

# Gears MS Scan 1.0

# STARTING GUIDE



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# Contents

1. Tł	HE WORKFLOW	
1.1.	INPUT	5
1.2.	Processing	6
1.3.	PLUGINS	7
1.4.	DESIGN	
1.5.	Оитрит	9
2. Tł	HE OUTPUT FOLDER	
3. RI	ESULTS REVIEW WITH THE MGEARS VIEWER	

For researchers working with LCMS data in the field of metabolomics, a common task is to quantify the masses found within a certain retention time window. Such an analysis can be unexpectedly difficult in instrument vendor software, especially when the list of masses is long or the number of samples large. MS Scan is a plugin for Mnova Gears that has been developed to solve this problem, it automatically extracts mass spectra and corresponding chromatograms from a batch of TIC (total ion current) files. The software:

- reads a TIC from an input document
- extracts up to three mass spectra in the previously set Retention Time ranges
- it then reads the peaks, extracts the chromatograms, and quantifies peaks for each of the masses that pass the specified threshold.

MS Scan generates automatic reports with results that can be easily reviewed and edited via the Mgears viewer.

This document is intended to help you configure and run a typical MS Scan analysis. For more details about Mgears options, please refer to the Mgears manual.

So, let's get you started!

#### **1. The Workflow**

Before starting your analysis, go to Mnova **File** > **Preferences** > **Mass** to configure the mass analysis preferences. MS Scan creates quite a lot of graphics, so it is highly recommended to save the Mnova results with the minimum possible "weight".

To do so, in the **General** tab > **Saving Options**, check the **Item Only** option to save the minimum data required.

$\left( \leftarrow \right)$	O Preferences	? ×
New	General Riveries Import Database NMR Mars Malasile Science Drawing Tech	Publications
Recent	General Plug-ins · Database Nivirk Wass Molecule scripting Drawing loois	Publications
<ul> <li>Save</li> <li>Save As</li> <li>Export to PDF</li> <li>Save To</li> </ul>	General       Setup       Import       Verify         Saving Options       Item Only       Crosshairs Behavior:         Item Only       Item Only       Item Compression         Your choice will affect only to newly imported items.       Live Mass Spectrum Preview	
<ul> <li>Open</li> <li>Open Directory</li> <li>Open From</li> </ul>	Scales Behavior:       Isotope Cluster Selection         Image: Synchronized Chromatogram's Time Scale       Minimum Intensity Ratio: 0.0100 *         Maximum Distance:       10.000 Da *         Image: Relative Macross Visibility       Relative Macross Visibility	
🖶 Print 👔 Page Setup		
Help Preferences Advanced Plug-ins		
() Exit	OK Save 👻 Load.	Cancel



In the **Setup** tab, uncheck the **Open TAC** option to avoid opening the PDA total absorbance chromatogram at the top of each document.

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General Plug-i	ns Import	Database	NMR	Mass	Molecule	Scripting	Drawing Tools	Publications
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Selab which data to	show when loadir	ng a new mass it	em					+
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Now launch Mgears from the **Tools** ribbon.

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File Ho	me V	iew Mo	olecule	Predictio	on Tools	Data Ar	nalysis	Database	Ver	ification	Elucidatio	n Chemometrics	Tools	Analysis	Quantitation
*	5	G		-	1	63	M	00		4			Screen	Screen	
Chemical Shift Perturbation	t Edit Script	Run Script	Stop Script	Import E	port Report	Loaded Scripts <sub>*</sub>	NMR Tools 🗸	Mgears	Mgears Viewer	MyGears	MyGears Workflows	Reaction Monitoring	Mnova Screen 1H	Mnova Screen 19F	View Mnova Screen Result
Binding		Scripts			Script	Tools			N	lgears		Reaction Monitoring		Screen	



The MS Scan analysis follows the general Mgears workflow based on the five-step setup.

