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Gas chromatographic detection of Sesquiterpenoids in Dodecane using Perkin Elmer GC 580

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1

Works for me

dx.doi.org/10.17504/protocols.io.kj2cuqe

CyanoWorld



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ABSTRACT

This is a quick guide for routine quantitative analysis of sesquiterpenoids in dodecane using an autosampler-assisted gas chromatograph. This protocol has been established in the Lindberg lab at Ångström laboratory (Uppsala University) for direct analysis of dodecane-based *ex-situ* extracts from cyanobacterial strains producing different types of sesquiterpenoids. The basic protocol was originally described in the master thesis of Sara Nilsson for (*E*)- α -bisabolene quantification [1], and has been extended to (-)- α -bisabolol and (-)-patchoulol.

This protocol describes the pipeline from the general setup to the generation of quantitative data output for external data processing (e.g. in Excel). Qualitative data analysis is not described.

[1] Nilsson, S. (2017). (*E*)- α -bisabolene production in *Synechocystis* sp. PCC 6803 (Master thesis). Retrieved from <http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-325811>

EXTERNAL LINK

http://www.perkinelmer.com/CMSResources/Images/44-74577MAN_TotalChromWorkstationVolume1.pdf

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Dienst D., Wichmann J., Mantovani O., Rodrigues J., Lindberg P. (2019) High density cultivation for efficient sesquiterpenoid biosynthesis in *Synechocystis* sp. PCC 6803.

MATERIALS

NAME	CATALOG #	VENDOR
Alfa Aesar Bisabolene, mixture of isomers	A18724	Fisher Scientific
β -Caryophyllene $\geq 80\%$	W225207	Sigma Aldrich
(-)- α -Bisabolol; analytical standard	95426	Alfa Aesar
Dodecane Reagent Grade $\geq 99\%$	D221104	
Patchouli alcohol primary reference standard	5986-55-0	Sigma Aldrich

MATERIALS TEXT

Perkin Elmer GC 580 gas-phase chromatograph (Perkin Elmer), Elite-Wax Polyethylene Glycol Series Capillary (Perkin Elmer), Carrier gas: N₂, TotalChrom Workstation (Perkin Elmer), 1.5 mL clear glass GC vials with a 9 mm Silicone/PTFE closure (VWR, Art.# 548-1488)

1 General Setup

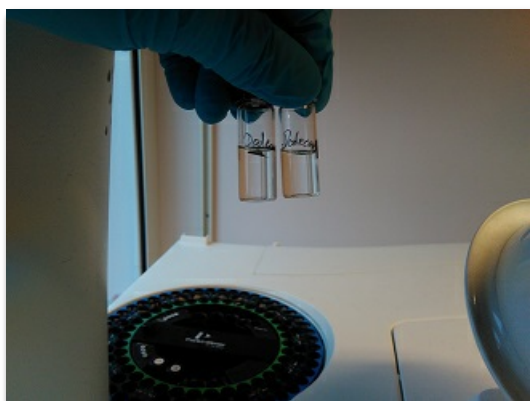
- fill two wash bottles w/ each 3 mL Dodecane (solvent)
- place bottles into 'wash' positions 1 & 2 of autosampler platform
- place empty wash bottles into 'waste' positions 1 & 2
- place GC vials (max. 108) into corresponding positions of autosampler platform

For sample preparation check the following protocols:

[\(-\)-patchoulol GC sample preparation](#)

[\(E\)- \$\alpha\$ -bisabolene GC sample preparation](#)

