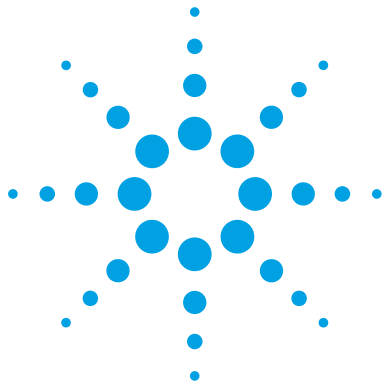




Comprehensive Test Mix for MassHunter Forensics and Toxicology Triggered MRM Database and Library

Method Setup Guide

- Step 1. Set up the LC part of the method 2
- Step 2. Set up LC/MS ion source parameters 3
- Step 3. Set up the MRM method 5
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NOTE

The Comprehensive Forensics and Toxicology Test Mix is included with the G1734BA Forensics and Toxicology Triggered MRM Application Kit.

Agilent does not provide the actual acquisition methods to use with the Comprehensive Test Mix, due to the large number of instrument configurations that are possible.

Instead, Agilent provides this guide to explain how to create MRM methods that are used to create dMRM and tMRM methods for the test mix.

You will copy the values from the file **MRM_Methods_ForTox.xlsx**, found on the *Support Disc*, to set up your MRM methods.

Before you begin, make sure that your system meets the installation requirements that are described in the *MassHunter Forensics and Toxicology Triggered MRM Database and Library Quick Start Guide*.

For more detailed instructions, see the *Quick Start Guide* for this database, and the *MassHunter Data Acquisition for 6400 Series Triple Quadrupole LC/MS Familiarization Guide* and *online Help*.



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Step 1. Set up the LC part of the method

Step 1. Set up the LC part of the method

1 Set up the solvent.

This step is identical for all LC configurations.

- Solvent A: 5 mM ammonium formate in 0.01% formic acid in water
- Solvent B: 0.01% formic acid in methanol

2 Set up the gradient.

The gradient setup is dependent upon the LC configuration. Some examples follow.

1290 Infinity LC system

1290 Infinity LC system with Agilent Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm ZORBAX LC column (p/n 959758-902), included in the G1734BA Forensics and Toxicology Triggered MRM Application Kit.

Time [min]	△	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00		95.00	5.00	0.400	1200.00
0.50		95.00	5.00	---	---
1.50		70.00	30.00	---	---
6.50		40.00	60.00	---	---
9.00		5.00	95.00	---	---

Stop time is 9 minutes with a post time of 3.5 minutes.

1260 Infinity LC system

The 1260 Infinity LC system can have a lower backpressure (up to 600 bar) and a higher dead volume than the 1290 Infinity LC system.

Time [min]		A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00		95.00	5.00	0.400	600.00
1.50		95.00	5.00	---	---
2.00		70.00	30.00	---	---
8.50		40.00	60.00	---	---
11.00		5.00	95.00	---	---

Stop time is 11 minutes with a post time of 3.5 minutes.

These settings are optimized over the whole Comprehensive Test Mix. For best sensitivity of SubMix 5, use pure water and methanol in negative mode.

Step 2. Set up LC/MS ion source parameters

- Set up the ion source parameters in the MS part of the method.

For a multi-component method, the ion source parameters shown in the next figure are used to achieve the best overall sensitivity for all of the compounds in the Comprehensive Test Mix. You can make adjustments to optimize for individual compounds or submixes.