#### 1.1. Input

First, in the **Input** tab, select the data files for analysis by Mgears. Input files can come from **Disk** directories, a database (**DB**), or from **Real-Time** acquisition. These modes are detailed in the Mnova Gears manual.

In this guide, we will use data from a **Disk** directory. So, in the **Disk** section, click on .... and choose the directory from which your MS data files will be retrieved.

9	nova Gears	?	×	
•	Input 🥂 Processing 🐞 Plugins 🖺 Design 📑 Output 🔅 Settings			
1	2 Automatic Detection			
8	Main Directory: C:/Users/Usuario/Desktop/Datasets/TIC		<u></u>	5
	Optional Directory:		V	М

Use the Automatic Detection mode, with which Mgears will use the saved masks to find experiment files.



**Watch out!** Your dataset masks ("MSD1.MS" in this example) must be correctly configured to be recognized by Mgears. Go the **Settings** tab and click on the **Mask Manager** button to view current masks. If your file mask is not entered, you can simply type it and click on **OK** to save the changes.

<ul> <li>Mnova Gears</li> <li></li></ul>	Design Output Settings	?	×
General Masks Manager Retry on Error Opening Dataset  Autoresume on Critical Error (Not Available in D Allow Events Auto Attach Traces Verbose Log Expert Debug Mode (Execute All Process in Foregrour Debug Log CSV Separator ;  Notifications	Masks For Automatic Detection  Masks For Automatic Detection  MMR:  fid, ser, *.jdf, *.mnova, *.exp  Raw NMR:  Ir, 2rr  GC/LC/MS:  FUNC001.DAT, MSD1.MS, analysis.baf, _FU  Chromatography:  UV/IR/Raman/Fluorescence:  ,jdx, *.spa, *.spc  Structures:  *.mol, *.sdf Back to Default		¥
▶ Resume 👔 Load Settings 👔 Save Settings	OK Cancel	Cancel	Run



Going back to the **Input** section, you can click on **Experiments Selection** and check the **GC/LC/MS** experiment type to avoid Mgears attempting to recognize and analyze other types of experiment files.

<b>*</b>	Inova Gears	?	×
+	Input 🥂 Processing 🐞 Plugins 🖺 Design 📑 Output 🔅 Settings		
Real Time	Automatic Detection       Experiments Selection         Main Directory:       C:/Users/Usuario/Desktop/Datasets/TIC         Optional Directory:		
_	Advanced Options		
	Allow More than One Experiment of Each Type 🗌 Join Folders with Similar Name	_	
	Experiment Selection Mode:     FID     From:     31 Dec 1969     To:     31 Dec 1969       Preview Subset Only     1000 <ul> <li>Regular Expression to Adapt Match /</li> <li>Regular Expression to Adapt Match /</li></ul>	•	Ø
Filter	Filtering String:       Regular Expression Filter b         Mapping File:       V Substring N	oy Name Natching	Only

If needed, configure the **Advanced Options** or use the **Filtering Options** to refine the detection of your input data files.

**Top Tip!** Before moving to the next step, you can use the **Automatic inspection** button **a** to check if the detected experiments correspond to your selection criteria.

😵 Experiments Found: 2		?	×
List of detected experiments:			
Experiment: SMPL1 MS: C:/Users/Usuario/Desktop/Dat	asets/TIC/SMPL1/MS	SD1.MS	
Experiment: SMPL2 MS: C:/Users/Usuario/Desktop/Dat	asets/TIC/SMPL2/ms	sd1.ms	•
	ОК	Canc	el

#### 1.2. Processing

In the **Processing** tab, you can upload a processing script to apply your own customized processing settings (please refer to the <u>Mnova Gears manual</u> for more details about this option).



#### 1.3. Plugins

In the **Plugins** tab, select and add the MS Scan plugin.



The added plugin will appear on the right-hand side of the dialog. You should now click on **MS Scan Plugin Settings** to configure the **Analysis** and **Output** parameters.

