JASCO-HPLC Operating Manual

(Analytical HPLC)

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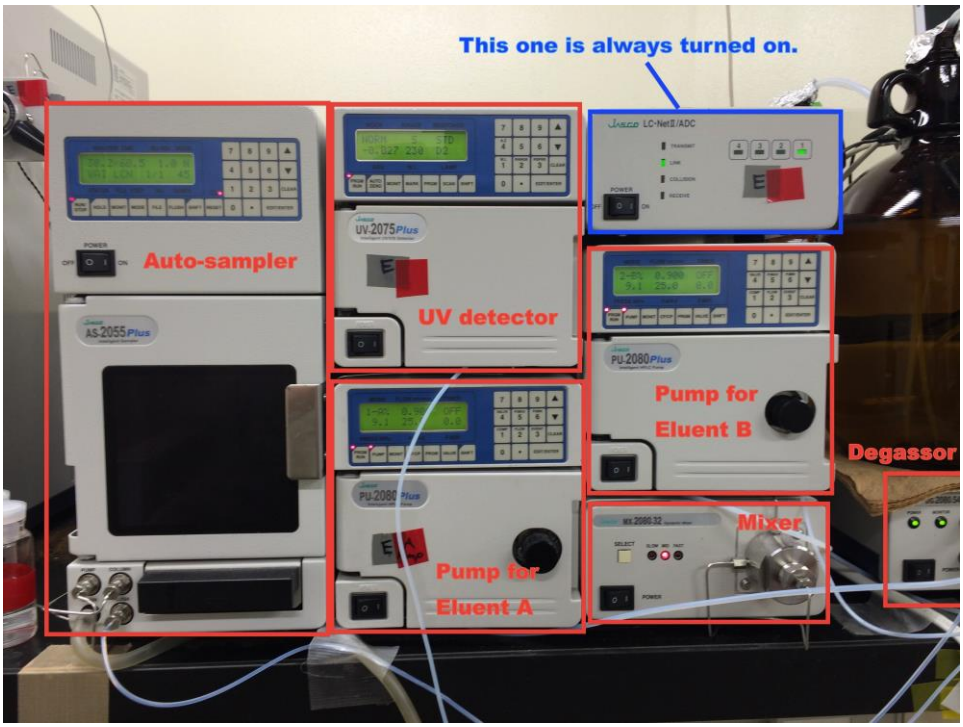
A) Turning on Equipment and Starting ChromNav

When you want to use JASCO-HPLC, please turn on the following apparatus.

- ✓ UV detector
- ✓ Pump for Eluent A
- ✓ Pump for Eluent B
- ✓ Mixer
- ✓ Degassor (Sorry, the photo doesn't show the picture of it totally…)
- ✓ Mobile Phase Mixer
- ✓ Auto-sampler (when you want to carry out automatic measurement)



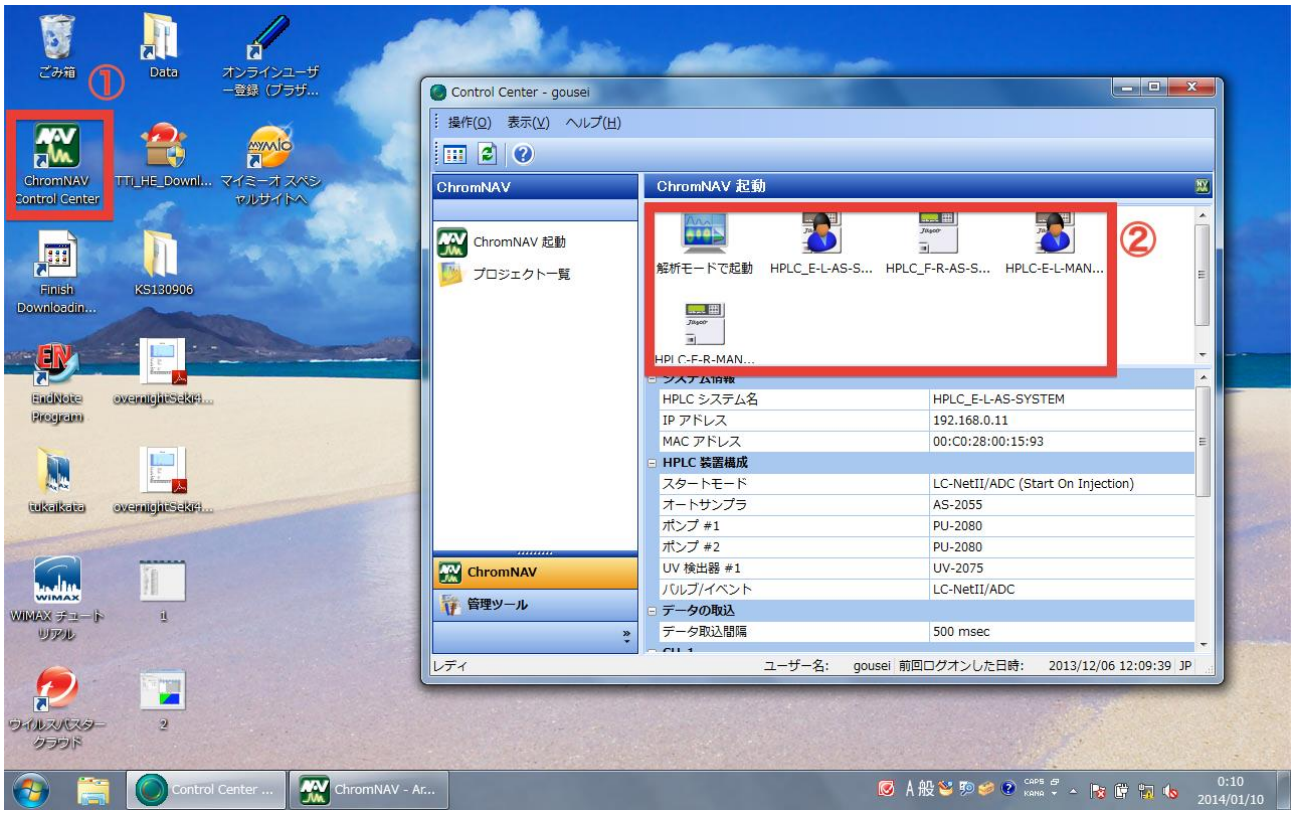
Then, please double-click "ChromNav" icon and start "ChromNav".

Log-in ID and password is same: "gousei".

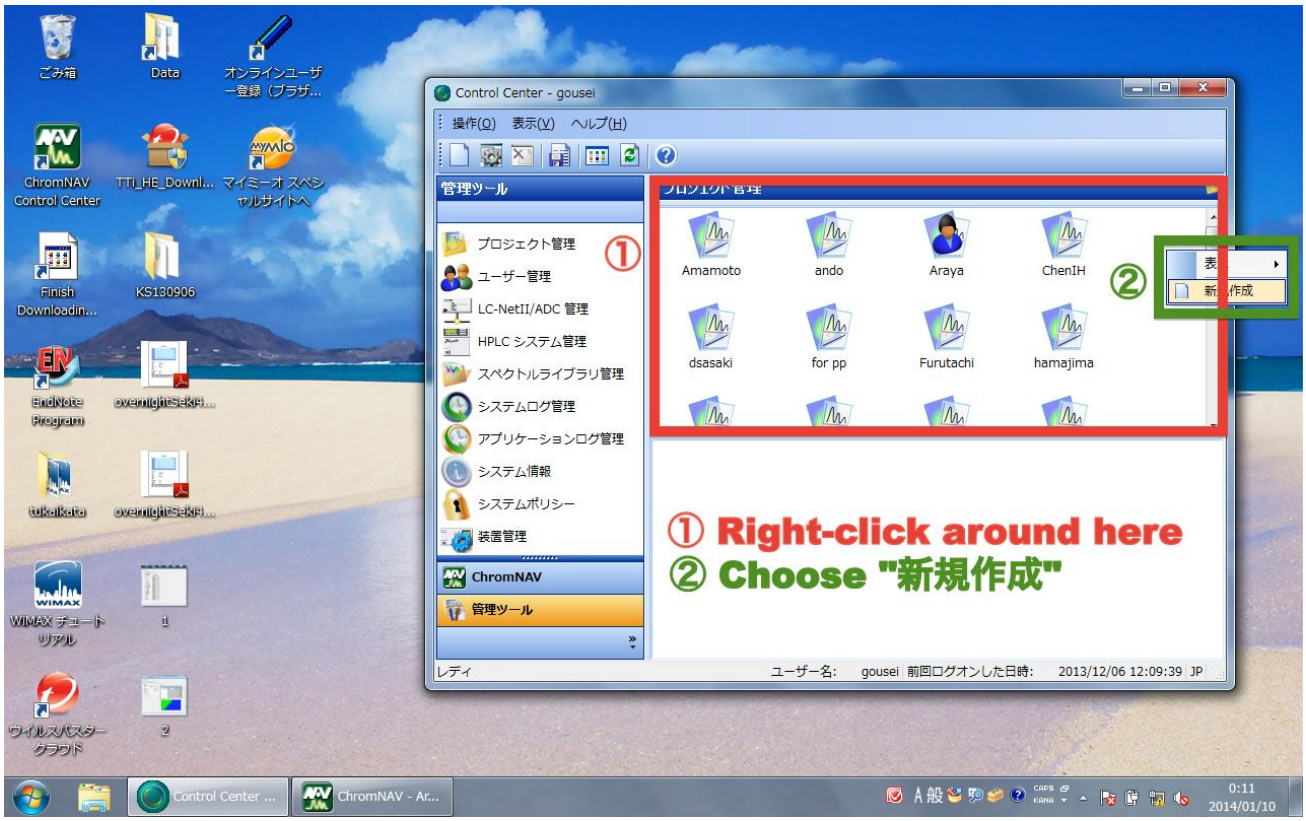
There are 5 icons:

- ✓ 解析モードで起動: for Analysis Mode
- ✓ HPLC_E-L-AS-SYSTEM: use HPLC-E with auto-sampler (automatic measurement)
- ✓ HPLC_F-R-AS-SYSTEM: use HPLC-F with auto-sampler (automatic measurement)
- ✓ HPLC-E-L-MANUAL-SYSTEM: use HPLC-E (manual measurement)
- ✓ HPLC-F-R-MANUAL-SYSTEM: use HPLC-F (manual measurement)

Please double-click the suitable icon and choose the project you need.

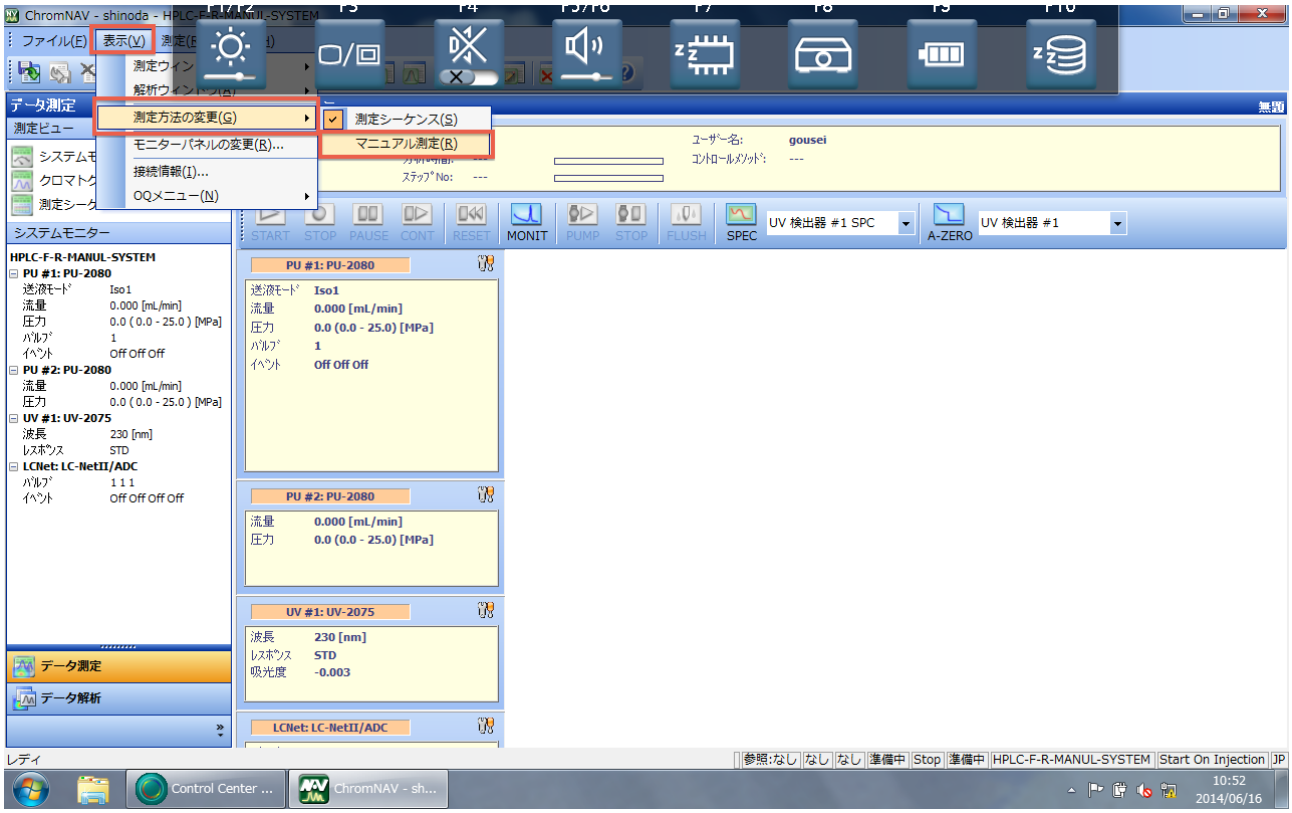


If it is your first time to use this apparatus, please create your own project newly as follows.



B) For Manual Measurement

For manual measurement, you first need to set the view mode as “マニュアル測定”.



(1) Making Control Method

You can set the measurement details by setting “Control Method”.

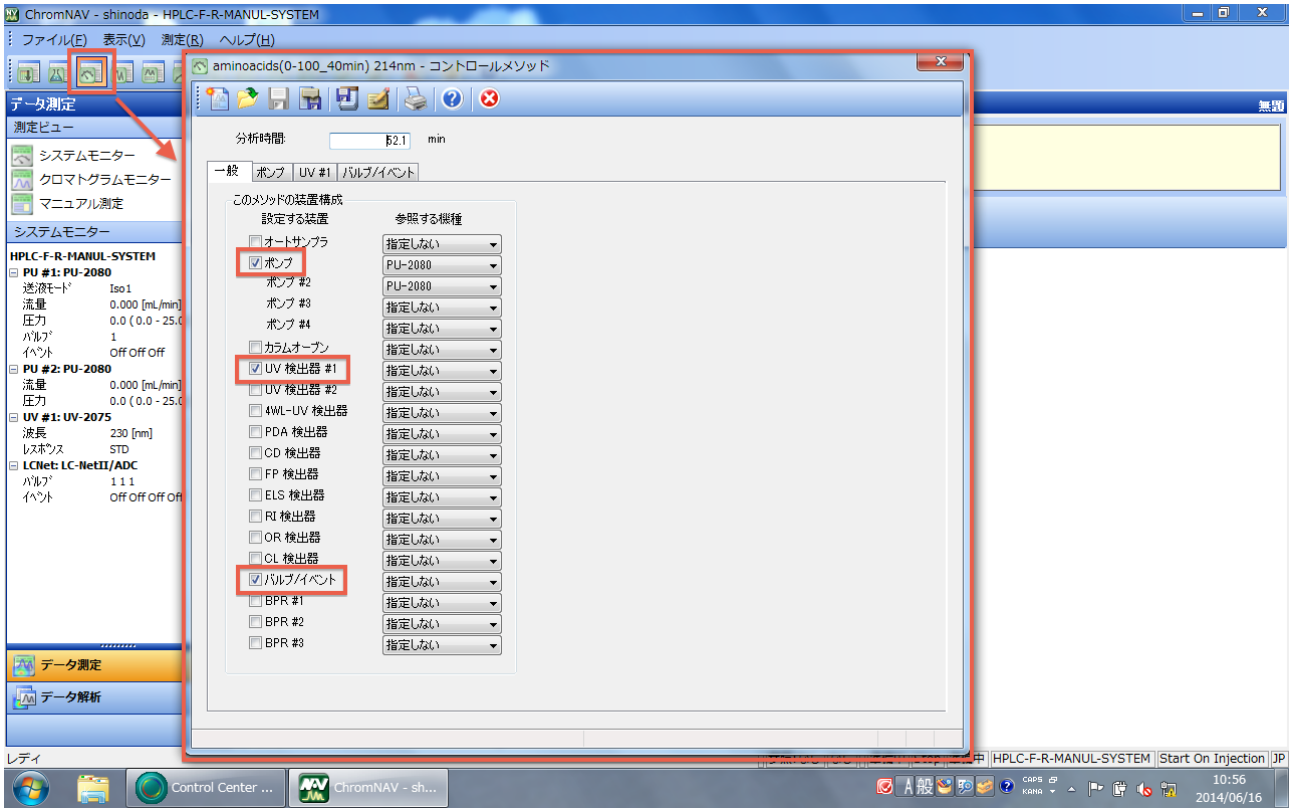
Following 3 boxes should be checked:

✓ ポンプ: PU-2080

✓ UV 検出器 #1: 指定しない

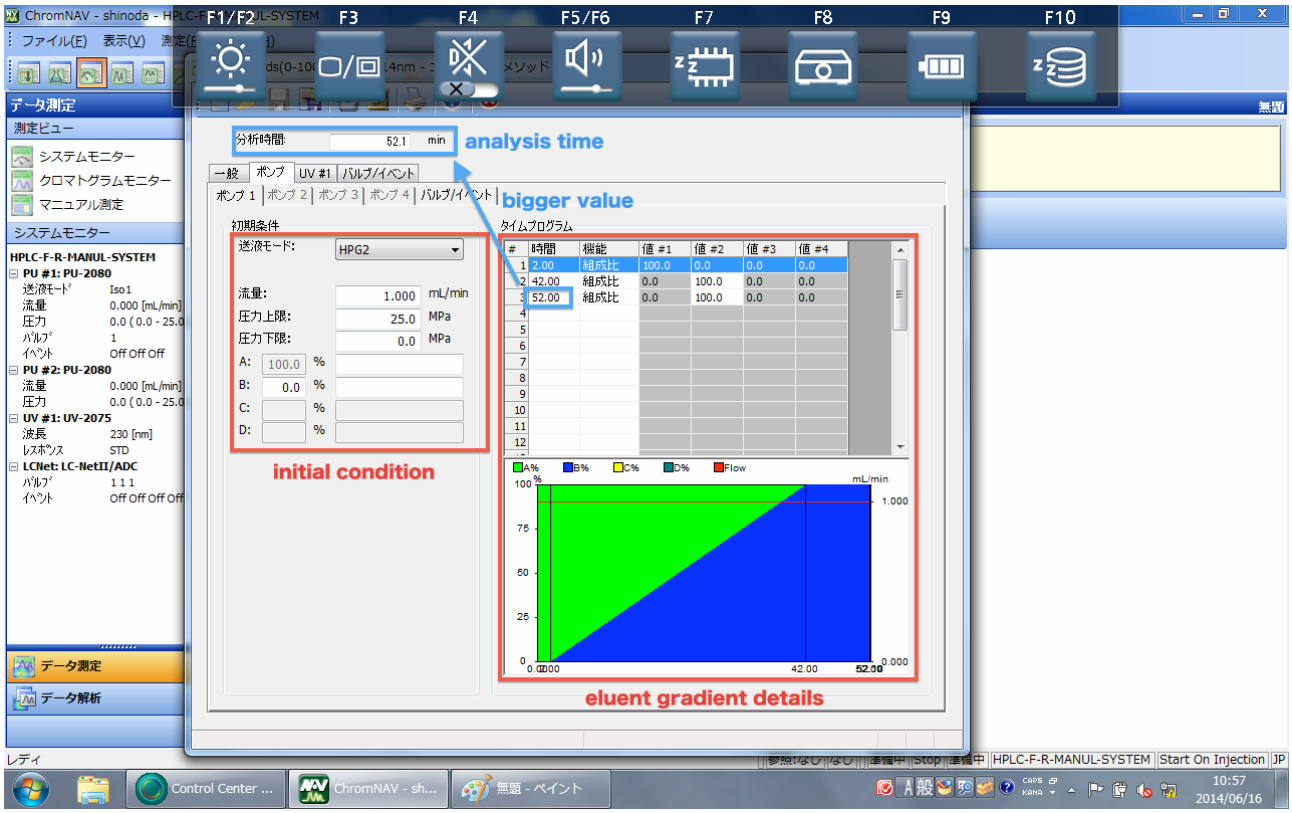
✓ バルブ/イベント*: 指定しない

*The reason this box should be checked is not clear; the supplier checked this box at explanatory meeting.



”ポンプ” tab … you can set the mobile phase gradient.

In following figure, standard gradient setting for measurement is shown.



(Eluent A: aqueous TFA (0.1%), Eluent B: MeCN)

You also need to set the washing method; please fill each box with following values.

- ✓ initial condition → A: 0%, B: 100%; other values should not be changed
- ✓ eluent gradient details → don’t fill in anything
- ✓ analysis time → 20.0 min

”UV #1” tab … you can set the wave length for measurement.

You can set the wave length from 190nm.

Finally, please save your method with appropriate name and close this window.

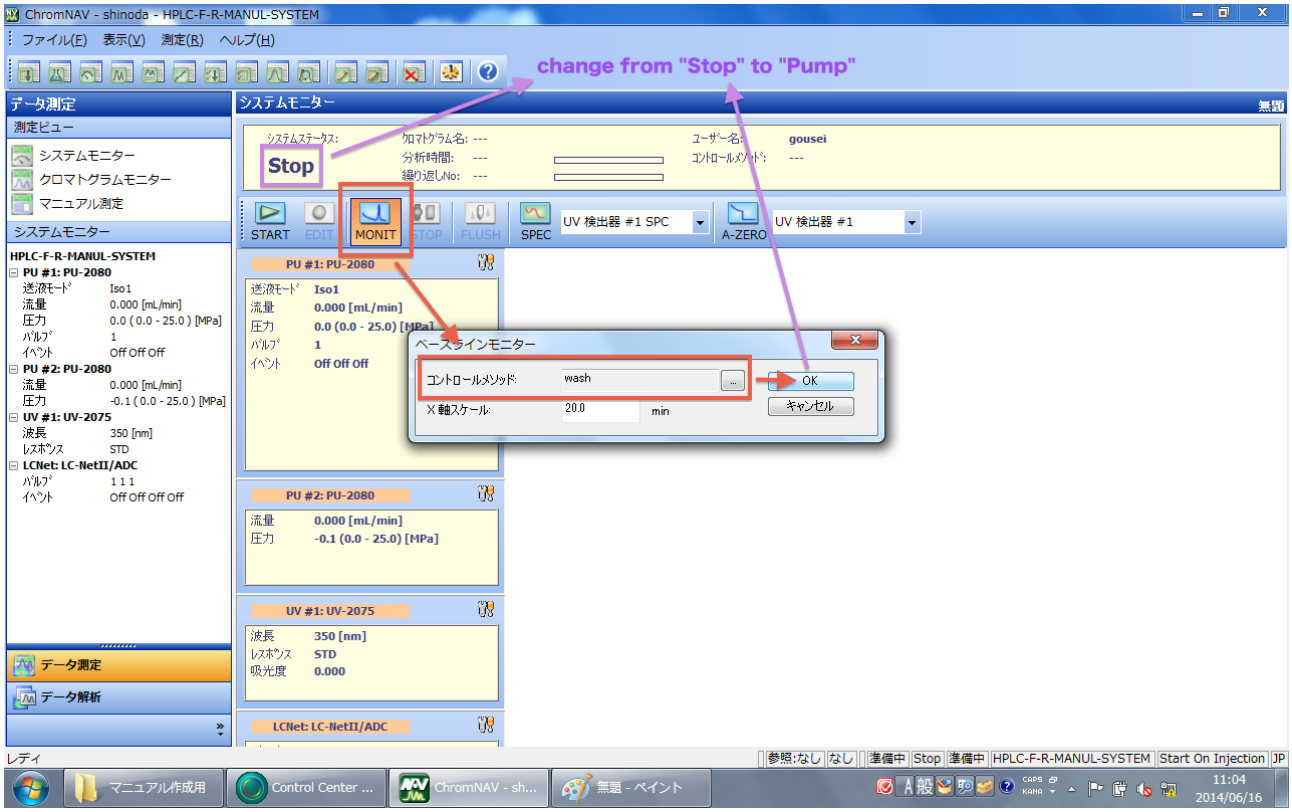
(ex. “0-100_40min_1mLmin_230nm” or “wash_MeCN_1mLmin”)

”バルブ/イベント” tab … you don’t need to do anything.

(2) Preparation for Measurement

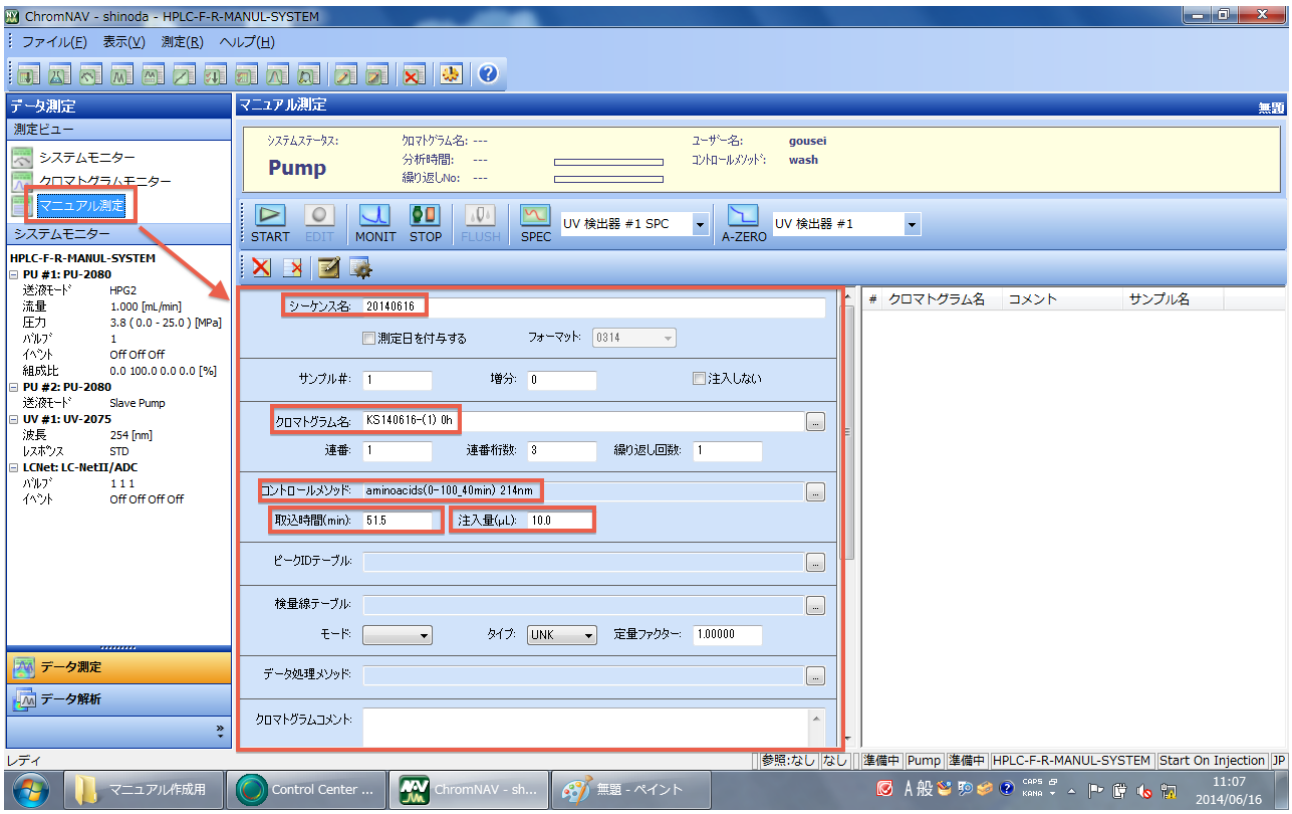
At first, you need to wash the column with MeCN. Washing takes 10 minutes usually.

Please select washing method you set before and start washing as follows.

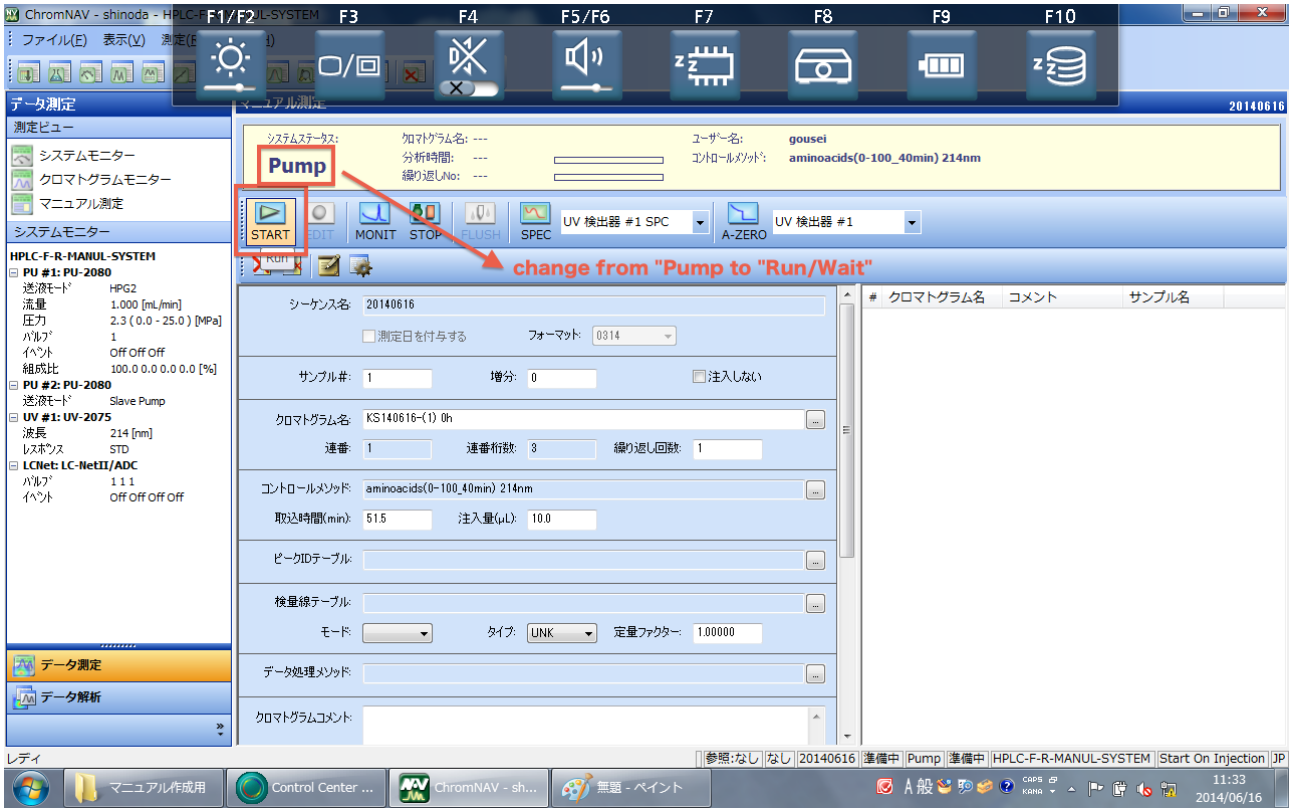


During washing, it is recommended to set the measurement information as follows.

- ✓ シーケンス名 → today's date
- ✓ クロマトグラム名 → experiment number
- ✓ コントロールメソッド → select the control method for analysis
- ✓ 取込時間(min) → −0.5 min from the analysis time of the selected control method
- ✓ 注入量(μL) → injection volume



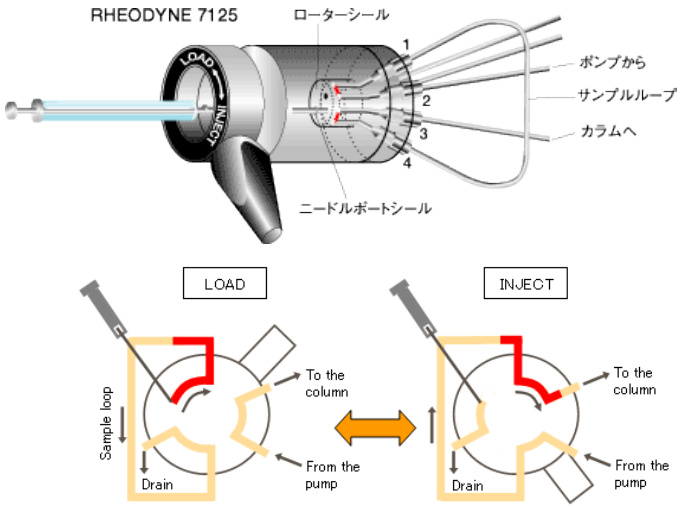
After 10 minutes, please start pre-run as follows. Pre-run takes 10 minutes usually.



