.

Step 3

Open the *Chromatogram* window (it can be opened by clicking on the menu *Window - Chromatogram*). In the lower part of the *Chromatogram* window, you can see the new *DHA* tab. Open the chromatogram N_ PARAFFINS_ FOR_ ASTM6730.PRM. This file is a calibrated n-paraffins standard.

Step 4

Select the *DHA* tab, and on the right side of the bottom pane, you will see the *DHA settings* pane. There you can choose a norm for your *DHA Method* and create a new *DHA Calibration*.

The settings from the *DHA Method* field are saved in a special .DHA (*DHA Method*) file, which can be used for multiple chromatograms. Click the *Open…* button to browse the Clarity Installation directory (by default C:\CLARITY\DATAFILES). In the DEMO_DHA subdirectory, select the ASTM6730.DHA file. The *DHA Method* will be loaded, and the active chromatogram results will be automatically recalculated according to the set norm and *Group Results* parameters. Note that the linked calibration ASTM6730_C5-C15-RI.CAL is a non-DHA calibration that serves only for the calibration of the present n-paraffin sample peaks. Due to this, the *DHA Results table* header contains a warning.

For more details see the chapter **DHA Settings** on pg. **6**.

Step 5

The norm selected in the *DHA Settings* pane will be used when creating a *DHA Calibration*. Click the *Create DHA Calibration* button, and the *Create DHA Calibration* dialog will be displayed. Retention times from the opened calibration standard will be automatically imported. After clicking the *Create* button, the *Calibration* window will open. Save the created calibration.

Step 6

Close the *Calibration* window, and in the *Chromatogram* window, open an unknown sample from the DEMO_DHA project, for example ASTM6730_455.PRM. Click the

DHA tab, and in the lower part of the *DHA Settings* pane, you will see that this chromatogram already has a linked calibration file *ASTM6730*. Note that this is the same file as set in the *Calibration File* field in the *Results* tab of the **Chromatogram** window. You can change it to the calibration created in **Step 5**.

Step 7

In the *DHA Results* sub-tab of the *DHA* tab, you can see the calculated results of compounds identified in the chromatogram. You can also switch to the *DHA Group Results* tab, where the results are presented in a pivot table. The table contains the sums of all compounds per group or carbon number.

If there is a compound in the *Result Table* that cannot be identified by the selected norm, the *Status* column displays a warning. This can be caused by the compound name as set in the calibration not corresponding to the compound name in norms. If you need to link a compound from the chromatogram with a matching compound name in norms, click *Edit Link Table…*, and in the displayed dialog, link the compound name from the chromatogram with the name in norms.

6 How to work with the DHA Extension

6.1 Measuring DHA data

Step 1

See the chapter **"Specification"** on pg. **2**. for an overview of specifications of the supported DHA norms. It is necessary to prepare your instrument and method according to the specifications of the particular norm to obtain valid results.

Step 2

In the *System Configuration* window (menu *Clarity - Configuration…*), open the *Instrument Type Setting* dialog via the ⋯ button, and check the *DHA* option on *Instrument 1.*

For more details, see also the chapter Installation.

Step 3

After closing the *System Configuration* window, open *Instrument 1*. Now, create a new project: in the *Login Dialog*, select *<New Project>* in the *Select Project* drop-down list and click the *OK* button. In the *Create Project or Save as...* dialog, name your new project, and click *OK*.

Step 4

Now, you have to create a *DHA Calibration* according to the selected norm. *DHA Calibration* is a calibration that contains parameters required for the DHA calculations. Proceed as follows:

Measure the known n-paraffin samples. It is best to measure all the possible C1-C15 n-paraffins to have an exact interpolation of the calculated retention indexes. In order to create a full calibration with all n-paraffins, it is necessary to measure two standards (first for C1-C4 light hydrocarbons and then for C5-C15 hydrocarbons). As a minimum, you have to use C5-C11, however, extrapolations usually do not give precise results.

Step 5

If you measured the retention times of C1-C4 compounds, open this chromatogram and note down the four retention times.