🎭 Mnova Gears	?	>	<
Finput N Processing Plugins E Design Output Settings			
SQA   Verify   Concentration   Purity   DB Search   SMA   SMA   QC Profiling   Multiplet Report   Multiplet Report   New   LUPAC Name   MS Scan   Peak Report   Peak Report   Pack Report   B 2ADF Converter   Multiplet Report   Multiplet Report   Multiplet Report   MS Scan   Multiplet Report   Multiplet Report   MS Scan   Multiplet Report   Multiplet Report   Multiplet Report   MS Scan   Multiplet Report   Multiplet Report   Multiplet Report   MS Scan   Plugin Settings			
▶ Resume       Image: A constraint of the second section of the second sec	ancel	C R	un



A **Settings** dialog will appear. In the **Analysis** tab, you should choose the mass spectrum **Retention time (RT)** ranges from which you want to extract chromatograms. You can enter up to three RT ranges.

You should also define the Minimum Relative Abundance % for the Mass spectrum extraction.

You can then choose the **Tolerance** and **Minimum Area Threshold** for the peaks of the mass chromatogram extraction.

🐄 Settings	?	$\times$
Analysis Output		
Mass Spectrum Extraction		
✓ RT from: 1.00 <sup>+</sup> to: 3.00 <sup>+</sup> min		
✓ RT from: 3.00 <sup>↑</sup> to: 5.00 <sup>↑</sup> min Minimum Relative Abundance: 5.00	% ‡	
RT from: 10.00 <sup>+</sup> to: 15.00 <sup>+</sup> min		
Mass Chromatogram Extraction		
Tolerance: 0.250 <sup>+</sup> Da • Minimum Area Threshold: 10.00	% 🗘	
OK	Cance	el

In the **Output** tab, you can configure the variables to display in the CSV and HTML result files that will be automatically generated by Mgears. The **RT**, **m/z**, and **Total Relative Area** of the peaks are included in the reports by default, whereas other parameters, such as **Relative area**, peak **Height**, etc., are entirely optional.

🎭 Settings			?	×
Analysis Output				
✓ RT	✓ Rel Area (%)	✓ Rel Height (%)		
✓ m/z	✓ Area (Abs)	Scan		
✓ Total Rel Area (%)	Height (Abs)	Start/End Time		
		ОК	Cance	!

#### 1.4. Design

In the **Design** tab, you can upload an Mnova layout templates or a Script to produce a final customized report.

4	🎭 Mnova Gears	?	×			
	🔶 Input 🕂 Processing 🐞 Plugins 🖺 Design 📑 Output 🔅 Settings					
Layout Template: C:/Users/Usuario/Desktop/Datasets/MS Scan template.mnova						
	Script:					
	Autoliceu		>			



### 1.5. Output

In the **Output** tab, click on ..... and choose a directory in which to save your analysis results.

🎭 Mnova G	ears	?	$\times$
🔶 Input	N Processing 🏟 Plugins 🖺 Design 📑 Output		
Disk			
Directory:	C:/Users/Usuario/Desktop/Results/MS Scan		
			/^2

As for all Mgears plugins, you can also choose to create an Mnova or PDF document with your results and save your results to a connected DB.

### 2. The output folder

The results folder, which is named according to the date and time at which your analysis was run, is saved under the directory specified previously. This folder contains all the output generated in the current evaluation.