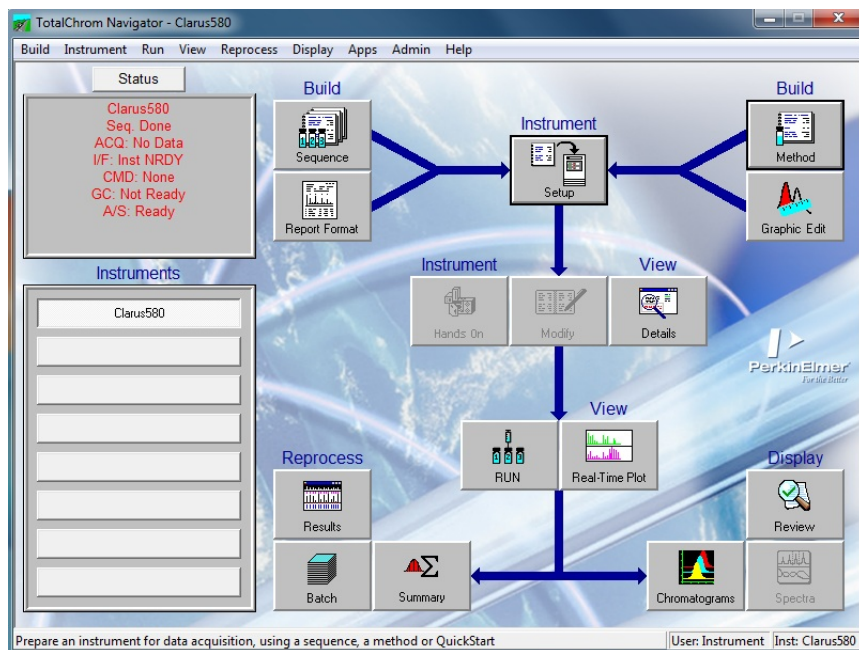
[\(-\)- \$\alpha\$ -Bisabolol GC sample preparation](#)



Wear gloves and eyeshields! Dodecane is hazardous to health (GHS08)

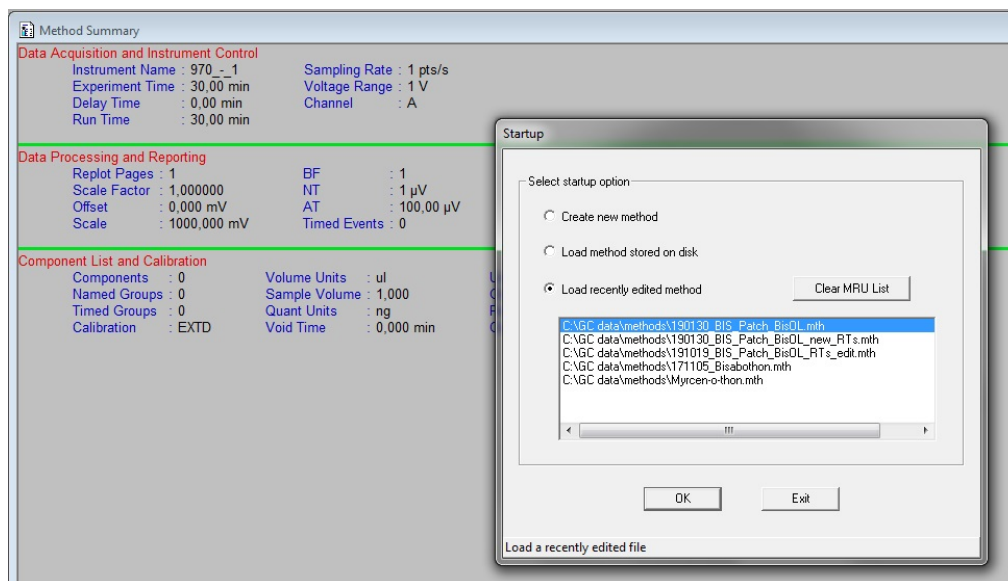
<http://www.sigmaaldrich.com/catalog/product/sial/297879?lang=en&ion=SE>

2

Open the Method Editor from TotalChrom Workstation

3 Define the GC settings in the Method editor

If not yet established - follow the 'Startup' wizard under 'Create new method' using the following settings



Startup wizard in Method editor

⇒ use the following settings

- Injection T = 250 °C
- Injection Vol.: 1 µL
- 1 min, 100 °C
- ramp 5 °C * min⁻¹ to 160 °C,
- 2 min hold,
- ramp 10 °C * min⁻¹ to 240 °C
- Carrier gas: N₂
- Flow rate: 50 mL * min⁻¹

Go to Step 4

4 Define Components

Go to 'Components' → 'New Component...' in the **Method Editor**

Define retention times (RT) of target compounds (e.g. bisabolene, bisabolol and patchoulol) and internal standard (IS) compound (here: β-caryophyllene). The RT and reasonable window settings might be slightly different, due to various factors like carrier gas quality, flow stability, or the wear of the inlet liner (incl. degree of its contamination).