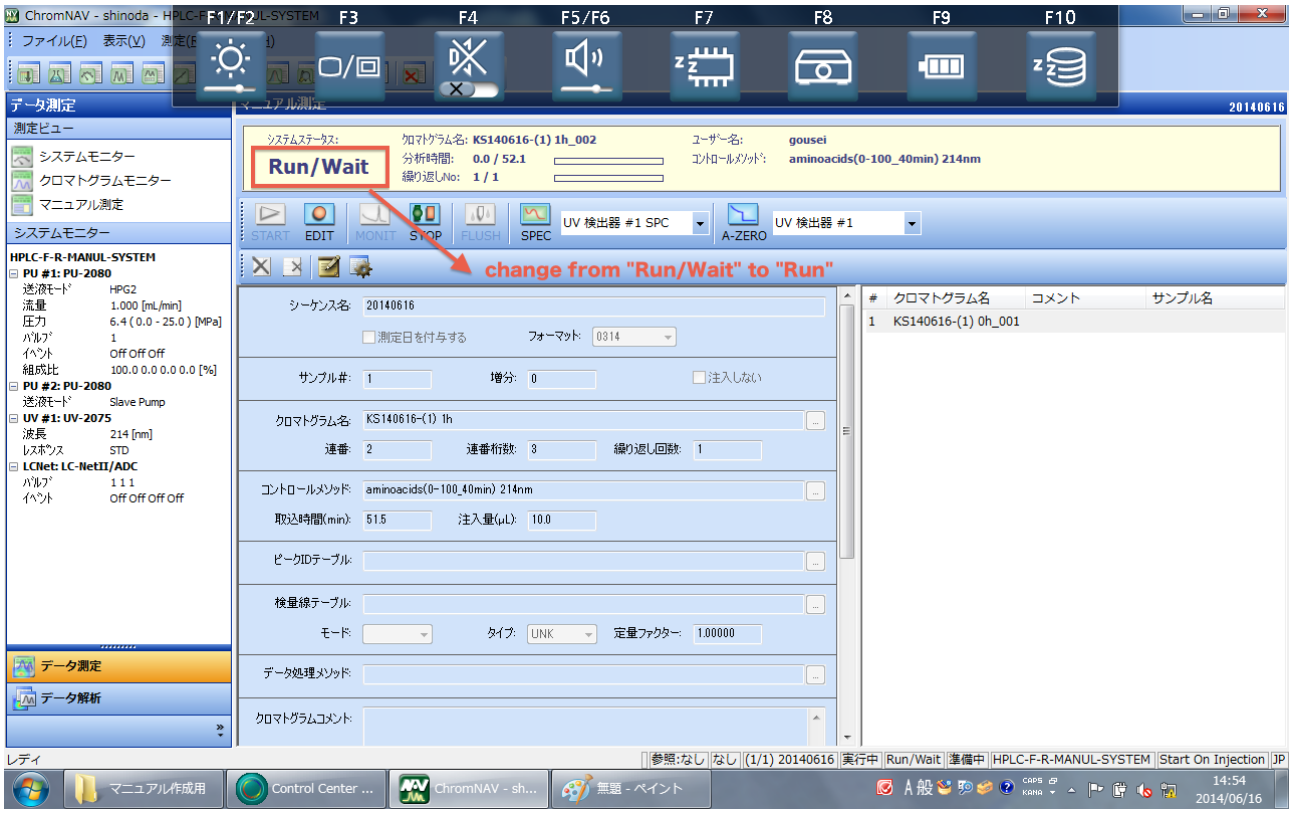
(3) Measurement

After 10 minutes, please inject your sample with 50 µL syringe from injection port as follows.

The measurement starts automatically when the following process is carried out.



1. inserting syringe
2. “INJECT” → “LOAD”
3. injecting sample
4. “LOAD” → “INJECT”
5. extracting syringe



You can see the measurement proceeding in “クロマトグラムモニター”.

(4) After Measurement

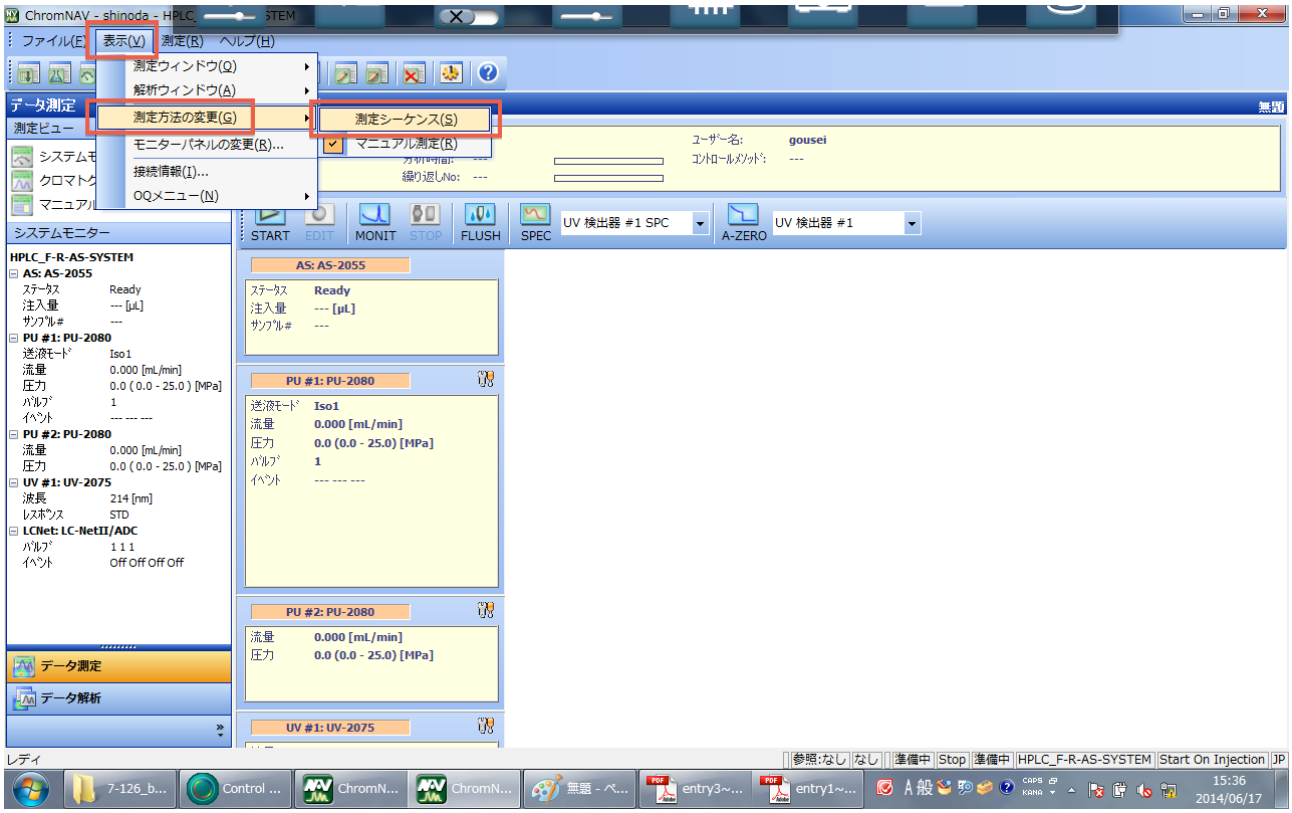
When the measurement is finished, the display changes from “Run” to “Run/Wait” and Eluent A is flowed. As acidic solution has bad effect on column, you are recommended to fill column with MeCN.

Please click the “STOP” icon and click the “MONIT” icon and select wash method.
Solvent changing will take 10 minutes.

After 10 minutes, you can stop the washing.

C) For Automatic Measurement Using Auto-Sampler

For manual measurement, you first need to set the view mode as “測定シーケンス”.



(1) Making Control Method

You can set the measurement details by setting “Control Method”.

Following 4 boxes should be checked:

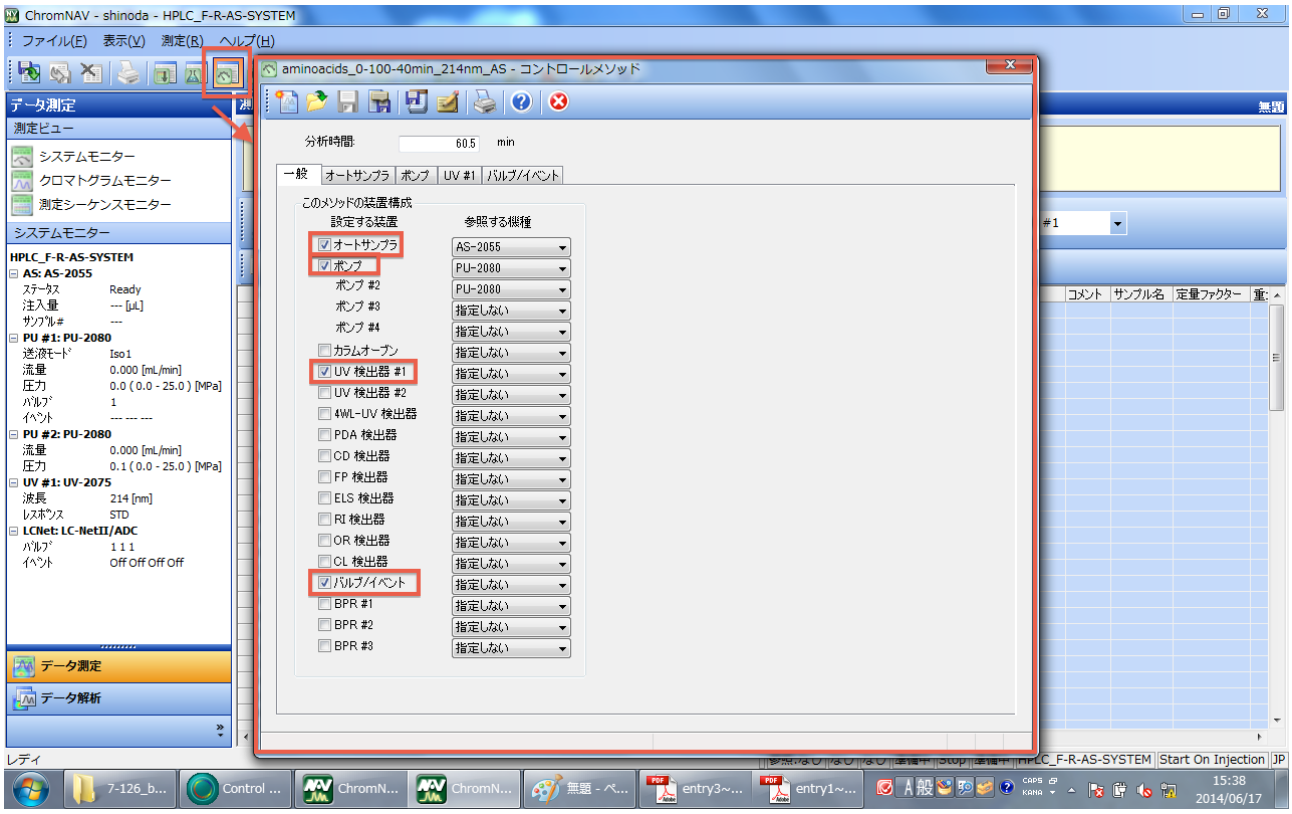
✓ オートサンプラー: auto-sampler

✓ ポンプ: pump

✓ UV 検出器 #1: UV detector No. 1

✓ バルブ/イベント*

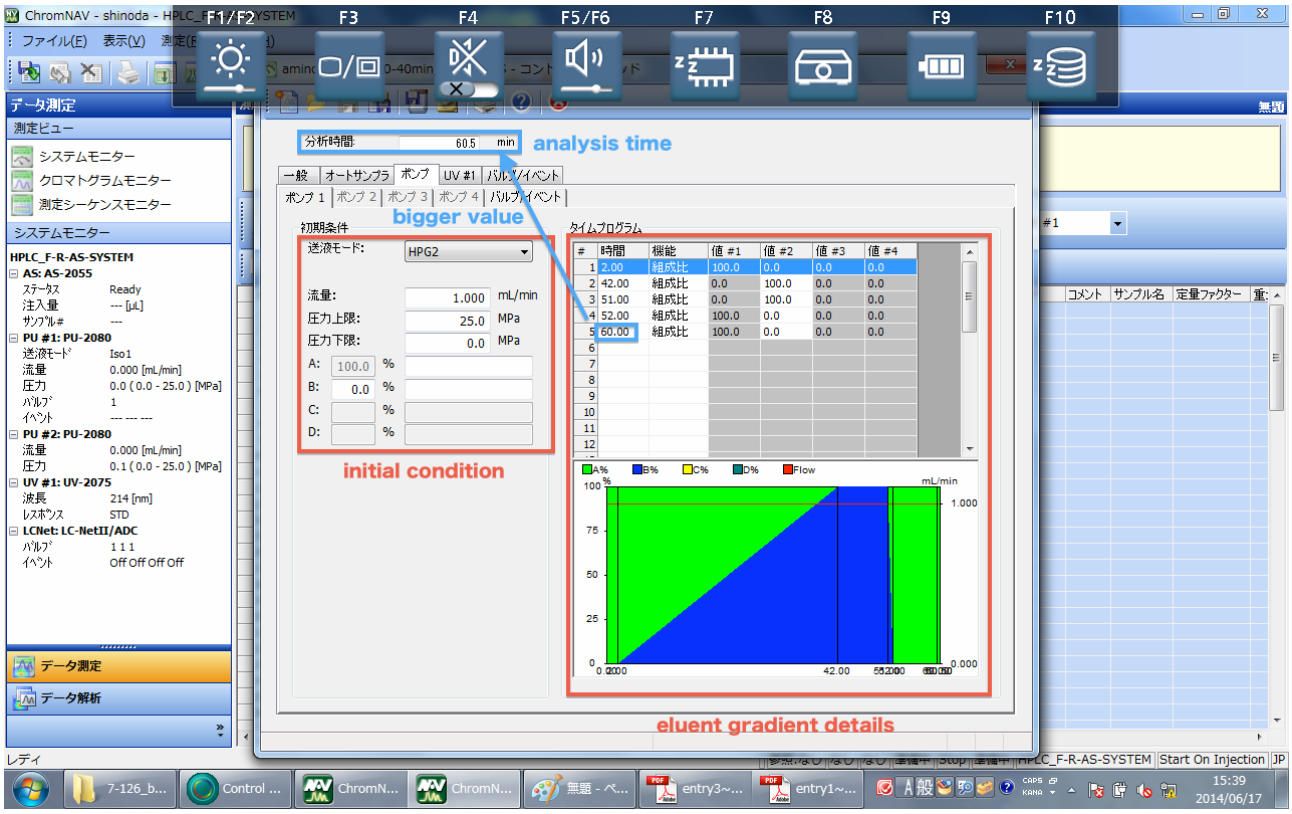
*The reason this box should be checked is not clear; the supplier checked this box at explanatory meeting.



“オートサンプラー” tab … you don’t need to do anything.

”ポンプ” tab … you can set the mobile phase gradient.

In following figure, standard gradient setting for measurement is shown.

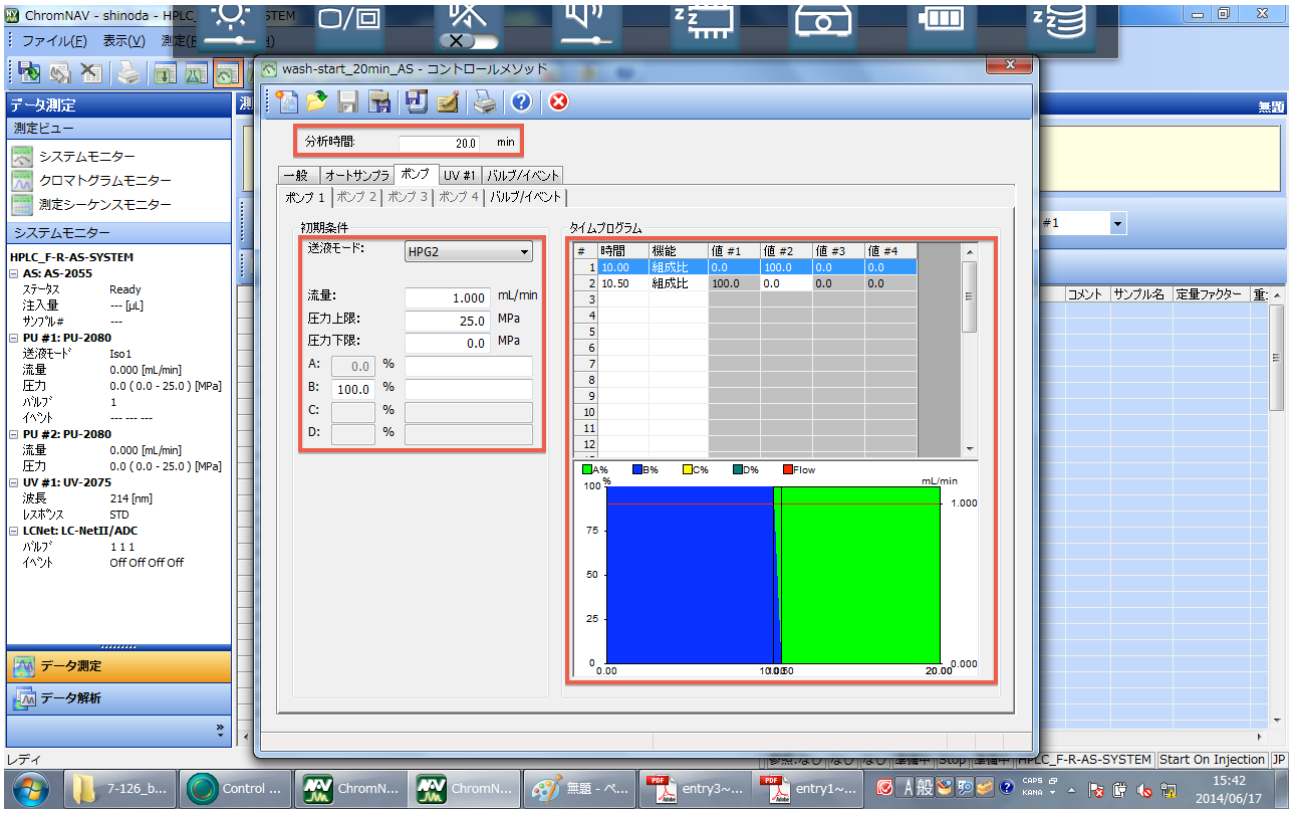


(Eluent A: aqueous TFA (0.1%), Eluent B: MeCN)