MS Scan results and analysis parameters are reported:

• in a CSV document:

Tolerance [Da]	Minimum Relative Abudance [%]	Minimum Area Threshold [%]			
0.25	5	10			
m/z [Da]	RT [min]	Total Rel Area [%]	Rel Area [%]	Abs Area	Rel Height [%]
537.3	1.28	10.09	100	14523	100
552.2	1.96	70.13	100	100985.5	100
568	1.4	0.84	73.99	1216	58.06
568	2.66	0.3	26.01	427.5	41.94
614	1.76	16.77	100	24147.5	100
537.3	4.24	0.14	48.38	198.112	49.36
537.3	4.66	0.15	51.62	211.4	50.64
552.2	3.2	0.11	28.65	162	17.4
552.2	3.48	0.08	20.34	115	18.62
552.2	4.46	0.13	32.63	184.5	24.41
552.2	4.5	0.07	18.39	104	39.56
568	3.94	0.05	10.94	75	50.08
568	4.14	0.42	89.06	610.5	49.92
614	3.22	0.16	22.79	234.5	25.58
614	3.66	0.24	33.14	341	31.82
614	3.9	0.19	27.11	279	21.87
614	4.2	0.12	16.96	174.5	20.73

• and in an HTML file:

# Mgears MSScan Results

#### Parameters

Parameter	Value
Results Directory	C:/Users/Usuario/Desktop/Results/MS Scan/2021-03-12T09.59.15
Started On	2021-03-12T09:59:15
Completed On	2021-03-12T09:59:20
Minimum Relative Abundance	5%
Tolerance	0.25 Da
Minimum Area Threshold	10.00 %

#### **Detailed Results**

Show 100 v entries		Сору	CSV	Columns	PDF	Print	\$	Search:	
Output csv	Output mnova	m/z [Da] 🔅	RT [I	min] 🍦 To	tal Rel A	rea [%] 🔅	Rel Area [%] 🔶	Abs Area	Rel Height [%] 🕴
MSScanResults_SMPL1.csv	SMPL1.mnova	37.3000	1.28	10.0	9		100.00	14523.000	100.00
MSScanResults_SMPL1.csv	SMPL1.mnova 5	52.2000	1.96	70.1	13		100.00	100985.500	100.00
MSScanResults_SMPL1.csv	SMPL1.mnova	68.0000	1.40	0.84	1		73.99	1216.000	58.06
MSScanResults_SMPL1.csv	SMPL1.mnova 5	68.0000	2.66	0.30	)		26.01	427.500	41.94
MSScanResults_SMPL1.csv	SMPL1.mnova	14.0000	1.76	16.7	77		100.00	24147.500	100.00
MSScanResults_SMPL1.csv	SMPL1.mnova	37.3000	4.24	0.14	1		48.38	198.112	49.36
MSScanResults_SMPL1.csv	SMPL1.mnova	37.3000	4.66	0.15	5		51.62	211.400	50.64
MSScanResults_SMPL1.csv	SMPL1.mnova	52.2000	3.20	0.11	1		28.65	162.000	17.40
MSScanResults_SMPL1.csv	SMPL1.mnova 5	52.2000	3.48	0.08	3		20.34	115.000	18.62
MSScanResults_SMPL1.csv	SMPL1.mnova	52.2000	4.46	0.13	3		32.63	184.500	24.41
MSScanResults_SMPL1.csv	SMPL1.mnova	52.2000	4.50	0.07	7		18.39	104.000	39.56

Each row in the CSV and HTML reports represents an extracted chromatogram peak with its m/z, RT, and Total Rel Area (%). Rel Area (%), Abs Area, and Rel Height (%) are also displayed for each peak, as previously defined in the plugin settings in this example.

You will also find links to the CSV and Mnova results in the HTML report.

The advantage of this type of report is that it allows you to change the display of the **Columns** and adapt them to your preferences, then **Copy**, **Print**, or save your results into **CSV** or **PDF** format.