6460 LC/MS Ion source parameters for the 6460 LC/MS instrument:

Source parameters			
Gas Temp:	350	°C	
Gas Flow:	6	l/min	
Nebulizer:	40	psi	
Sheath Gas Temp:	375	°C	
Sheath Gas Flow:	11	l/min	
	Positive	Negative	
Capillary:	3500 V	3000 V	
Nozzle Voltage:	0 V	0 V	

Step 2. Set up LC/MS ion source parameters

6490 LC/MS Ion source parameters for the 6490 LC/MS instrument:

Acquisition	Source	Chromatogram	Instrument
Source parameters			
Gas Temp: 120 °C			
Gas Flow: 15 l/min			
Nebulizer: 35 psi			
Sheath Gas Temp: 375 °C			
Sheath Gas Flow: 12 l/min			
Positive Negative			
Capillary: 3500 V 3000 V			
Nozzle Voltage: 300 V 500 V			
iFunnel parameters			
Positive Negative			
High Pressure RF 150 V 90 V			
Low Pressure RF 60 V 60 V			
Copy Paste			
Paste to All Segments			

The ion source parameters shown for the 6490 LC/MS also include the iFunnel parameters. These iFunnel parameters ensure the best overall sensitivity for all of the compounds in the Comprehensive Test Mix. You can use the Source and iFunnel Optimizer program to optimize for individual compounds or submixes. Refer to the MassHunter Data Acquisition for 6400 Series Triple Quadrupole LC/MS *Familiarization Guide* and *online Help*.

Step 3. Set up the MRM method

- 1 From the *Support Disc*, open the file **MRM_Methods_ForTox.xlsx**.

This spreadsheet file contains ten tabs, **SubMix 1** through **SubMix 10**, one for each of the ten standard mixes in the Comprehensive Test Mix.

	A	B	C	D	E	F	G	H	I	J	K	L
1	MRM											
2	Compound Group	Compound	ISTD?	Precursor MS1 Res	Product Ion MS2 Res	Dwell	Fragment	Collision E	Cell Accel	Polarity		
3	SubMix 1	HU-210	FALSE	387.3 Unit	43.1 Unit	10	151	56	3	Positive		
4	SubMix 1	HU-210	FALSE	385.3 Unit	367.2 Unit	10	285	24	3	Negative		
5	SubMix 1	HU-210	FALSE	385.3 Unit	301.2 Unit	10	285	36	3	Negative		
6	SubMix 1	JWH-200	FALSE	385.2 Unit	155 Unit	10	184	20	3	Positive		
7	SubMix 1	JWH-200	FALSE	385.2 Unit	114.1 Unit	10	184	28	3	Positive		
8	SubMix 1	JWH-018	FALSE	342.2 Unit	155 Unit	10	199	24	3	Positive		
9	SubMix 1	JWH-018	FALSE	342.2 Unit	127 Unit	10	199	52	3	Positive		
10	SubMix 1	JWH-250	FALSE	336.2 Unit	121 Unit	10	171	20	3	Positive		
11	SubMix 1	JWH-250	FALSE	336.2 Unit	91.1 Unit	10	171	52	3	Positive		
12	SubMix 1	CP 47,497	FALSE	331.3 Unit	313.2 Unit	10	247	24	3	Negative		
13	SubMix 1	CP 47,497	FALSE	331.3 Unit	259.2 Unit	10	247	32	3	Negative		
14	SubMix 1	JWH-073	FALSE	328.2 Unit	155 Unit	10	189	24	3	Positive		
15	SubMix 1	JWH-073	FALSE	328.2 Unit	127 Unit	10	189	52	3	Positive		
16	SubMix 1	CP 47,497	FALSE	317.2 Unit	299.2 Unit	10	232	24	3	Negative		
17	SubMix 1	CP 47,497	FALSE	317.2 Unit	245.2 Unit	10	232	32	3	Negative		
18	SubMix 1	CBN Cann	FALSE	311.2 Unit	293.2 Unit	10	126	16	3	Positive		
19	SubMix 1	CBN Cann	FALSE	311.2 Unit	223 Unit	10	126	20	3	Positive		
20	SubMix 1	CBN Cann	FALSE	309.2 Unit	279.1 Unit	10	209	32	3	Negative		
21	SubMix 1	CBN Cann	FALSE	309.2 Unit	222 Unit	10	209	48	3	Negative		
22	SubMix 1	Ketamine	FALSE	238.1 Unit	179.1 Unit	10	103	12	4	Positive		
23	SubMix 1	Ketamine	FALSE	238.1 Unit	125 Unit	10	103	28	4	Positive		
24	SubMix 1	HU-210	FALSE	387.3 Unit	71.1 Unit	10	151	24	3	Positive		
25												
26												
27												
28												
29												
30												
31												
32												
33												
34												
35												
36	SubMix 1	SubMix 2	SubMix 3	SubMix 4	SubMix 5	SubMix 6	SubMix 7	SubMix 8	SubMix 9	SubMix 10		
Ready Average: 127.0363636 Count: 277 Sum: 1												

- 2 Open the MassHunter Data Acquisition program.