- as for β-caryophyllene tick 'This component is an internal standard'
 - as for target compounds select β-caryophyllene as internal standard
 - save method settings and call method in the sequence setup (Step 5)
- a method file for sesquiterpenoid analysis is attached



In the example given below, three peaks for β -caryophyllene (BCP) are defined, because the commercial standard contains further isomers (>80% BCP purity). For IS calculation only the major peak (7.71 min) is used. The use of an alternative IS compound (e.g. α -humulene) is an option.

Setup for β -caryophyllene detection

Setup for (*E*)- α -bisabolene detection

Setup for (-)-patchoulol detection

Setup for (-)- α -bisabolol detection

☐ 190130_BIS_Patch_BisOL.mth

☐ 190130_BIS_Patch_BisOL.rpt

5

- open **Sequence Editor** from TotalChrom Workstation
- select 'Clarus 580'
- call Method (Step 4) of choice for Ch.A and select **base file name** and file path
→ the **base file name** is used for result (.RST) and raw data (.RAW) files
→ the file path should be fix as it will be important for data analysis (Step 8)

Spreadsheet:

- enter sample name into column 'Name'
- enter your samples numbers into column 'Number' (use alphanumeric numbers)
→ align sample numbers with vial numbers in column 'Vial'
→ the **'Smart Fill'** command is pretty helpful here
- the data path in columns 'Method' and 'Rpt Fmt File' (Report Format File) should be the same
→ the **Report Format File** will be crucial for a smooth data output
- double-check data path and **base file name** in column 'Data'
→ the **'Smart Fill'** command is pretty helpful here
- Save vial list as a sequence with .seq extension
- an .idx file will automatically be generated in parallel
- TotalChrom uses the **base file name** from the Setup dialog

Example .seq and .idx files are attached

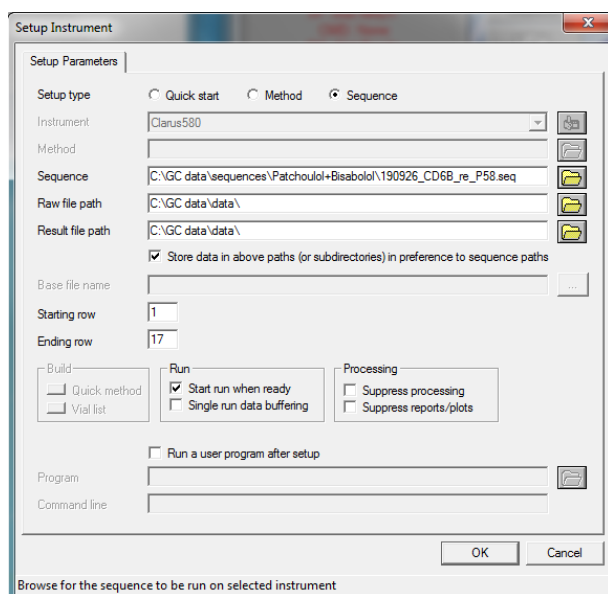
Sequence Information																		
Row	Type	Study name	Name	Note	Number	Vial	Method	Rpt Fmt File	Data	Colb Rpt	Col Level	Update RT	Sample Amt	Int Std Amt	Sample Vol	Dil Factor	Multiplier	Divisor
1	Sample	CellDeg	190705_CellDeg6B_Col-1		001	1	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
2	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		002	2	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
3	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		003	3	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
4	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		004	4	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
5	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		005	5	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
6	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		006	6	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
7	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		007	7	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
8	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		008	8	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
9	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		009	9	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
10	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		010	10	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
11	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		011	11	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
12	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		012	12	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
13	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		013	13	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
14	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		014	14	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
15	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		015	15	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
16	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		016	16	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
17	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		017	17	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
18	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		018	18	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
19	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		019	19	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
20	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		020	20	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
21	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		021	21	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
22	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		022	22	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
23	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		023	23	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
24	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		024	24	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
25	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		025	25	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
26	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		026	26	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
27	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		027	27	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
28	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		028	28	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
29	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		029	29	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
30	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		030	30	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
31	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		031	31	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
32	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		032	32	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
33	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		033	33	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
34	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		034	34	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
35	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		035	35	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000
36	Sample	CellDeg	190730_BIS_Peak_BioChrom_R11		036	36	method:190730_BIS_Peak_BioChrom_R11	Peaks_BioChrom_R11	c:\pgp\data\data\path\out\190705_CellDeg6B_Col-1				1.000000	1.000000	1.000	1.000000	1.000000	1.000000