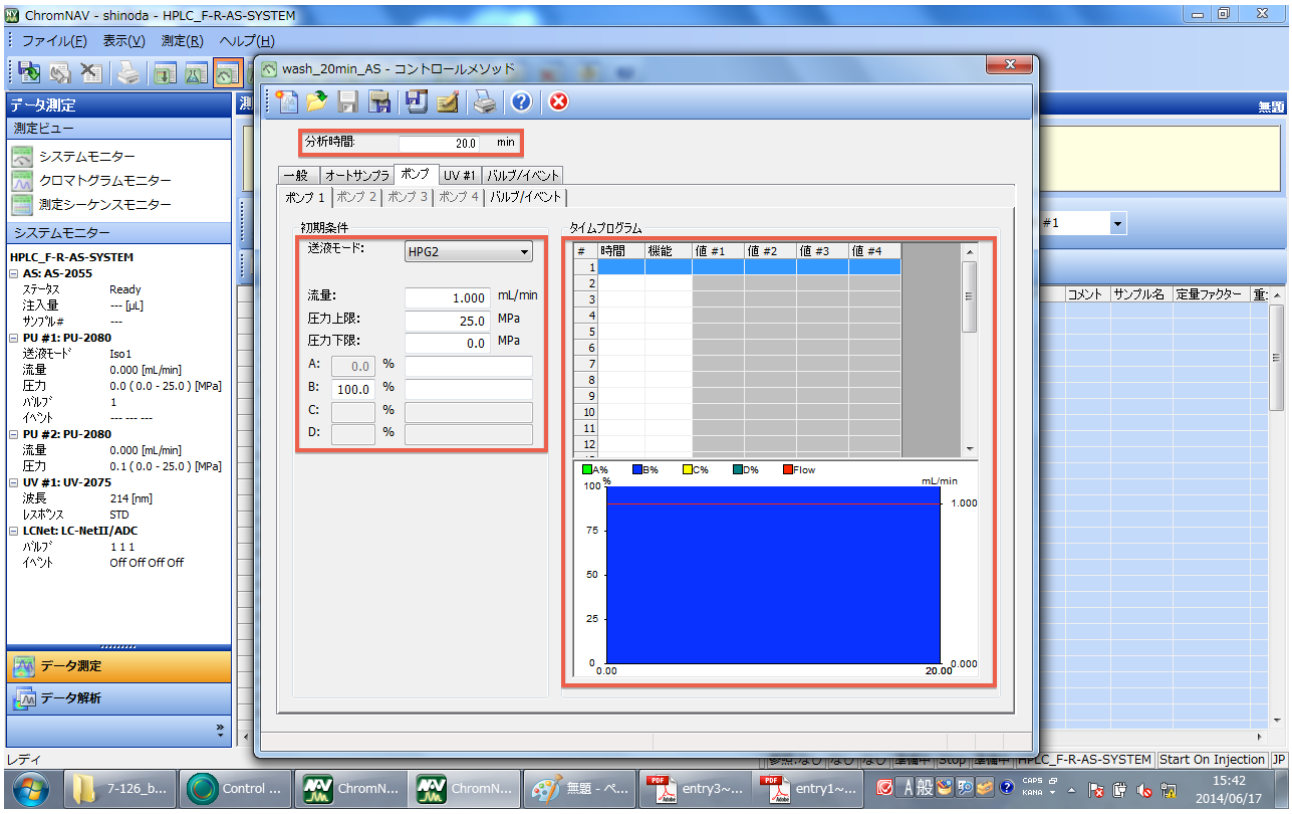
In order to carry out a measurement sequentially, initializing time (8 min, eluent A 100%) is set.

Also, you are recommended to set pre- and post-sequence washing method.

<pre-sequence>



<post-sequence>



”UV #1” tab ... you can set the wave length for measurement.
You can set the wave length from 190nm.

Finally, please save your method with appropriate name and close this window.
(ex. “AS_0-100_40min_1mLmin_230nm” or “AS_wash-start_1mLmin” or “AS_wash-end_1mLmin”)

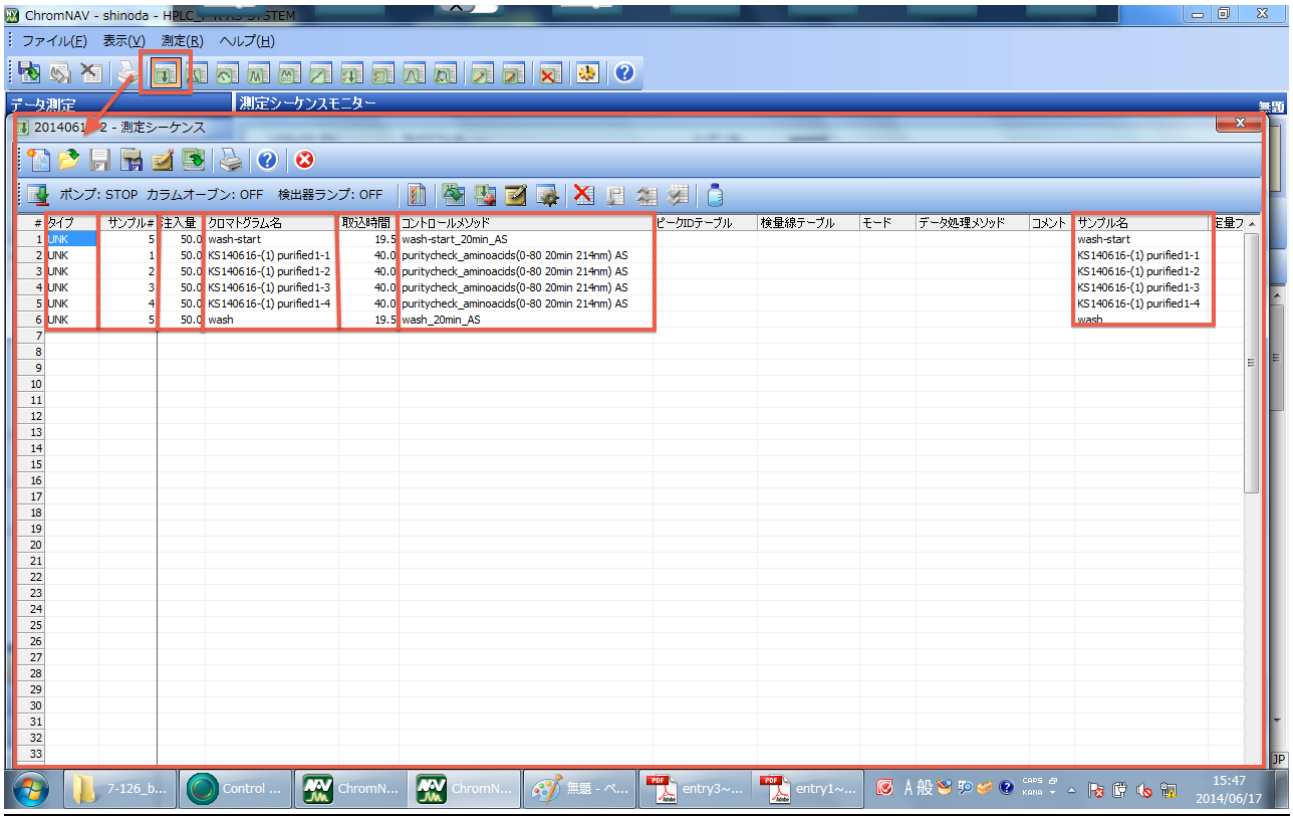
”バルブ/イベント” tab ... you don’t need to do anything.

(2) Preparation for Measurement

You need to set batch table for automatic measurement.

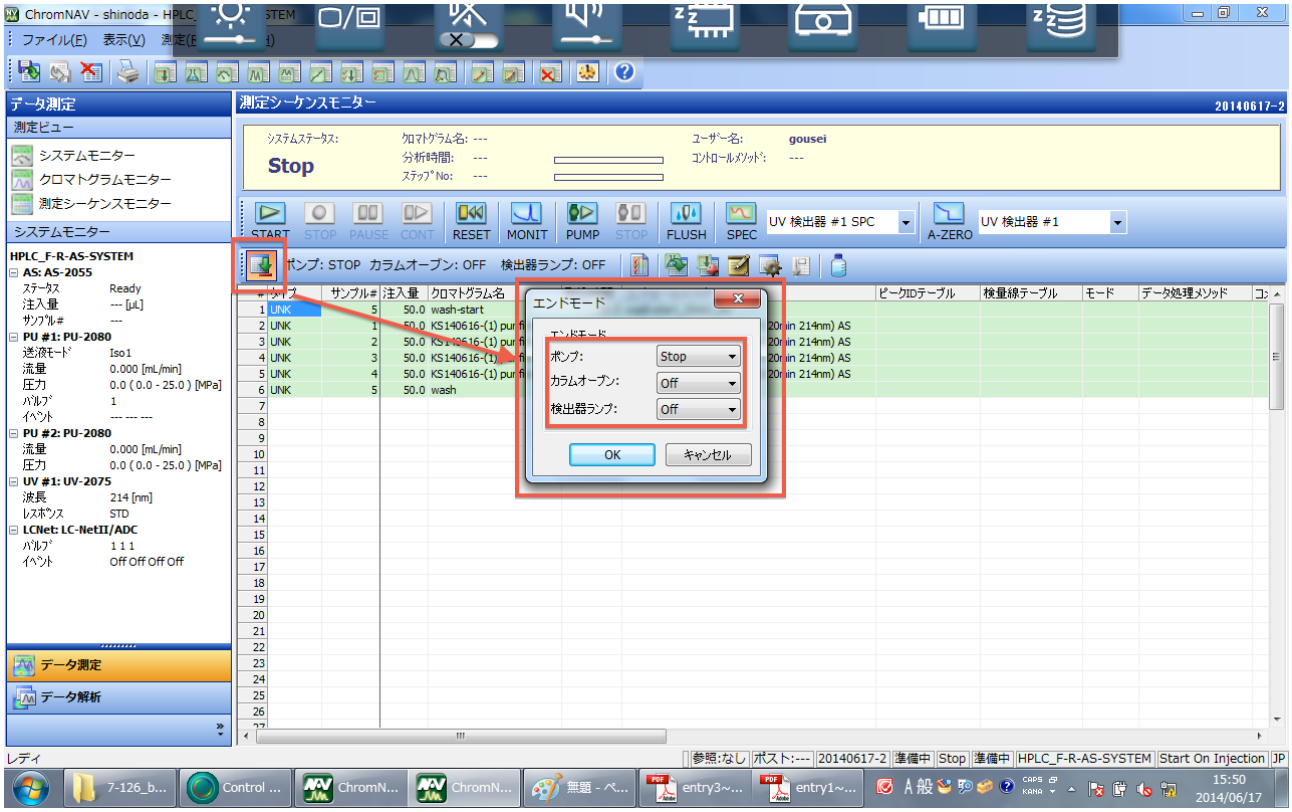
Following 6 blanks should be filled as follows:

- ✓ タイプ: UNK
- ✓ サンプル#: vial port number you used
- ✓ 注入量: injection volume, μL order
- ✓ クロマトグラム名: experiment number
- ✓ 取込時間: −0.5 min from the analysis time of the selected control method
- ✓ コントロールメソッド: appropriate control method
- ✓ サンプル名: experiment number



You are recommended to add “wash-start” line before your first sample and add “wash-end” line after your last sample, as shown in above.

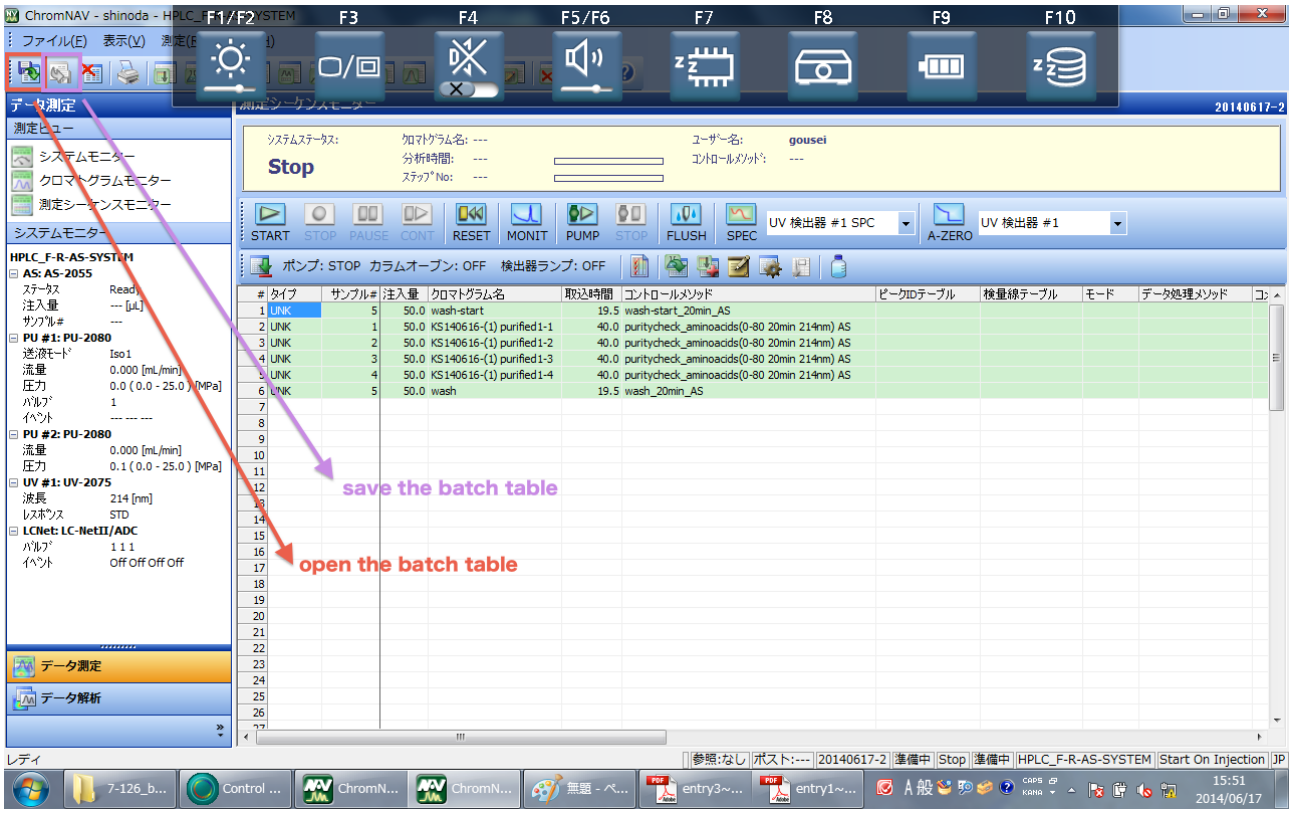
Please don’t forget to set “end mode” as follows:



When you finish setting the batch table, please save it. As for the filename, date will be suitable.

(3) Measurement & After Measurement

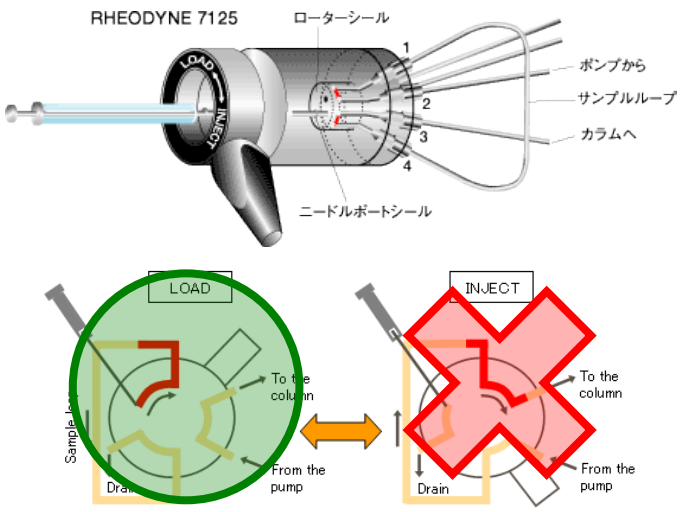
Please open the batch table you have set.