Step 3. Set up the MRM method

3 In the Method Editor window, click **QQQ > Acquisition**.

Acquisition | Source | Chromatogram | Instrument

Scan segments

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Compound1		☐	350 Unit		200 Unit		200	135	0	7	Positive

Time segments

#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
1	0	MRM	To MS	200	0	☒

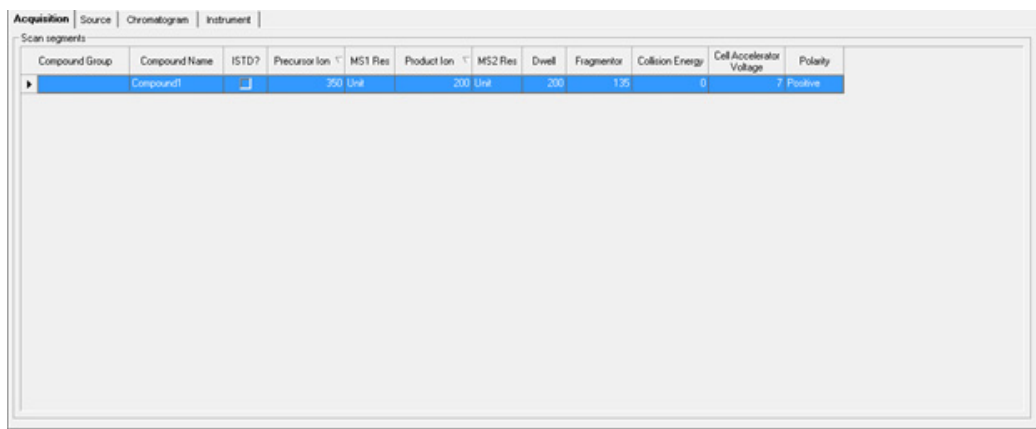
4.91 cycles/s 203.5 ms/cycle

4 In the spreadsheet file, in the **SubMix 1** tab, select all of the cells that contain MRM information. Make sure that you select the two header rows. *Do not select the entire table!*

	A	B	C	D	E	F	G	H	I	J	K	L
1	MRM											
2	Compound Group	Compound	ISTD?	Precursor	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
3	SubMix 1	HU-210	FALSE	387.3 Unit		43.1 Unit		10	151	56	3	Positive
4	SubMix 1	HU-210	FALSE	385.3 Unit		367.2 Unit		10	285	24	3	Negative
5	SubMix 1	HU-210	FALSE	385.3 Unit		301.2 Unit		10	285	36	3	Negative
6	SubMix 1	JWH-200	FALSE	385.2 Unit		155 Unit		10	184	20	3	Positive
7	SubMix 1	JWH-200	FALSE	385.2 Unit		114.1 Unit		10	184	28	3	Positive
8	SubMix 1	JWH-018	FALSE	342.2 Unit		155 Unit		10	199	24	3	Positive
9	SubMix 1	JWH-018	FALSE	342.2 Unit		127 Unit		10	199	52	3	Positive
10	SubMix 1	JWH-250	FALSE	336.2 Unit		121 Unit		10	171	20	3	Positive
11	SubMix 1	JWH-250	FALSE	336.2 Unit		91.1 Unit		10	171	52	3	Positive
12	SubMix 1	CP 47,497	FALSE	331.3 Unit		313.2 Unit		10	247	24	3	Negative
13	SubMix 1	CP 47,497	FALSE	331.3 Unit		259.2 Unit		10	247	32	3	Negative
14	SubMix 1	JWH-073	FALSE	328.2 Unit		155 Unit		10	189	24	3	Positive
15	SubMix 1	JWH-073	FALSE	328.2 Unit		127 Unit		10	189	52	3	Positive
16	SubMix 1	CP 47,497	FALSE	317.2 Unit		299.2 Unit		10	232	24	3	Negative
17	SubMix 1	CP 47,497	FALSE	317.2 Unit		245.2 Unit		10	232	32	3	Negative
18	SubMix 1	CBN Cann	FALSE	311.2 Unit		293.2 Unit		10	126	16	3	Positive
19	SubMix 1	CBN Cann	FALSE	311.2 Unit		223 Unit		10	126	20	3	Positive
20	SubMix 1	CBN Cann	FALSE	309.2 Unit		279.1 Unit		10	209	32	3	Negative
21	SubMix 1	CBN Cann	FALSE	309.2 Unit		222 Unit		10	209	48	3	Negative
22	SubMix 1	Ketamine	FALSE	238.1 Unit		179.1 Unit		10	103	12	4	Positive
23	SubMix 1	Ketamine	FALSE	238.1 Unit		125 Unit		10	103	28	4	Positive
24	SubMix 1	HU-210	FALSE	387.3 Unit		71.1 Unit		10	151	24	3	Positive