Screenshot of a typical 'sequence' spreadsheet using TotalChrom software (Perkin Elmer)

☐ 190705_CellDeg6B_0h.seq ☐ 190705_CellDeg6B_0h.idx

6 Start the run

- Make sure that no 'Editor' window is open
→ you cannot simultaneously edit and setup a run
- Open the 'Setup' menu
- Select 'Sequence' as Setup type
- Select **sequence file (.seq)** from Step 5
- Select the same path for 'Raw files' and 'Result files'
→ make sure that it matches the path in the Sequence editor's 'Data' column (Step 5)
- Tick 'Start run when ready'
- Press 'OK' button exit Setup
→ run will start automatically :-)



- each sample will take ~30 min
- the process can be monitored by clicking the 'Real-Time Plot' button

7 Summary Report File

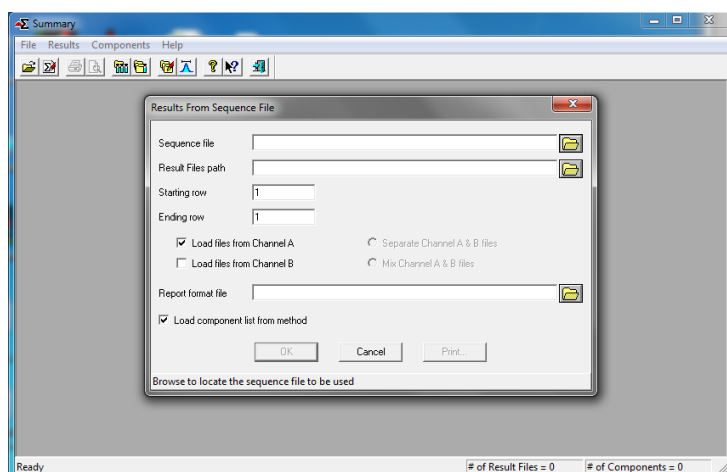
To generate a quantitative output file in **.csv format** for analysis (e.g. in excel), you first need to generate a '**Summary Report**' template:

- click the 'Summary' button in the main menu
 - click the 'edit format' button
 - the first two columns should be '1 File Name' and '2 Sample Name'
 - make sure that each component (including IS) is represented by each one column for:
 - retention time (Time [min]) and
 - peak area (Area [μ V·s])
 - Adjusted amount
 - a **template (.sum file)** for parallel analysis of (*E*)- α -bisabolene, (-)-patchoulol and (-)- α -bisabolol with β -caryophyllene (BCP) as internal standard is **attached**
- note that for the commercial BCP standard (>80%) three main peaks are annotated in that example, only one of which is used as **internal standard: RT = 7.71 min**

 Patch_BisOL.sum

8 Generate Output File

- Click 'Summary' button in main menu
- Select 'Results From Sequence File'
- Select Sequence File from Step 5
- Select 'Results File path' from Step 5
- Select Results Files path (according to path in Sequence file)
- Select 'Report format file' (.sum) from Step 7
- Go to 'Edit Format' → 'Options' → **tick 'Store report in an ASCII formatted file'**
 - select '**.CSV file**' for data analysis in Excel et al.