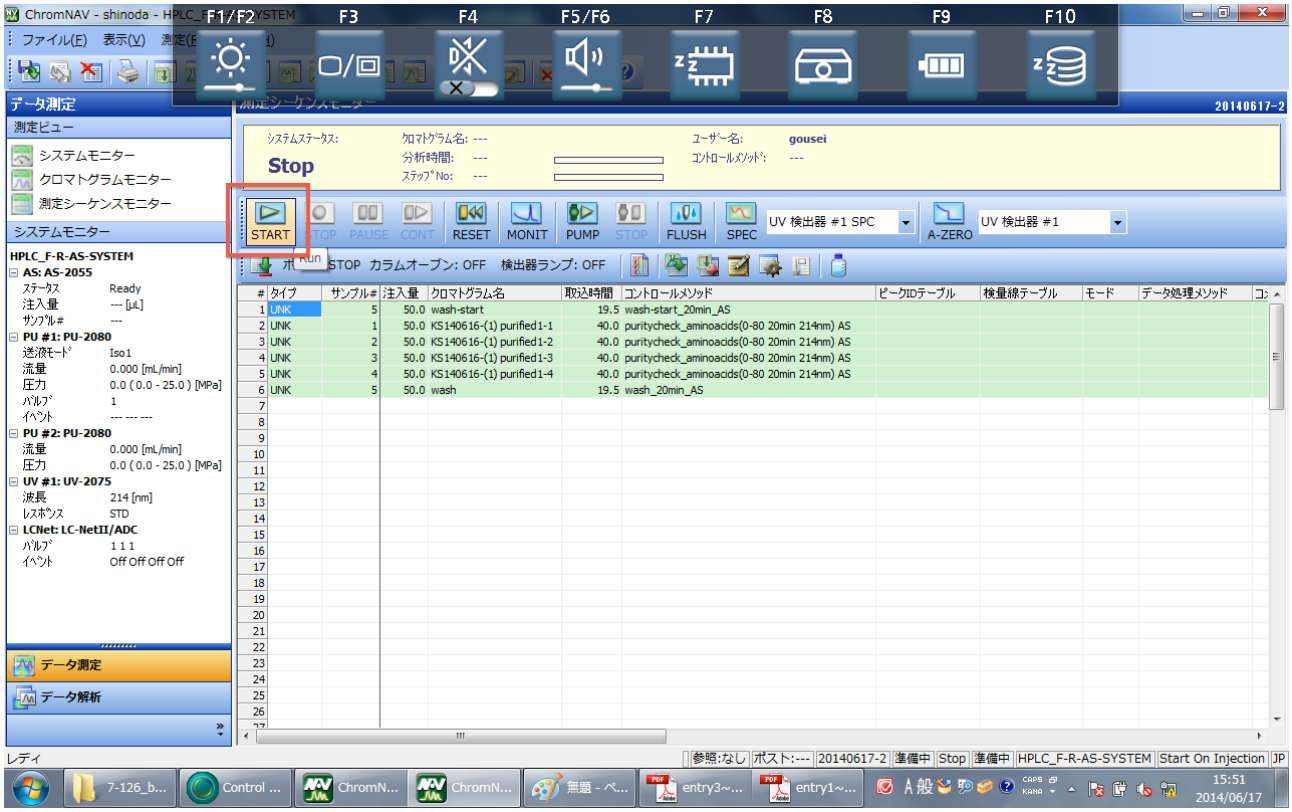
Further sample addition and sample delete can be done.

When the change is finished, please don't forget to save batch table.

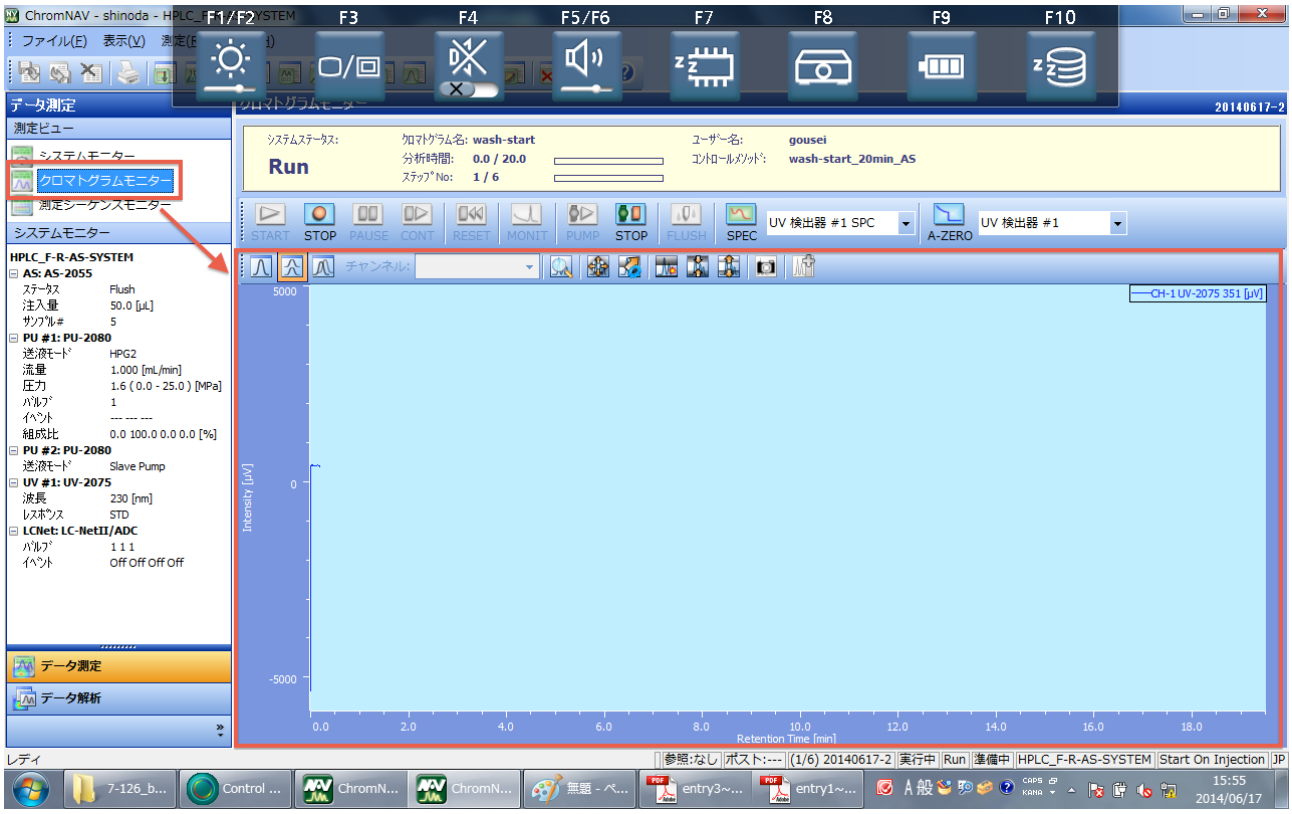
Please don't forget to set the injection port position as "LOAD". When measurement is carried out with "INJECT" position, data won't be saved.



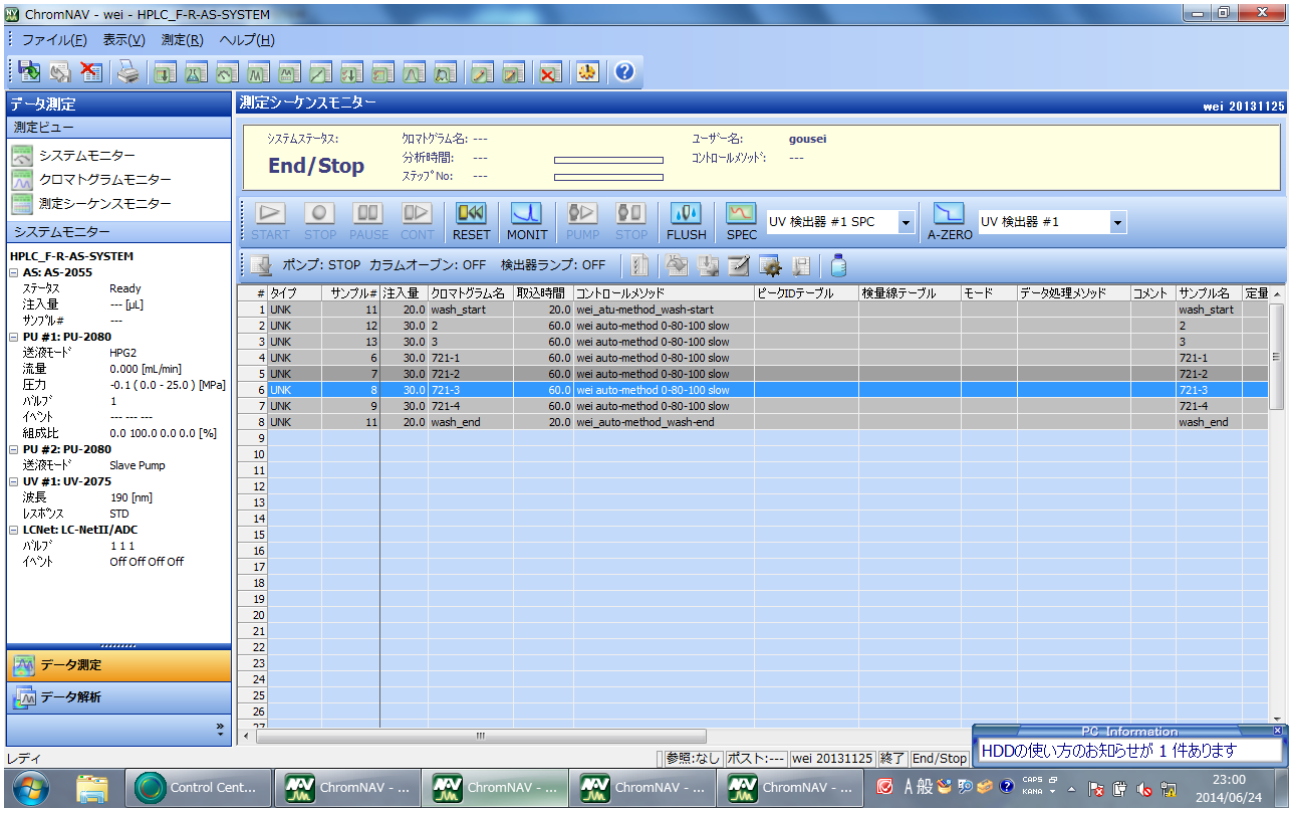
Automatic measurement starts when you click “START” icon.



You can see measurement proceeding in “クロマトグラムモニター” tab.



When the measurement is finished, you will see the following view.



Please go to the analysis mode (p. 25~).

D) For Overnight Measurement

For overnight measurement, you need to use auto-sampler.

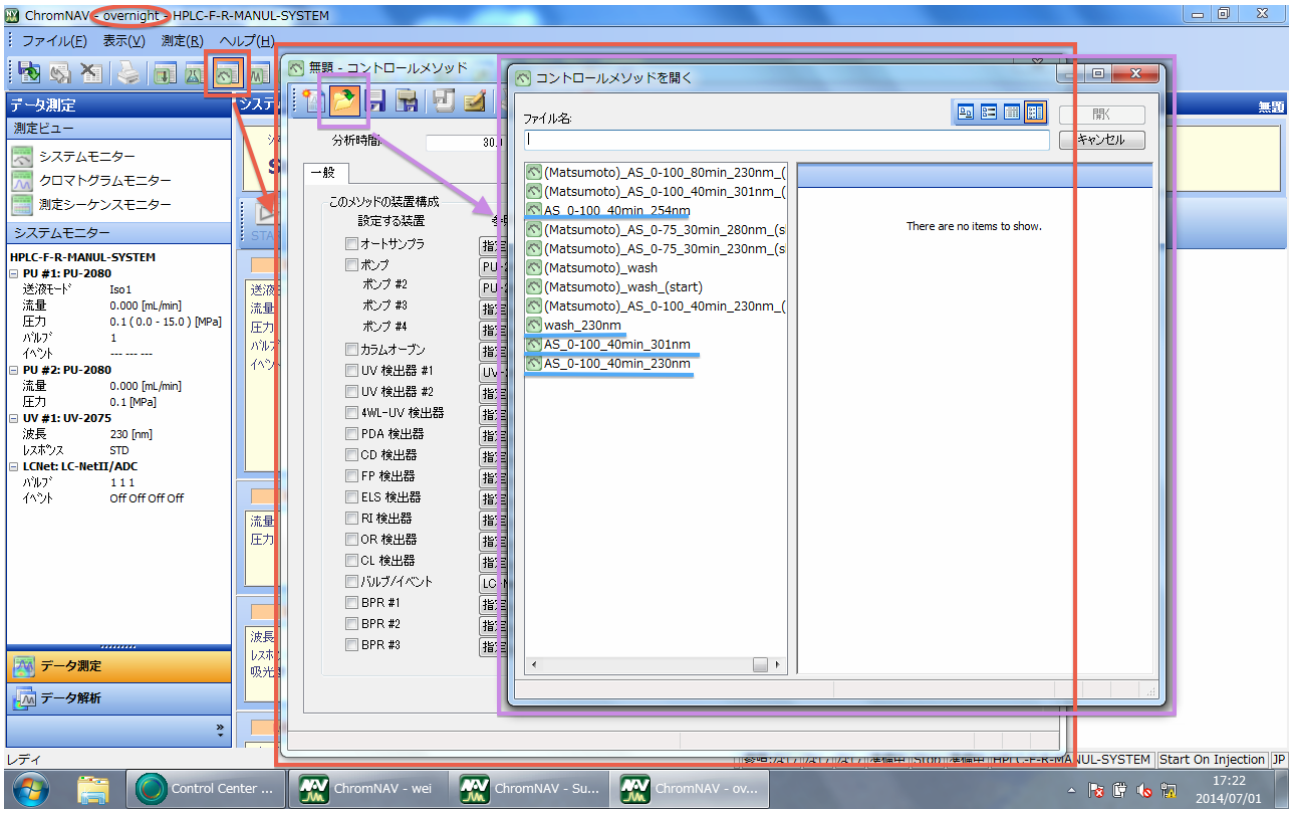
However, if you carry out overnight measurement in your own account, other person who also wants to carry out overnight measurement can't add his/her sample and conduct his/her measurement.

Therefore, for overnight measurement, please use the common account named "overnight".

In account named "overnight", there are following 4 common control methods:

- ✓ 0-100_40min_230nm (flow: 1 mL/min)
- ✓ 0-100_40min_301nm (flow: 1 mL/min)
- ✓ 0-100_40min_254nm (flow: 1 mL/min)
- ✓ wash_230nm (flow: 1 mL/min, MeCN 100%)

Please use these methods as much as you can in order not to increase unnecessary methods.

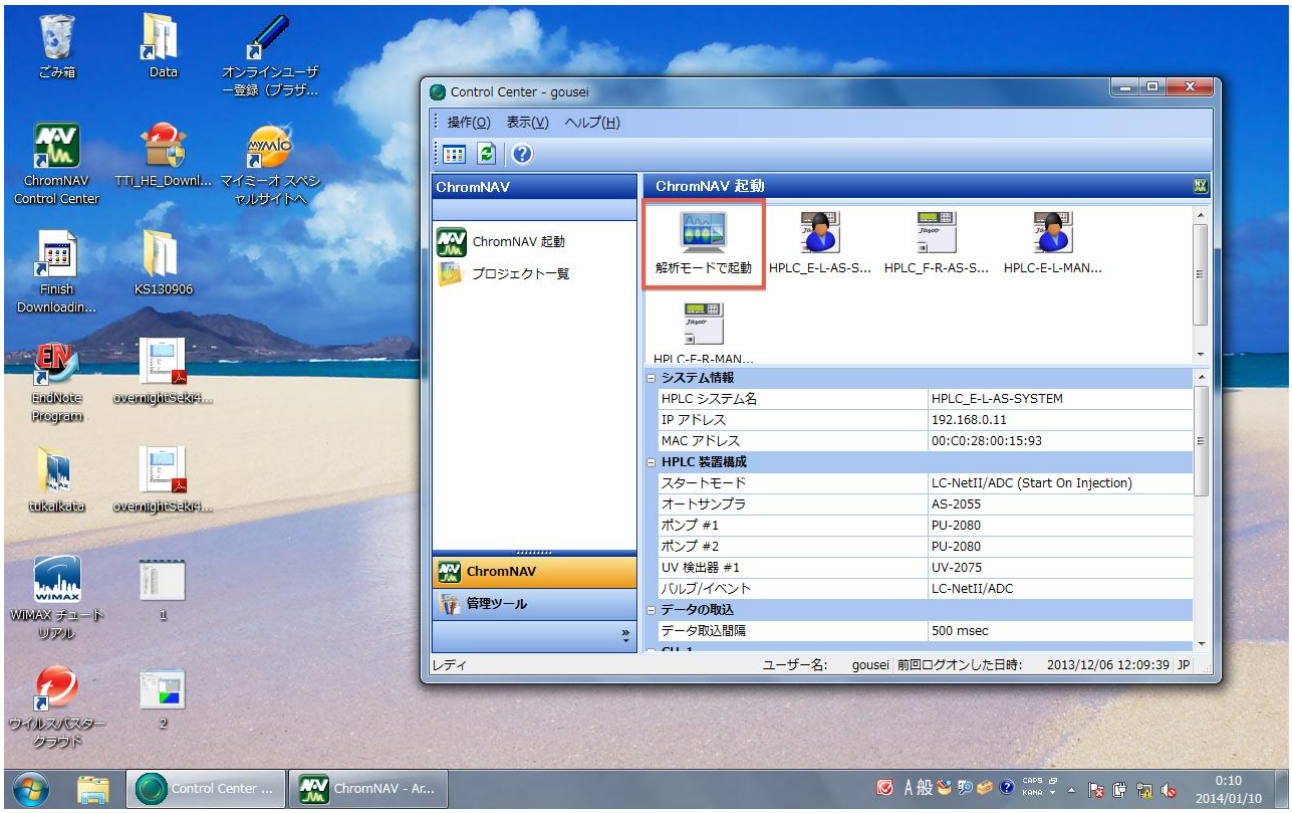


Other setting details are the same as shown in section C (automatic measurement using auto-sampler).