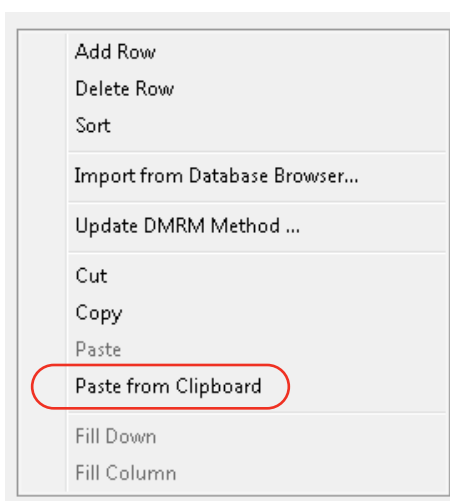
Step 3. Set up the MRM method

- 5 Copy the selected cells. (Press **Ctrl+C** or use the Copy command).
- 6 In the MassHunter Data Acquisition program, in the first line of the **Scan segments** table, click the leftmost column to select the first line.



Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Compound1		350 Unit		200 Unit		200	135	8		7 Positive

- 7 Right-click and click **Paste from Clipboard**.



Step 3. Set up the MRM method

The Scan segments table for instruments that are not equipped with iFunnel technology looks similar to the next figure.

Compound Group	Compound Name	ISTD?	Precursor Ion ▾	MS1 Res	Product Ion ▾	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Compound1	☐	350	Unit	200	Unit	200	135	0	7	Positive
SubMix 1	HU-210	☐	387.3	Unit	43.1	Unit	10	151	56	3	Positive
SubMix 1	HU-210	☐	385.3	Unit	367.2	Unit	10	285	24	3	Negative
SubMix 1	HU-210	☐	385.3	Unit	301.2	Unit	10	285	36	3	Negative
SubMix 1	JwH-200	☐	385.2	Unit	155	Unit	10	184	20	3	Positive
SubMix 1	JwH-200	☐	385.2	Unit	114.1	Unit	10	184	28	3	Positive
SubMix 1	JwH-018	☐	342.2	Unit	155	Unit	10	199	24	3	Positive
SubMix 1	JwH-018	☐	342.2	Unit	127	Unit	10	199	52	3	Positive
SubMix 1	JwH-250	☐	336.2	Unit	121	Unit	10	171	20	3	Positive
SubMix 1	JwH-250	☐	336.2	Unit	91.1	Unit	10	171	52	3	Positive
SubMix 1	CP 47,497-C8 homolc	☐	331.3	Unit	313.2	Unit	10	247	24	3	Negative
SubMix 1	CP 47,497-C8 homolc	☐	331.3	Unit	259.2	Unit	10	247	32	3	Negative
SubMix 1	JwH-073	☐	328.2	Unit	155	Unit	10	189	24	3	Positive
SubMix 1	JwH-073	☐	328.2	Unit	127	Unit	10	189	52	3	Positive

The Scan segments table for instruments that are equipped with iFunnel technology, such as the 6490, looks similar to the next figure.