Open the measured chromatogram with integrated C5-C15 n-alkanes. In the *DHA Settings*pane, fill out the *Unretained Peak Time* if known (usually the methane retention time). Select the norm you intend to use and click the *Create DHA Calibration* button. The displayed dialog automatically fills the retention times of n-alkanes from the standard, starting from C5. Fill out the retention times of C1-C4 n-alkanes manually, if measured beforehand (these values cannot be added later to the calibration, similarly to the *Unretained Peak Time*). Continue by clicking the *Create* button.

Save the created *DHA Calibration*. Note that in this type of calibration, the option *Retention Indexes use Log. Interpolation with Unretained Peak* in the **Calibration Options** is automatically enabled.

Step 6

Now, you can prepare a method for measuring the unknown samples.

If you want to link the *DHA Calibration* to your measured chromatograms automatically, select the calibration file created in **Step 5** using the *Set…* button in the **Method Setup - Calculation** window. Set other method parameters as necessary.

Step 7

Measure the unknown data using the **Method** created in the previous step and open the chromatograms.

In the **Chromatogram** window - *DHA* tab, you can find the DHA Results and DHA Group Results subtabs containing the desired results.

6.2 How to print or export results from the DHA Extension

Print

To print the data from the DHA Extension, open the *Report Setup* window by clicking the menu *File - Report Setup…* in the **Chromatogram** window.

On the left side, select the *DHA* tab. By checking the *Print* checkbox, the other controls on that pane are enabled. You can configure what information will be printed by choosing the corresponding options.

Settings from the *Report Setup* are used in the print output and preview, accessible from the *File* menu of the **Chromatogram** window.

You can also use the prepared DHA report style: in the *Report Setup* window, click *Open…*, and select the DHA_RESULTS.STY report style.

For more details, see also the chapter Report Setup

Export

Open the *Export Data* dialog via the *Setting - Export Data…* menu of the **Instrument** window. Check the *DHA Results* and *DHA Group Results* checkboxes to include them in the exports. Settings from this window are used in the **Single Analysis - Post-run Options** tab.

For more details, see also the chapter Export Data

6.3 How to create a custom norm

In addition to the available norms, the DHA Extension enables you to create your own set of custom parameters corresponding to the conditions of DHA data analysis in your laboratory.

DHA Extension uses the DHA.CSV file located in the COMMON subdirectory of the installation folder (by default C:\CLARITY\DATAFILES\COMMON\DHA.CSV). This file contains *Compounds*, *Retention Indexes*, and other information required for DHA calculations. It also includes the *RICustom* column, which contains *Retention Indexes* you need to measure while developing your custom norm.

Note that to prepare a custom norm, you first need to create an auxiliary calibration file, which will help you prepare the parameters for the custom norm. Once the

necessary values are in the DHA.CSV file, you can create a full calibration for the sample measurements. To achieve this, follow these steps:

Step 1

Open the DHA.CSV file in a spreadsheet application, such as **MS Excel**.

Caution: The file uses the *dot* as the decimal separator. If your local settings of **MS Excel** or **Windows** use a *comma* as the decimal separator, this may corrupt the DHA.CSV file when saving any changes. Before editing this file, change the local **Windows** setting to use the *dot* as the decimal separator.

Step 2

Measure the known n-paraffin samples. It is best to measure all the possible C1-C15 n-paraffins to have an exact interpolation of the calculated retention indexes. In order to create a full calibration with all n-paraffins, it is necessary to measure two standards (first for C1-C4 light hydrocarbons and then for C5-C15 hydrocarbons). As a minimum, you have to use C5-C11, however, extrapolations usually do not give precise results.

Step 3

If you measured the retention times of C1-C4 compounds, open this chromatogram and note down the four retention times.

Open the measured chromatogram with integrated C5-C15 n-alkanes. In the *DHA Settings*pane, fill out the *Unretained Peak Time* if known (usually the methane retention time). Select the *Custom (edit DHA.csv)* norm and click the *Create DHA Calibration* button. The displayed dialog automatically fills the retention times of n-alkanes from the standard, starting from C5. Fill out the retention times of C1-C4 n-alkanes manually, if measured beforehand (these values cannot be added later to the calibration, similarly to the *Unretained Peak Time*). Continue by clicking the *Create* button.