- a '**Summary Report**' table will be generated in .pdf format by default (see Fig. below)
- the creation of a .csv (ASCII) file is noted on the same sheet
- the .csv file can be imported into Excel via the 'Data' → 'From text' path

***** Summary Report *****

File Name	Sample Name	beta-Caryophyllene_6.1s			beta-Caryophyllene_7.71			beta-Caryophyllene_8.9			alpha-Bisabolene		
		Time [min]	Area [μV.s]	Adjusted Amount	Time [min]	Area [μV.s]	Adjusted Amount	Time [min]	Area [μV.s]	Adjusted Amount	Time [min]	Area [μV.s]	Adjusted Amount
bisabothon001.rst	wash	6,13	7502	4,98e-07	7,75	171893	0,00	8,98	15067	0,00	10,87	36	2,37e-06
bisabothon002.rst	wash	6,12	7905	5,04e-07	7,75	174084	0,00	8,95	14015	0,00	10,89	43	3,04e-06
bisabothon003.rst	wash	6,12	7398	5,22e-07	7,75	174388	0,00	8,98	14184	0,00	10,79	27	1,91e-06
bisabothon004.rst	wash	6,13	7046	4,98e-07	7,74	174122	0,00	8,95	14163	0,00	10,74	25	1,74e-06
bisabothon005.rst	0 μg-1	6,13	8848	4,80e-07	7,75	173946	0,00	8,95	14261	0,00	10,88	66	4,86e-06
bisabothon006.rst	25 μg-1	6,13	8722	4,43e-07	7,74	173375	0,00	8,95	15158	0,00	10,81	4773	3,15e-07
bisabothon007.rst	50 μg-1	6,13	8778	4,53e-07	7,75	173805	0,00	8,98	14945	0,00	10,81	9312	6,23e-07
bisabothon008.rst	100 μg-1	6,13	3476	2,28e-07	7,75	173958	0,00	8,98	15384	0,00	10,81	18198	0,00
bisabothon009.rst	150 μg-1	6,13	8573	4,29e-07	7,74	173804	0,00	8,95	15314	0,00	10,81	27049	0,00
bisabothon010.rst	200 μg-1	6,13	8593	4,19e-07	7,75	173878	0,00	8,98	15742	0,00	10,81	37488	0,00
bisabothon011.rst	0 μg-2	6,13	7456	5,30e-07	7,75	173249	0,00	8,98	14087	0,00	10,78	25	1,79e-06
bisabothon012.rst	25 μg-2	6,13	7428	4,88e-07	7,74	173972	0,00	8,98	15298	0,00	10,81	4899	3,07e-07
bisabothon013.rst	50 μg-2	6,12	12800	8,46e-07	7,74	172529	0,00	8,95	15123	0,00	10,81	9481	6,27e-07
bisabothon014.rst	100 μg-2	6,13	8657	4,41e-07	7,74	172082	0,00	8,95	15108	0,00	10,81	18202	0,00
bisabothon015.rst	150 μg-2	6,13	7257	4,66e-07	7,75	173528	0,00	8,98	15576	0,00	10,81	27105	0,00
bisabothon016.rst	200 μg-2	6,12	7235	4,81e-07	7,74	173506	0,00	8,95	15698	0,00	10,81	37119	0,00
bisabothon017.rst	0 μg-3	6,13	7840	5,05e-07	7,75	173557	0,00	8,98	15132	0,00	10,75	50	3,30e-06
bisabothon018.rst	25 μg-3	6,12	7112	4,65e-07	7,75	173421	0,00	8,95	15297	0,00	10,81	4804	3,01e-07
bisabothon019.rst	50 μg-3	6,13	10949	7,10e-07	7,75	173139	0,00	8,98	15424	0,00	10,81	9056	5,87e-07
bisabothon020.rst	100 μg-3	6,13	7970	5,10e-07	7,75	172890	0,00	8,98	15624	0,00	10,81	18539	0,00
bisabothon021.rst	150 μg-3	6,13	8879	4,29e-07	7,75	174003	0,00	8,98	16026	0,00	10,82	26904	0,00
bisabothon022.rst	200 μg-3	6,13	8861	5,45e-07	7,75	173594	0,00	8,95	15898	0,00	10,81	37542	0,00
Averages		6,13	7495	4,97e-07	7,75	173497	0,00	8,98	15114	0,00	10,79	13198	8,47e-07
%RSD		0,04	23	23,11	0,03	0	4,30	0,02	4	0,00	0,43	103	101,93

An ASCII file will be created



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