Compound Group	Compound Name	ISTD?	Precursor Ion ▾	MS1 Res	Product Ion ▾	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
SubMix 1	JwH-200	☐	385.2	Unit	114.1	Unit	10	380	28	3	Positive
SubMix 1	JwH-018	☐	342.2	Unit	155	Unit	10	380	24	3	Positive
SubMix 1	JwH-018	☐	342.2	Unit	127	Unit	10	380	52	3	Positive
SubMix 1	JwH-250	☐	336.2	Unit	121	Unit	10	380	20	3	Positive
SubMix 1	JwH-250	☐	336.2	Unit	91.1	Unit	10	380	52	3	Positive
SubMix 1	CP 47,497-C8 homolc	☐	331.3	Unit	313.2	Unit	10	380	24	3	Negative
SubMix 1	CP 47,497-C8 homolc	☐	331.3	Unit	259.2	Unit	10	380	32	3	Negative
SubMix 1	JwH-073	☐	328.2	Unit	155	Unit	10	380	24	3	Positive
SubMix 1	JwH-073	☐	328.2	Unit	127	Unit	10	380	52	3	Positive
SubMix 1	CP 47,497	☐	317.2	Unit	299.2	Unit	10	380	24	3	Negative
SubMix 1	CP 47,497	☐	317.2	Unit	245.2	Unit	10	380	32	3	Negative
SubMix 1	CBN Cannabinol	☐	311.2	Unit	293.2	Unit	10	380	16	3	Positive
SubMix 1	CBN Cannabinol	☐	311.2	Unit	223	Unit	10	380	20	3	Positive
SubMix 1	CBN Cannabinol	☐	309.2	Unit	279.1	Unit	10	380	32	3	Negative
SubMix 1	CBN Cannabinol	☐	309.2	Unit	222	Unit	10	380	48	3	Negative
SubMix 1	Ketamine	☐	238.1	Unit	179.1	Unit	10	380	12	4	Positive
SubMix 1	Ketamine	☐	238.1	Unit	125	Unit	10	380	28	4	Positive
▶ SubMix 1	HU-210	☐	387.3	Unit	71.1	Unit	10	380	24	3	Positive

Step 3. Set up the MRM method

Note that polarity switching is supported for MRM, but the transitions within each compound need to have the same polarity. Polarity switching (positive and negative transitions within a compound) is not supported.

8 Remove the first compound from the Scan segments table:

- a** Select the first line. For the first method that you create, the line contains the compound **Compound1**. For the other methods, the line contains a compound from the previous submix.
- b** Right-click and click **Delete Row**. See the next figure.

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
SubMix 1	CP 47-407	☐	350	Unit	200	Unit	200	135	0	7	Positive
		☐	387.3	Unit	43.1	Unit	10	151	56	3	Positive
		☐	385.3	Unit	367.2	Unit	10	285	24	3	Negative
		☐	385.3	Unit	301.2	Unit	10	285	36	3	Negative
		☐	385.2	Unit	155	Unit	10	184	20	3	Positive
		☐	385.2	Unit	114.1	Unit	10	184	28	3	Positive
		☐	342.2	Unit	155	Unit	10	199	24	3	Positive
		☐	342.2	Unit	127	Unit	10	199	52	3	Positive
		☐	336.2	Unit	121	Unit	10	171	20	3	Positive
		☐	336.2	Unit	91.1	Unit	10	171	52	3	Positive
		☐	331.3	Unit	313.2	Unit	10	247	24	3	Negative
		☐	331.3	Unit	259.2	Unit	10	247	32	3	Negative
		☐	328.2	Unit	155	Unit	10	189	24	3	Positive
		☐	328.2	Unit	127	Unit	10	189	52	3	Positive

The final method now looks like the next figure.