Save the created *DHA Calibration*. Note that in this type of calibration, the option *Retention Indexes use Log. Interpolation with Unretained Peak* in the **Calibration Options** is automatically enabled.

Step 4

In the *Calibration Summary Table*, display the *Reten. Index* column. You can see that the retention indexes of C1-C15 n-paraffins are automatically filled.

Step 5

The next step is measuring the *Retention Times* of compounds listed in the first column of the DHA.CSV file to obtain the necessary *Retention Indexes* for your custom norm. To link the prepared *DHA Calibration* to the measured chromatograms automatically, select the calibration file created in **Step 3** using the *Set…* button in the **Method Setup - Calculation** window. Set other method parameters as necessary.

Step 6

Open the measured chromatogram(s). The *Reten. Index* column can be displayed in the *Chromatogram* window - *Result Table*via the *Setup Columns* dialog (accessible from the context menu). The *Retention Indexes* are automatically

calculated by **Clarity** based on the *Retention Indexes* of the n-paraffins in the linked calibration and the measured *Retention Times*.

Once you have the *Retention Indexes* of the compounds in the DHA.CSV file, enter them manually into the last *RICustom* column.

Step 7

Now, a complete *DHA Calibration* can be created and used for the sample data. You can follow the procedure in the chapter **"How to work with the DHA Extension"** on pg. **21**., except for setting the *Custom (edit DHA.csv)*norm before clicking the *Create DHA Calibration* button. The resulting *DHA Calibration* will contain extrapolated *Retention Times* calculated based on the *DHA Custom norm* parameters. Note that the DHA.CSV file is applied when opening the **Clarity** station. You need to restart **Clarity** for the modifications to take effect.

7 Appendix - Mathematical Formulas

7.1 Retention Index

Also known as the Kováts retention index. For detected compounds, the *Retention Index* (RI) is calculated according to the following formula:

$$RI_i = 100 \cdot \left(\frac{\log t_{r,i} - \log t_{r,n}}{\log t_{r,n+1} - \log t_{r,n}} + n \right), \text{ where } t_{r,j} = tj - t_0$$

where $t_{r,j}$ is the reduced retention time of compound *j*, calculated as the difference between the retention time of the compound (t_j) and the column dead time (t_0 = *Unretained Peak Time*). The subscipt *i* denotes the unknown compound, *n* denotes the calibrated *n*-alkene eluting before the unknown compound (*n* is the number of carbon atoms in this compound), and *n*+1 is the *n*-alkene eluting after the unknown compound.

Note that when creating a *DHA Calibration* from a DHA standard using the *Create DHA Calibration...* button in the *Chromatogram* window, it is necessary to fill in there the *Unretained Peak Time* (which is usually the retention time of methane), so that it is then saved in the *DHA Calibration*.

7.2 Corrected Area

The *Corrected Area* is calculated from the chromatogram peak *Area* multiplied by the relative response factor (*RRF*) as defined by the selected norm. *RRF* of a compound not present in the norm is considered as *1*:

$$CorrectedArea_i = Area_i \cdot RRF_i$$

7.3 % Area

% Area corresponds to the percentage of the calculated *Corrected Area* of a compound divided by the sum of *Corrected Areas* of all *N* detected compounds:

$$\%Area_i = \frac{CorrectedArea_i}{\sum\limits_{j=1}^{N} CorrectedArea_j} \cdot 100\%$$

7.4 % Weight

% Weight corresponds to the weight percentage of a compound in the sample. The calculation takes into account the *Undetected Compounds* amount:

$$\%Weight_i = \frac{CorrectedArea_i \cdot (1 - UndetectedCompouds)}{\sum\limits_{j=1}^{N} CorrectedArea_j} \cdot 100\%$$

7.5 % Volume

% Volume corresponds to the volume percentage of a compound in the sample. It is calculated by dividing the calculated *% Weight* by the relative density (*RD*) as defined by the selected norm, and subsequently normalizing this ratio to the sum of *100%*:

$$\%Volume_i = \frac{\%Weight_i \cdot RD_i}{\sum\limits_{j=1}^{N} \%Weight_j \cdot RD_j} \cdot 100\%$$