E) Data Analysis

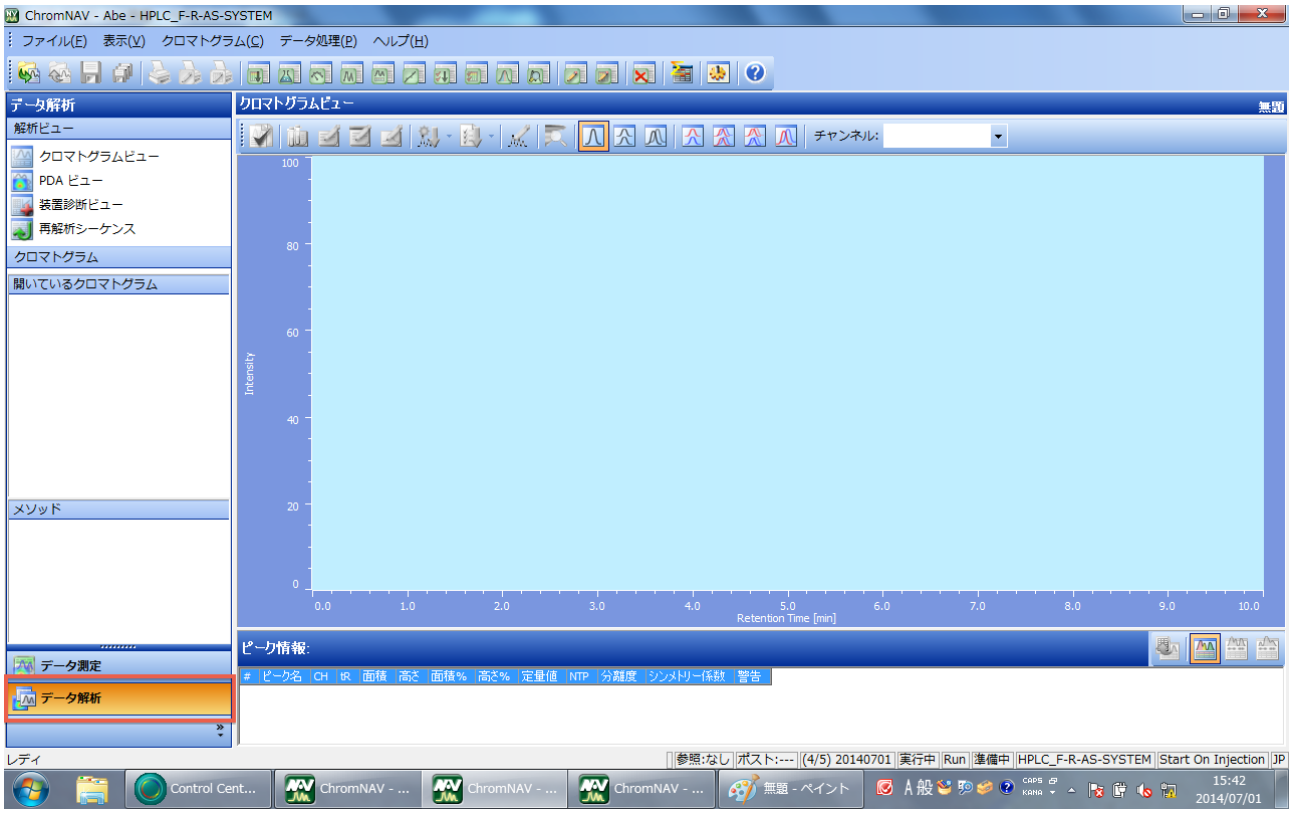
You can analyze your data by opening the analysis mode.

Please double-click the icon named “解析モードで起動”.



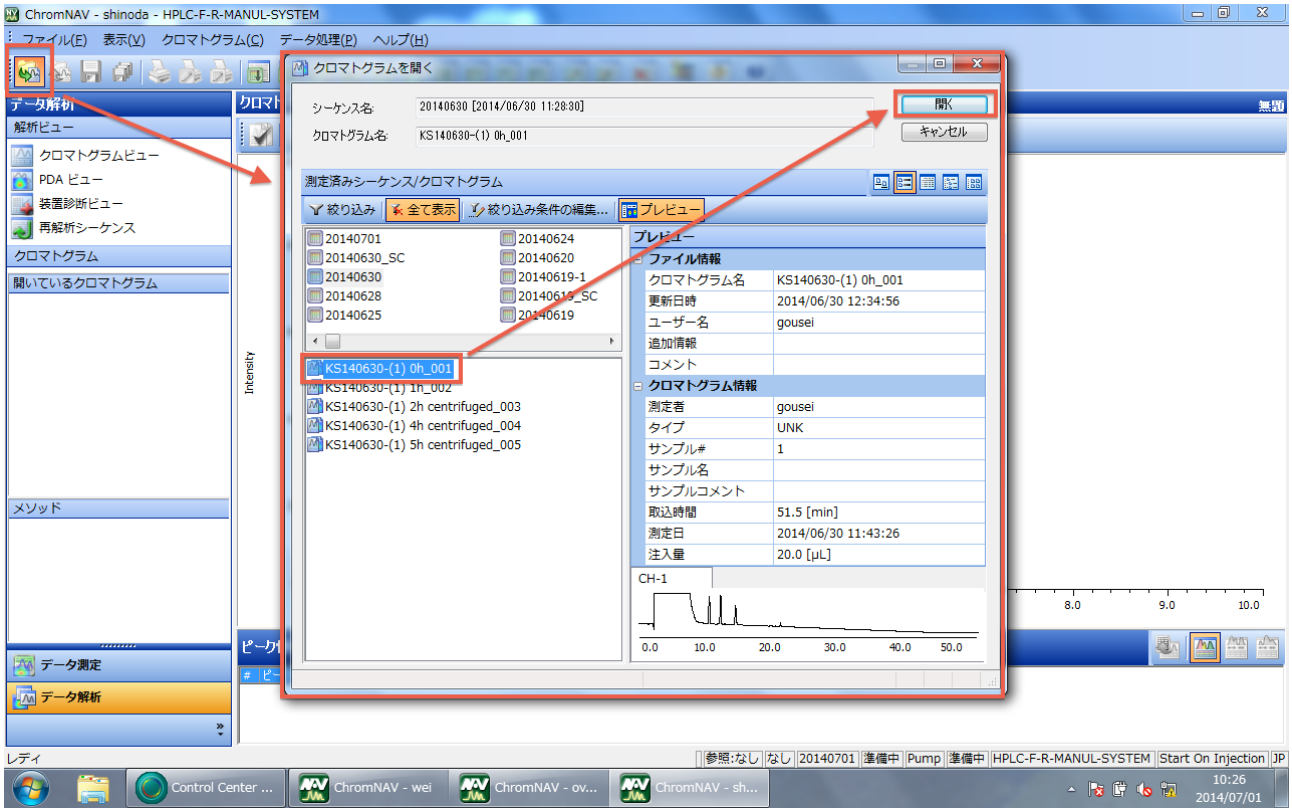
During measurement, you can also analyze your data.

Please click “データ解析” to change the analysis mode.



(1) Opening your Data

You can open the list of your data files as follows.

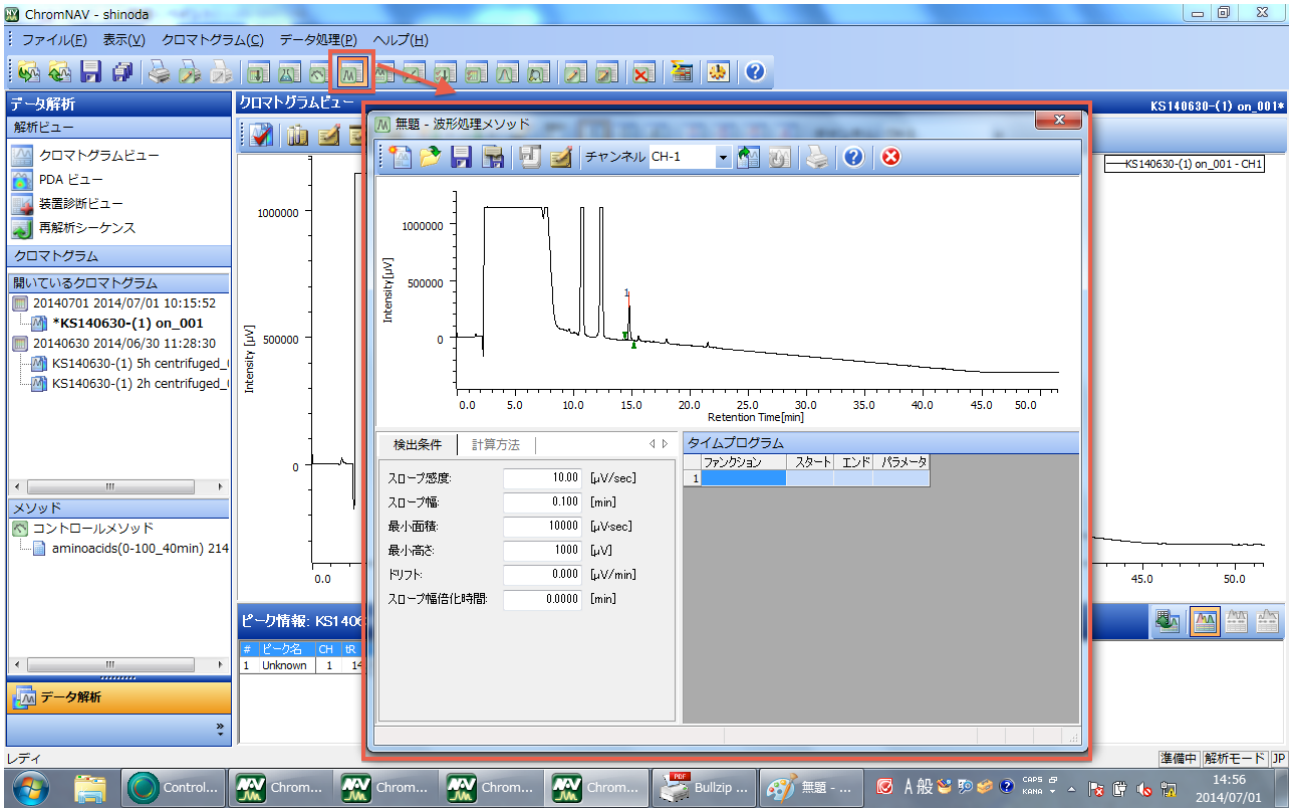


Please select the data and click “開く”, and your data will be displayed.

You can also open your data file by double-click.

(2) Automatic Peak-Picking

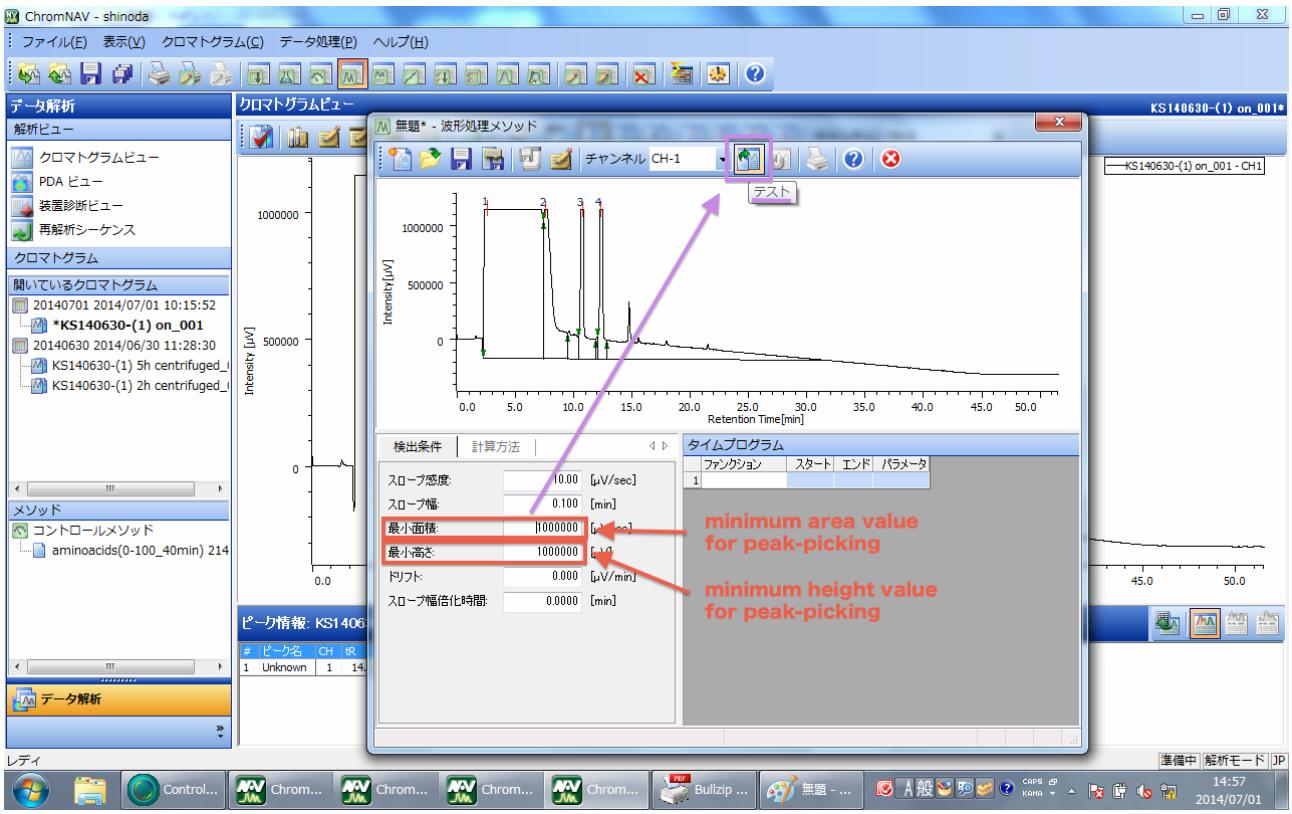
Please click the icon named “波形処理メソッドの編集”, and the new window will be opened as follows.



Please change the following values. These values are the criteria for automatic peak-picking.

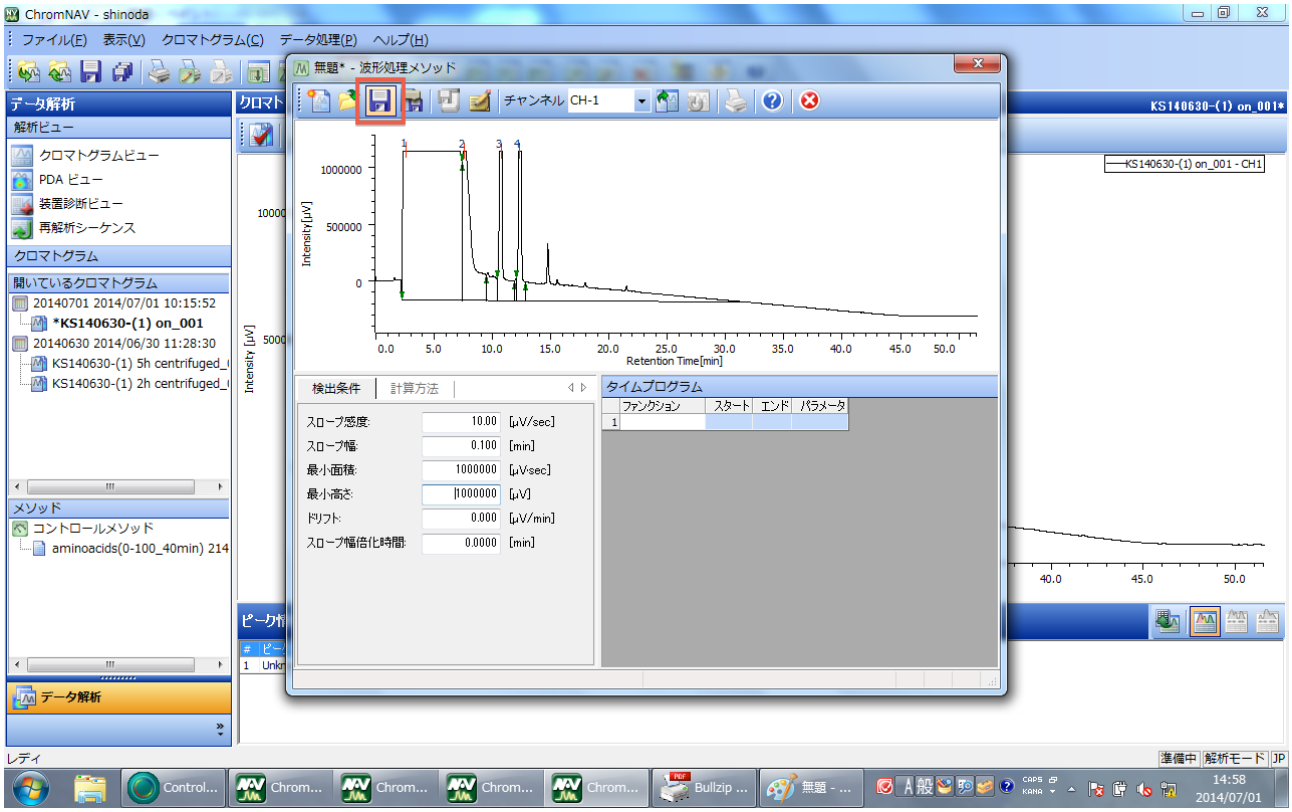
- minimum area value
- minimum height value

In order to check whether those values are suitable for your data or not, please click the icon named “テスト” and check the automatic peak-picking result.