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
SubMix 1	HU-210	☐	387.3	Unit	43.1	Unit	10	151	56	3	Positive
SubMix 1	HU-210	☐	385.3	Unit	367.2	Unit	10	285	24	3	Negative
SubMix 1	HU-210	☐	385.3	Unit	301.2	Unit	10	285	36	3	Negative
SubMix 1	JwH-200	☐	385.2	Unit	155	Unit	10	184	20	3	Positive
SubMix 1	JwH-200	☐	385.2	Unit	114.1	Unit	10	184	28	3	Positive
SubMix 1	JwH-018	☐	342.2	Unit	155	Unit	10	199	24	3	Positive
SubMix 1	JwH-018	☐	342.2	Unit	127	Unit	10	199	52	3	Positive
SubMix 1	JwH-250	☐	336.2	Unit	121	Unit	10	171	20	3	Positive
SubMix 1	JwH-250	☐	336.2	Unit	91.1	Unit	10	171	52	3	Positive
SubMix 1	CP 47,497-C8 homok	☐	331.3	Unit	313.2	Unit	10	247	24	3	Negative
SubMix 1	CP 47,497-C8 homok	☐	331.3	Unit	259.2	Unit	10	247	32	3	Negative
SubMix 1	JwH-073	☐	328.2	Unit	155	Unit	10	189	24	3	Positive
SubMix 1	JwH-073	☐	328.2	Unit	127	Unit	10	189	52	3	Positive

Step 4. Set up a worklist to run the submixes

9 Click **Method > Save As** (or click  in the Method Editor toolbar) and save the method as **ForTox_MRM_Mix1.m**.

10 Delete all but one compound from the Scan segments table.

The Scan segments table cannot be empty. You need to leave one compound in the table.

11 Repeat [step 4](#) through [step 10](#) for each of the submixes.

When you save each method, use a name that reflects the submix name, such as **ForTox_MRM_Mix2.m** for the values in the **SubMix 2** tab.

Step 4. Set up a worklist to run the submixes

- Set up the worklist as shown in the next figure. Include all submixes. Inject the first standard twice to allow the system to come to equilibrium.

	✓	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name
1	✓	SubMix_01	P1-A1	ForTox_MRM_Mix_1.m	to_delete.d	Calibration	1
2	✓	SubMix_01	P1-A1	ForTox_MRM_Mix_1.m	SubMix_01.d	Calibration	1
3	✓	SubMix_02	P1-A2	ForTox_MRM_Mix_2.m	SubMix_02.d	Calibration	1
4	✓	SubMix_03	P1-A3	ForTox_MRM_Mix_3.m	SubMix_03.d	Calibration	1
5	✓	SubMix_04	P1-A4	ForTox_MRM_Mix_4.m	SubMix_04.d	Calibration	1
6	✓	SubMix_05	P1-A5	ForTox_MRM_Mix_5.m	SubMix_05.d	Calibration	1
7	✓	SubMix_06	P1-A6	ForTox_MRM_Mix_6.m	SubMix_06.d	Calibration	1
8	✓	SubMix_07	P1-A7	ForTox_MRM_Mix_7.m	SubMix_07.d	Calibration	1
9	✓	SubMix_08	P1-A8	ForTox_MRM_Mix_8.m	SubMix_08.d	Calibration	1

To create the dMRM and tMRM methods from the MRM methods that you just created, refer to the *Quick Start Guide* for this database, or the *MassHunter Data Acquisition for 6400 Series Triple Quadrupole LC/MS Familiarization Guide* or *online Help*.

Step 4. Set up a worklist to run the submixes

In this Book

The *Method Setup Guide* describes how to create MRM methods for your specific LC/MS set up. The MRM methods are used to create Dynamic MRM (dMRM) and Triggered MRM (tMRM) methods for the Comprehensive Test Mix.

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