C	сору	CSV	Columns	PDF	Print
m/z [	Da] 🍦	RT [r	Output cs	v	[%] 🔶
537.30	000	1.28	Output m m/z [Da]	nova	
552.20	000	1.96	RT [min]		
568.00	000	1.40	Total Rel. Rel Area	Area [%] [%]	
568.00	000	2.66	Abs Area	[,0]	
614.00	000	1.76	Rel Heigh	nt [%]	

#### 3. Results review with the Mgears Viewer

MS Scan is compatible with the **Mgears Viewer**, which allows you to review your results. Start this example by opening the **Mgears Viewer** from the **Tools** section in Mnova:

M 🔚 🔿	C+ 🖶												Μ	lestReNova	М	ASS	Y L
File I	Home	View	Mole	ecule	Predicti	on	Tools	Data Ar	nalysis	Database	Ver	ification	Elucidatio	n Chemometrics	Tools	Analysis	Quantitation
1	5	2	Q					w		00		4			Screen	Screen	
Chemical SI	hift Ed	lit I	Run	Stop	Import E	xport	Report	Loaded	NMR	Mgears	Mgears	MyGears	MyGears	Reaction	Mnova	Mnova	View Mnova
Binding	on sci	Sc	ripts	Script	•	•	Script	Fools	10015 -		M	gears	WOIKIIOWS	Reaction Monitoring	Screen In	Screen	Screen Result

Click on 🔎 and select the MS Scan analysis output folder.

Mgears Viewer	
<b>-</b>	



The experiment results will be loaded in the **Mgears Viewer** dialog. Select a sample in the table or well plate. The detailed results will appear in the **Results** section, and the corresponding Mnova document will open.

Mg	ears	Viewer					8×
Γ			🌣 🛃 🎟				Analyze Again
	#	Title	Document			Locatio	on 🔺
1			SMPL1.mnova	C:/Users/Usuari	o/Desktop/R	esults/MS Scan/2021	-03-12T09.59
2 ∢		SMPL2 S	SMPL2.mnova	C:/Users/Usuari	o/Desktop/R	esults/MS Scan/2021	-03-12T09.59 👻
	Set	Mass Spectrum E Minimum Rela	Extraction	Mass C : 5 % Toler	hromatogram ance : 0.25 Da	Extraction —	r <b>eshold :</b> 10 %
		RT ranges:			from 1 to 3 r	nin	<b>_</b>
		m/z:			EIC 2: 552.2	000 Da	<b>*</b>
		m/z [Da]	RT [min]	Total Rel	Area [%]	Rel Area [%]	Abs Area
		1 552.2000	1.96	70.13		100.00	100985.500
		4					



In the **Results** section, you can select the **RT range** and **m/z** you want to inspect. Detailed results are displayed in the table below, and the corresponding extracted-ion chromatogram (EIC) plot is set as active in the Mnova document.