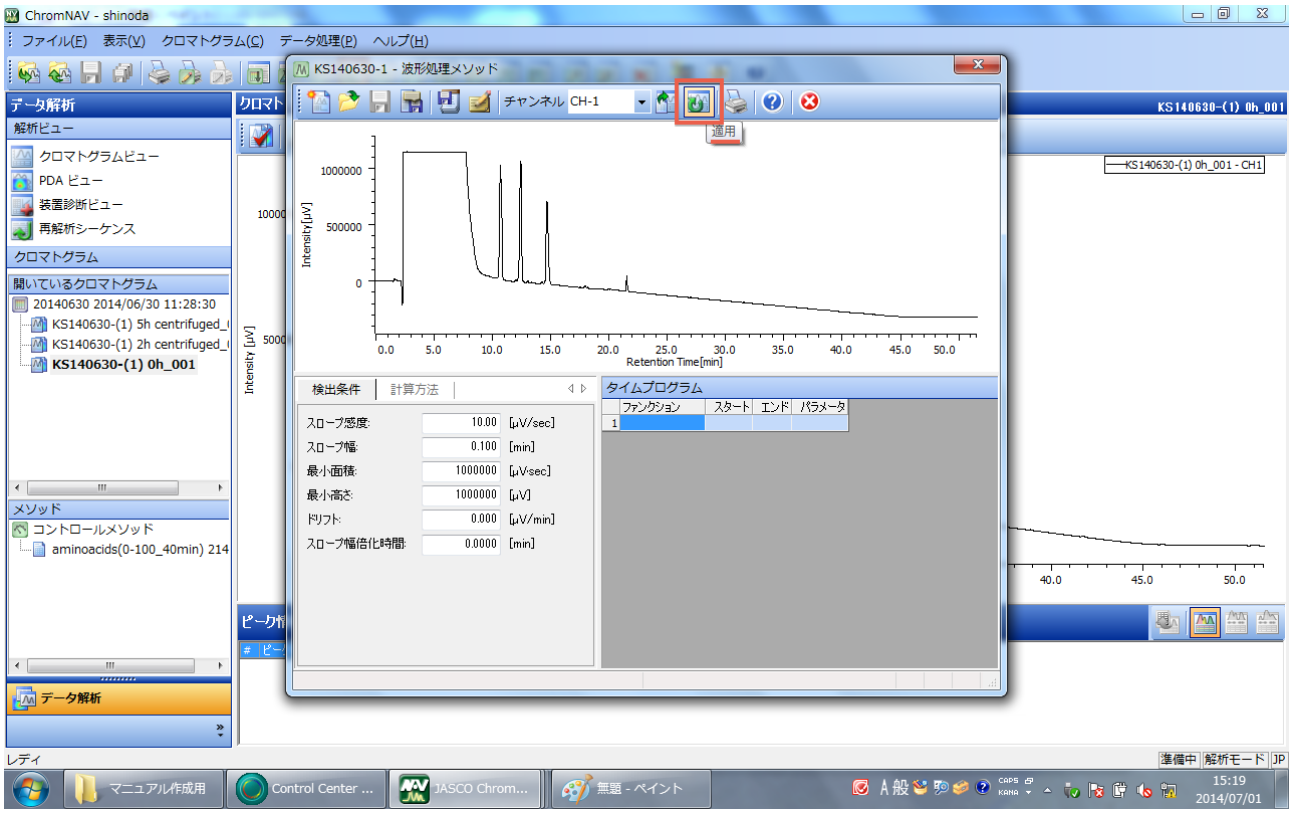


When you feel that peak-picking is not good, please change the criteria above further.

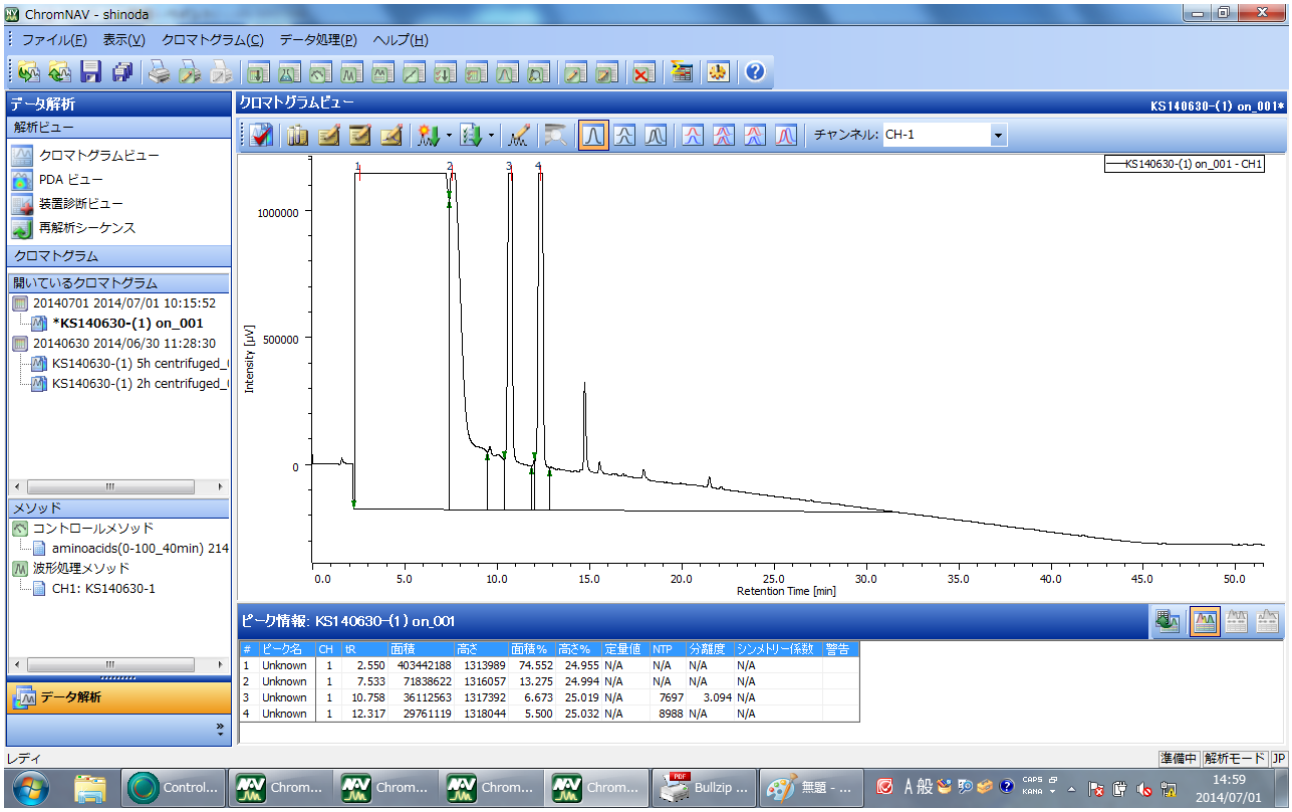
When you can obtain the suitable criteria, please click the save icon and save these values.



After that, the icon named “適用” will become active. Please click this icon.

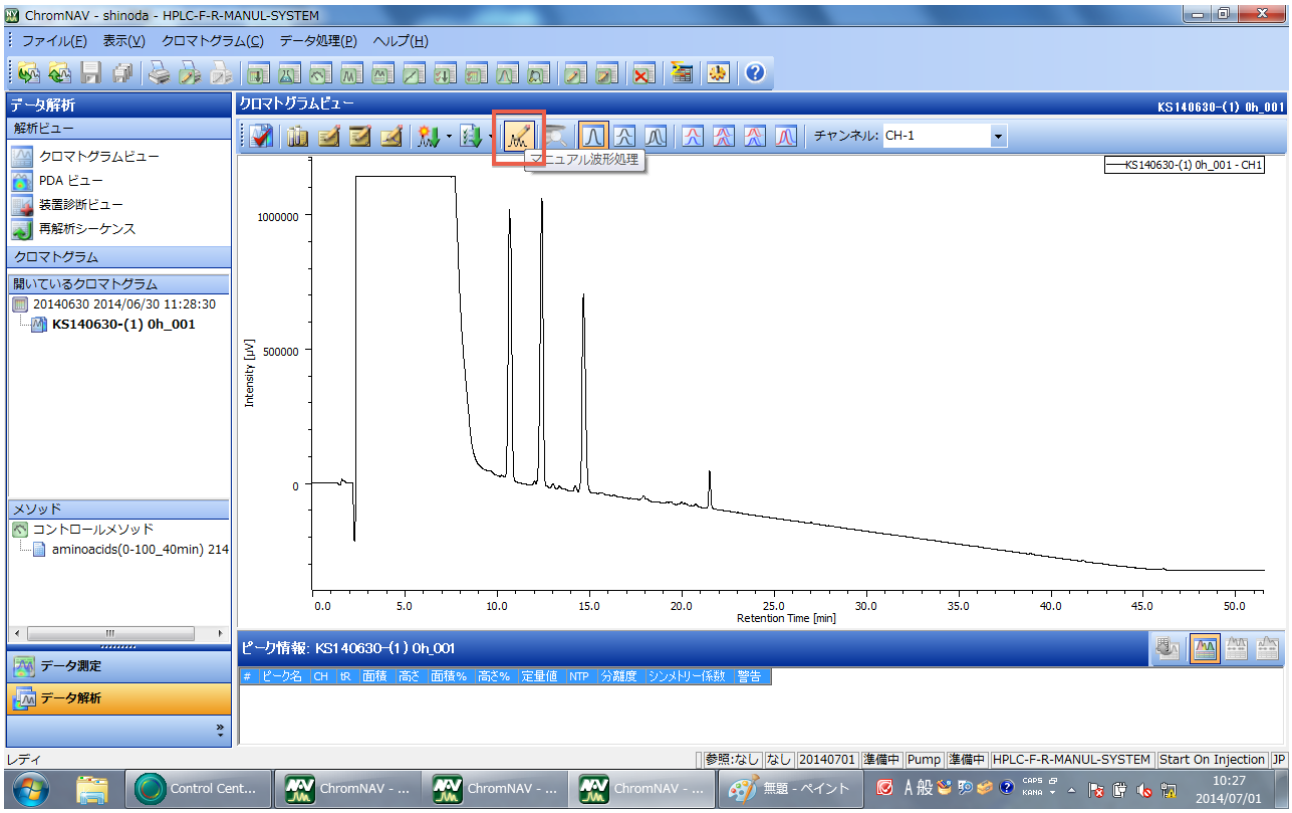


Automatic peak-picking will be carried out and you will see the following view.

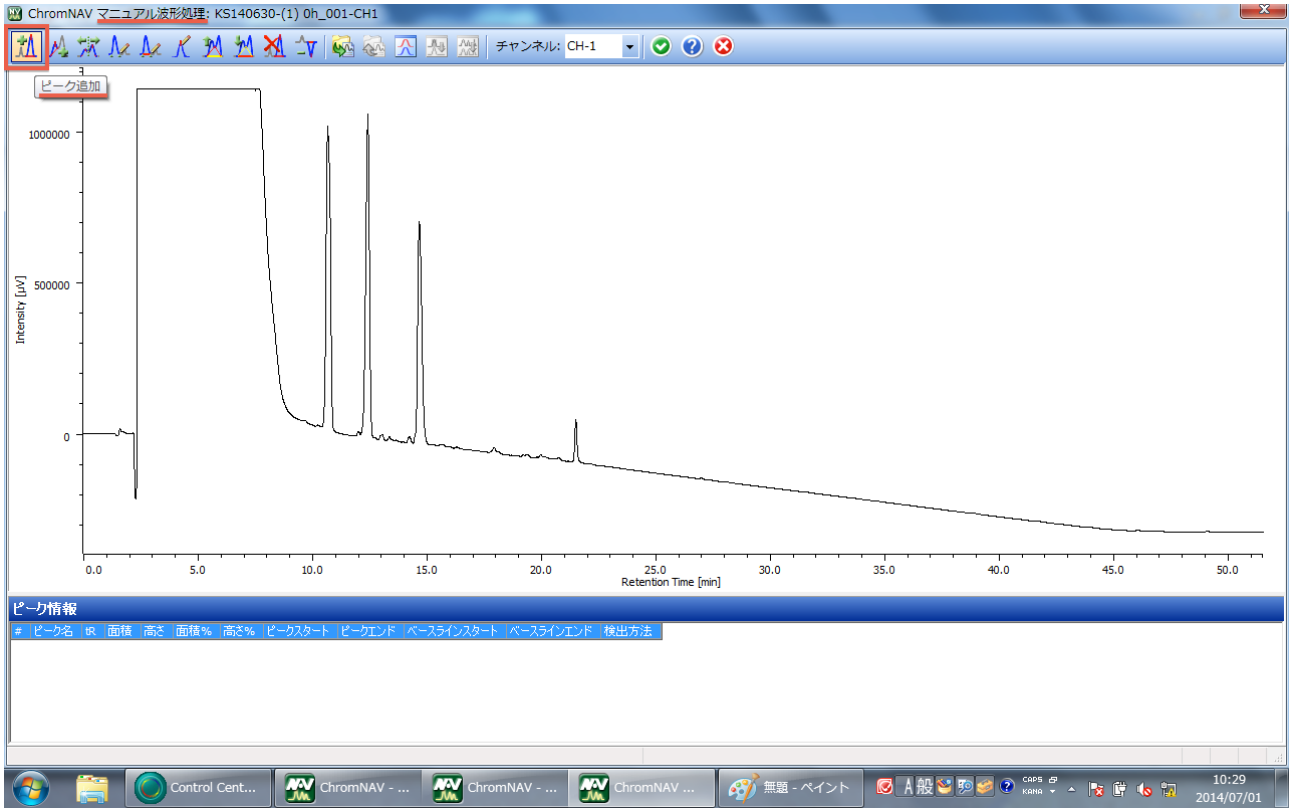


(3) Manual Peak-Picking

Please click the icon named “マニュアル波形処理”, and the new window will be opened.



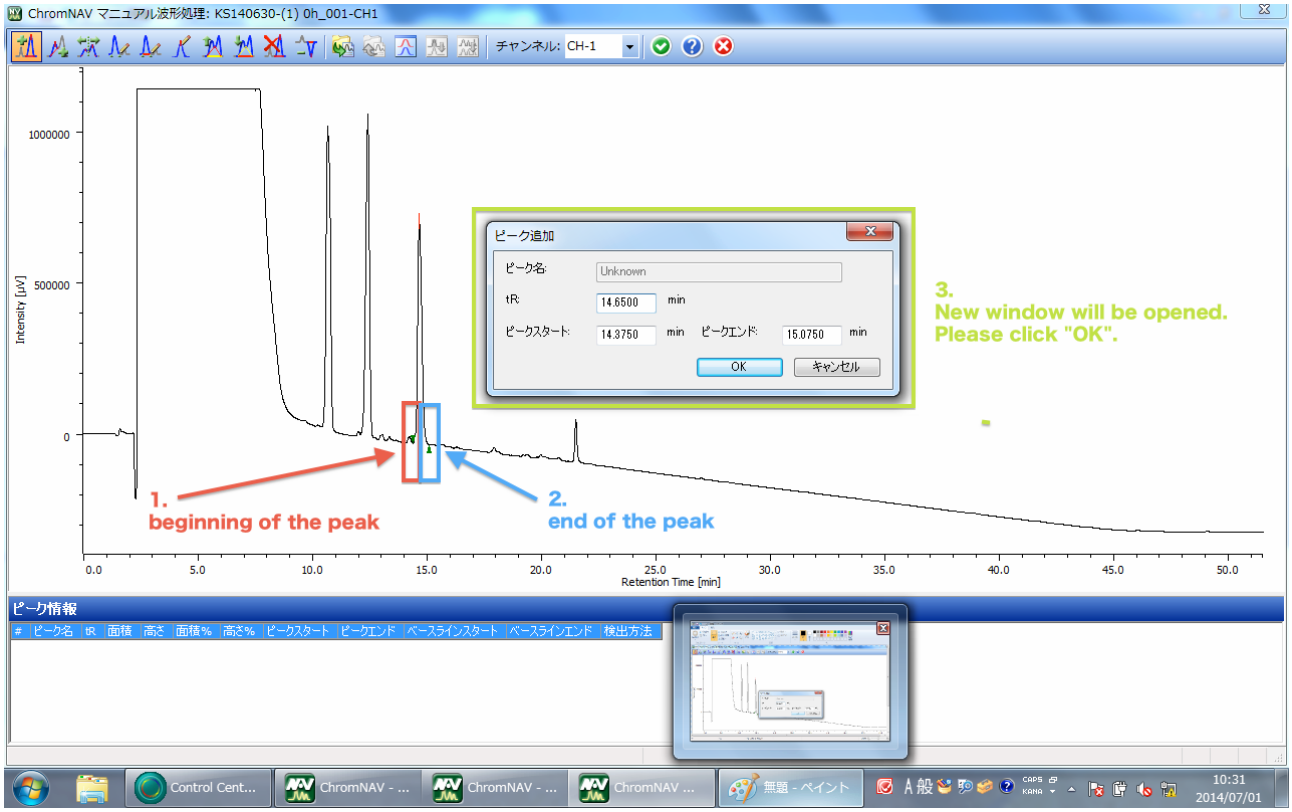
Please click the icon named “ピーク追加”, and the mouse icon will be changed to the pen icon.



First, please select the beginning of the peak.

Second, please select the end of the peak.

After that, the new window will be opened as follows.

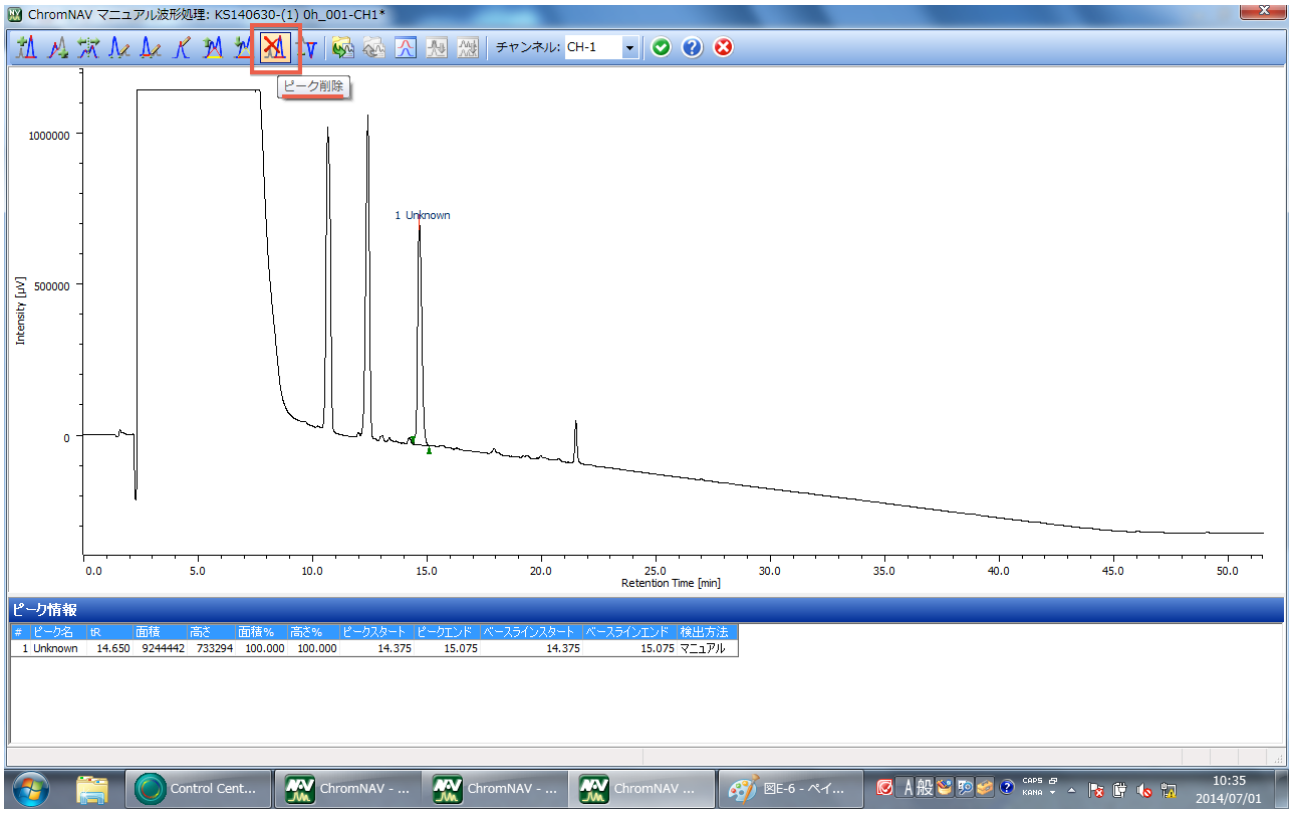


Please click OK, and the peak-picking will be finished.

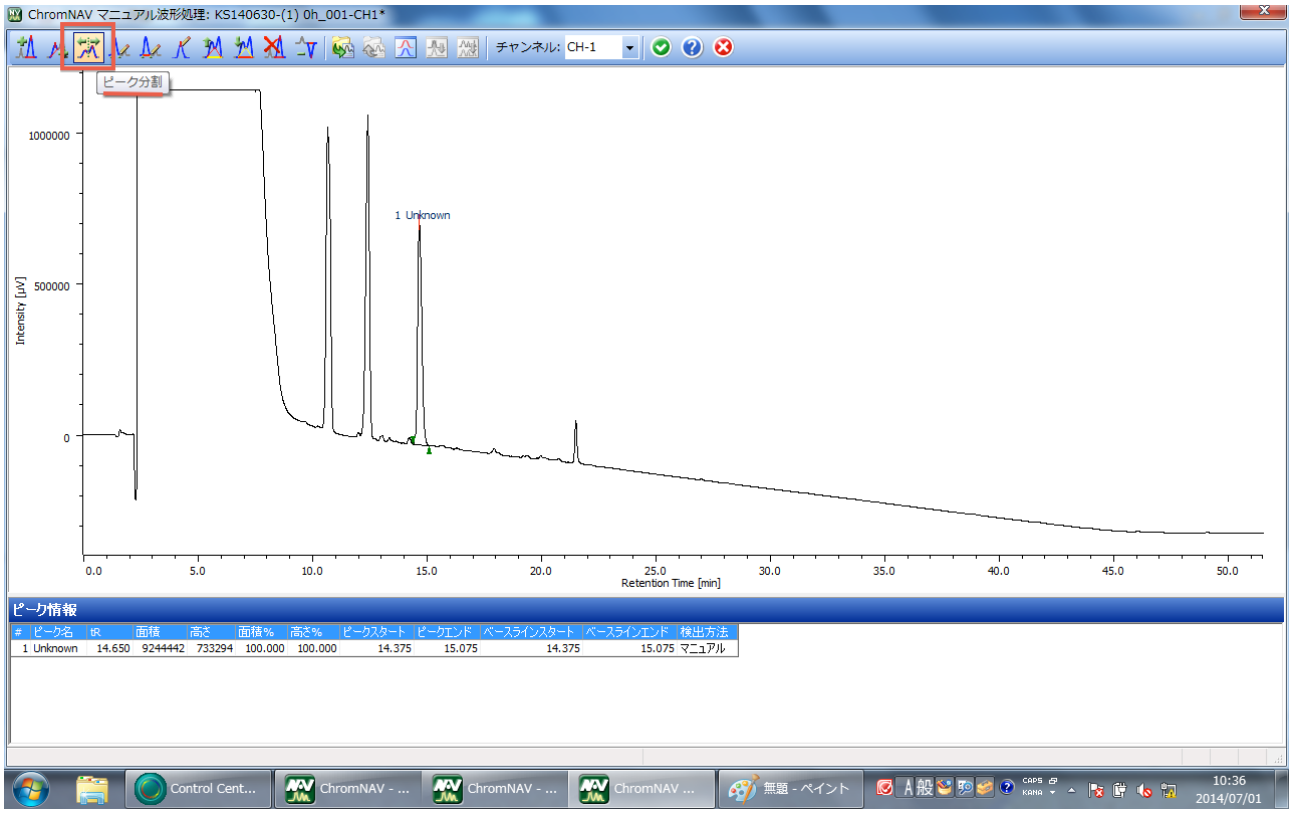
When you want to enlarge the display, please drag and select the region you want to enlarge using left button.

You can finish peak-picking mode when you click the right button.

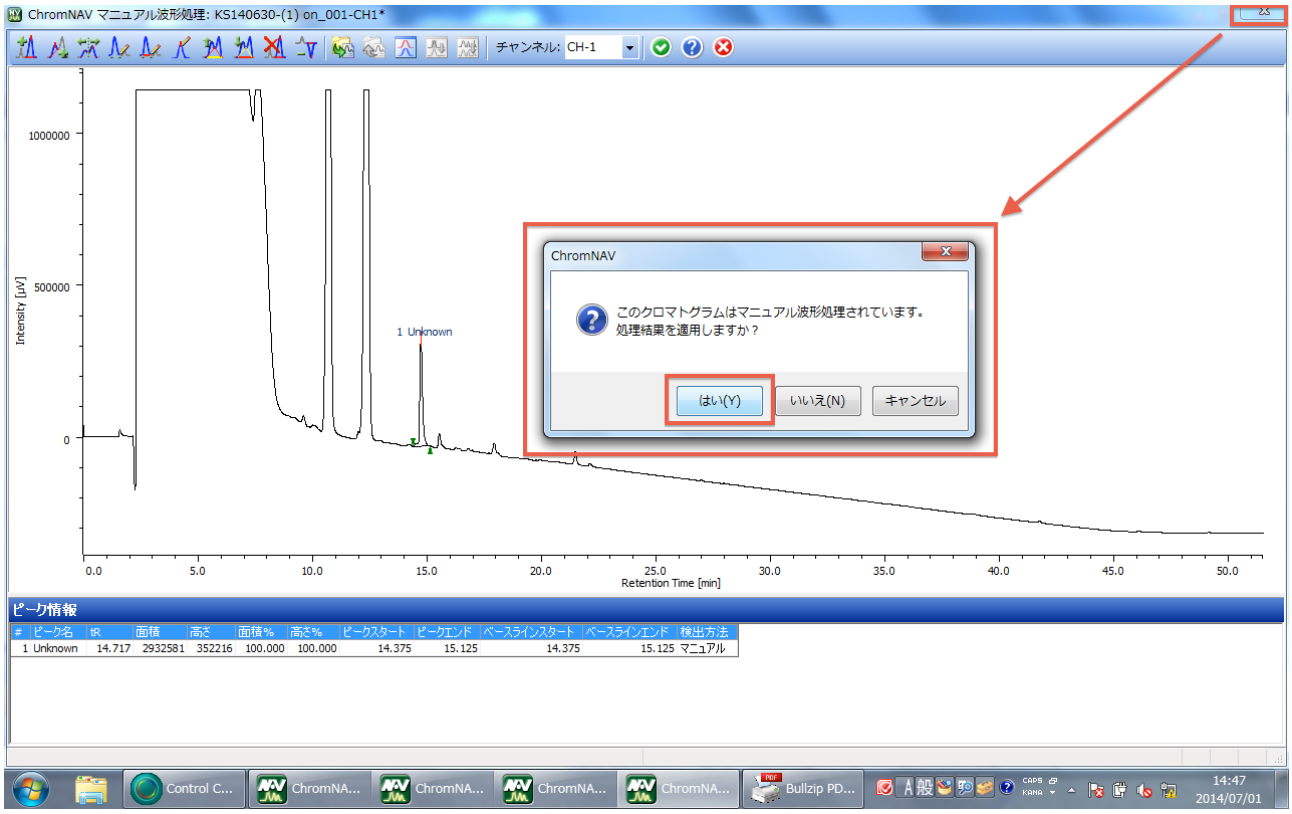
When you want to delete the picked peak, please click the icon named “ピーク削除” as follows and select the peak you want to delete.



When you want to separate the picked peak in two, please click the icon named “ピーク分割” as follows and click the separation point.

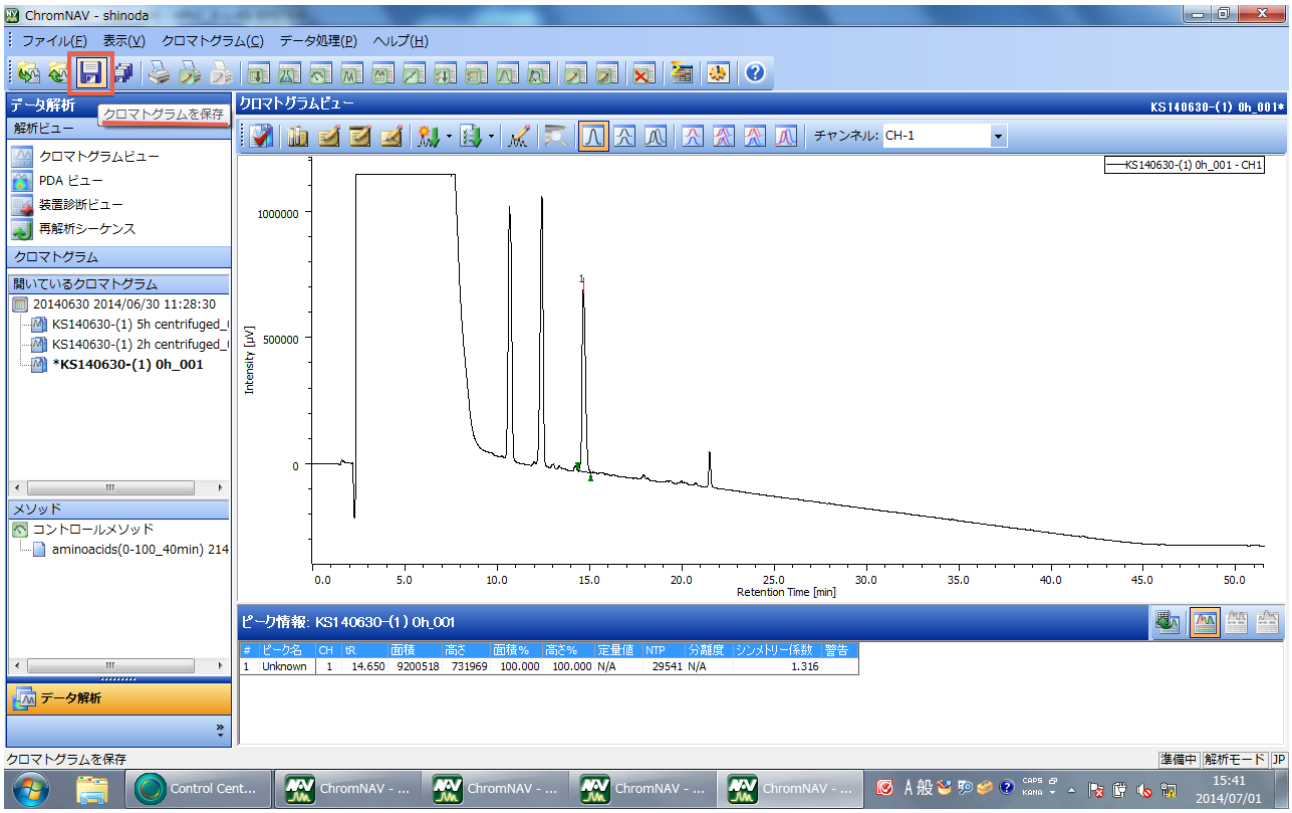


In order to save the peak-picking data, please click close icon and choose “はい(Y)” in the newly opened window as follows.



(4) Save your data

Please click the icon named “クロマトグラムを保存” and save the peak-picking data.



When you click the adjacent right icon, you can save peak-picking data of all chromatograms.

F) Preparation of Eluents

(1) Eluent A: How to prepare it?

First, using Milli Q generator, please fill the empty 3L gallon bottle (previously used as Eluent A bottle) with 3 L of Milli Q.

Using plus button and minus button, please adjust liquid volume and push start button.

Then, please add 3 mL of TFA (HPLC grade) using measuring pipette (please don't use Pipetman as much as you can!!).

The HPLC-grade TFA is in refrigerator at Eisai 1F (If you open the last one, please order it!!).

At last, please put it around the analytical HPLC (JASCO).

(2) Eluent B: How to order it?

When you open the 2nd box of acetonitrile (LC/MS grade) and the number of remaining bottles becomes three, please order it.

In Gousei Reagent Ordering Sheet (Google), you can find the following past order:

Acetonitrile -Plus-, 3L*6, 01033-76, ¥19,000*6

Please copy that line and paste it to order new bottles.

分取 HPLC 使用の際の注意

2013.9.26　HPLC 係：篠田 清道

- 圧力の上限について

分取 HPLC を使う際の圧力の上限は，製造者より以下のように指定されています。

	YMC	Mightysil	Cosmosil
圧力上限(MPa)	10 程度	20	15

カラムの劣化を防ぐのに役に立つので，ぜひご検討いただきたいと思います。

可能であれば，コントロールメソッドを作成する段階において，最大圧力を**「上記の値+5」**に設定していただきたく思います。

流速の目安は以下のとおりです。

	YMC	Mightysil	Cosmosil
10 mm	3~4 mL/min	4~6 mL/min	3~4.5 mL/min
20 mm	8~10 mL/min	10 mL/min	10 mL/min

なお，カラムの状態によっては，流速を落とさなければならないこともあると思います。

不明な点などありましたら，ご遠慮無く篠田までお知らせください。