- 1			911			°.;;		15		Analysis result
		] 🗘 🕅 🎟				Analyze Again		C:\Users\UsuarC\S	MPL1\MSD1.MS Injection 1 Fu	unction 1 (1000) TIC
#	Title	Document			Location		20000-	1.960 1.759		
	SMPL1	SMPL1.mnova	C:/Users/Usuario/[	Desktop/Results/MS Sca	n/2021-03-121	T09.59	1	.0 1.2 1.4 1.6 1.8 2	2.0 2.2 2.4 2.6 2.8 3.0 3.2 3 Retention time (mi	.4 3.6 3.8 4.0 4.2 4.4 4.6 4.8 in)
	SMPL2	SMPL2.mnova	C:/Users/Usuario/[	Desktop/Results/MS Sca	n/2021-03-121	T09.59 👻	l l j	C:\Users\UsuarC\S 552.200	MPL1\MSD1.MS Injection 1 Fu	unction 1 (1000) MS + spectrum
							70000-	537.300 100.00% 30.15%	568.000 19.76%	36.76%
Set	tings Mass Spectrur	n Extraction	Mara Char				4000-	1.278 1.00.00%	MPLI (MSDI.MS Injealon I Pu	unction 1 (1000) EIC 537.3 Da
Res	Minimum Re	elative Abundance	::5% Mass Chro	ce : 0.25 Da Minimum An	ea Threshold	: 10 %	20000-	0 1.2 1.4 1.6 1.8 2 C:\Users\UsuarC\S 1.960 100.00%	2.0 2.2 2.4 2.6 2.8 3.0 3.2 3 Retention time (mi MPL1\MSD1.MS Injection 1 Fu	1,4 3,6 3,8 4,0 4,2 4,4 4,6 4,8 in) unction 1 (1000) EIC 552.2 Da
Res	Minimum Re ults RT ranges:	elative Abundance	e : 5 % Mass Chro	ce : 0.25 Da Minimum Ar	ea Threshold	: 10 %	20000-	0 1.2 1.4 1.6 1.8 2 C:\Users\UsuarC\S 1.960 100.00%	2.0 2.2 2.4 2.6 2.8 3.0 3.2 3 Retention time (mi MPL1\MSD1.MS Injection 1 Fu 0.0 2.2 2.4 2.6 2.8 3.0 3.2 3 Retention time (mi	14 3.6 3.8 4.0 4.2 4.4 4.6 4.8 in) inction 1 (1000) EIC 552.2 Da
Res	Minimum Ra ults RT ranges: m/z: m/z [D	a) RT [min]	r: 5 % Tolerand	rom 1 to 3 min IC 2: 552.2000 Da	ea Threshold	: 10 %	0-1 20000-1 0-1 1 270- 180-	0 1.2 1.4 1.6 1.8 2 :\Users\UsuarC\S 1.960 100.00% 0 1.2 1.4 1.6 1.8 2 :\Users\UsuarC\S 23.99%	20 22 24 2.6 2.8 30 3.2 3 Retention time (m MPL1/MSD1.MS Injection 1 Fu 2.0 2.2 2.4 2.6 2.8 30 3.2 3 Retention time (m MPL1/MSD1.MS Injection 1 Fu MUMANANANANANANANANANANANANANANANANANANA	14 3.6 3.8 4.0 4.2 4.4 4.6 4.8 in) unction 1 (1000) EIC 552.2 Da 4 3.6 3.8 4.0 4.2 4.4 4.6 4.8 in) unction 1 (1000) EIC 568 Da MMMMMMMMMMMMMM
Res	Minimum R ults RT ranges: m/z: m/z [D 1 552.2000	a) RT [min]	Tolerand	rom 1 to 3 min IC 2: 552.2000 Da IC 1: 537.3000 Da IC 2: 552.2000 Da IC 3: 568.0000 Da IC 4: 614.0000 Da	Abs 100985	: 10 %	0- 1 20000- 0- 1 1 270- 180- 1 3000-	0 1.2 1.4 1.6 1.8 2 :(Users)(Usuar(SS 1.560) 10000% 0 1.2 1.4 1.6 1.8 2 :(Users)(Usuar(SS 1.580) 0 1.2 1.4 1.6 1.8 2 :(Users)(Usuar(SS 1.759) 100.00%	20 2:2 2:4 2:6 2:8 3:0 3:2 3 Retention time (m) MPL1(MSD1MS Injection 1 Fo 2:0 2:2 2:4 2:6 2:8 3:0 3:2 3 Retention time (m) MPL1(MSD1MS Injection 1 Fo 2:0 2:2 2:4 2:6 3:0 3:2 3 Retention time (m) MPL1(MSD1MS Injection 1 Fo	14 3.6 3.8 4.0 4.2 4.4 4.6 4.8 in) inction 1 (1000) EIC 552.2 Da 14 3.6 3.8 4.0 4.2 4.4 4.6 4.8 in) inction 1 (1000) EIC 568 Da MMM MMM MMM MMM MMM 14 3.6 3.8 4.0 4.2 4.4 4.6 4.8 in) inction 1 (1000) EIC 614 Da

You can review your results, change peaks colors, etc., using the standard tools from Mnova, then save the changes by clicking on **Save**  $\square$ .

Note that, in this version of MS Scan (1.0), the **Analyze Again** option is not available, therefore a recalculation cannot be performed.

For more details on the Mnova Gears options, please refer to the Mnova Gears manual.