1.Check Solvent Agilent A:MeOH B:CH3CN

Gilson H2O/IPA etc

2.Turn On HPLC modules

-Agilent Dagasser -Agilent Binary Pump -Agilent Column Oven -Gilson 215 Autosampler(Behind instrument) -Gilson 819 Valve(Behind instrument)

3.Start micrOTOF Control

4.Start Hystar

## 5.Select Sample Table from Hystar



6.Select Template file or existing file.

| 7.Modify parematers on General Tab<br>-Sample Identifier<br>-Vial Position<br>-Number of Injections<br>-Volume<br>-prerun :normally 0 min<br>-Subdirectory   |                    |
|--|--------------------|
| D:\Methods\Sample_Table\template.xml   |                    |
| Elle View Edit Help  |                    |
|  |                    |
| Upen SaveAs Acquisition Print Reload UB Gel Results           Upen SaveAs Acquisition Print Reload UB Gel Results           Upen SaveAs Acquisition In The Reload UB Gel Results           Upen SaveAs Acquisition Print Reload UB Gel Results   | Data Dataŭ palurid |
| Tome vian status sample to 10, volume (µ) whoand (µ) cata sam wernou 10 wernou 10 ward at autosampler wernou sampler wernou sam | nant DataAnaiysis  |
|  |                    |
|  | F                  |
| Sample description Sample Identifier: test (max. 30 letters) Sample Weight [mg]; 0 Dilution [m]; 1 Internal Standard [mg]; 0   |                    |
| Autosampler Parameters     Mumber of 1     Volume [µ]: 5     Prerun [min]: 0       Vial     1     Impections:     1     Volume [µ]: 5     Prerun [min]: 0       Select in Tray     Amount [µg]:     0     0  |                    |
| Result Data Path<br>Standard Path: D:\Data<br>Subdirectory: Test   |                    |

8.Select Methods on Methods Tab

-Check out Use Method

-Select LC Method D:/Methods/LC\_Methods/lc\_200ul\_20120209.m A/B=50/50, 200ul/min, 1min acquisition -Autosampler :Standard Wash -MS Method D:/Methods/MS\_Methods LCMS\_esi\_pos\_low.m LCMS\_esi\_pos\_wide.m LCMS\_esi\_neg\_low.m LCMS\_esi\_neg\_low.m LCMS\_esi\_neg\_wide.m LCMS\_esi\_neg\_high.m

9.Add sample if necessary. Fisrt sample should be Calibrant. ie. Tuning Mix or NaFormate Follow the Samples after calibrant.

| )pen              |                                | Save As                             | Acquisition                         | d P          | nint Reload             | 08 Gel      | "Au<br>Results  |            |   |   |   |            |
|-------------------|--------------------------------|-------------------------------------|-------------------------------------|--------------|-------------------------|-------------|---|------------|---|---|---|------------|
| e \               | vial [                         | Status                              | Sample ID                           | Inj.         | Volume [µl]             | Amount [µg] | Data Path   | Method     | LC Method Part  | Autosampler Method Part                         | MS Acquisition Method Part  | DataAnaly: |
| 1 1<br>2 1<br>3 1 | 1                              |                                     | calibration<br>sample<br>sample     | 1 1 1        | 5.000<br>5.000<br>5.000 | 0.000       | bdal\pm20131125<br>bdal\pm20131125<br>bdal\pm20131125 |            | lc_method\lc_200ul_20120209.m<br>lc_method\lc_200ul_20120209.m<br>lc_method\lc_200ul_20120209.m | Standard:Wash<br>Standard:Wash<br>Standard:Wash | ms_method\lcms_esi_pos_wide.m<br>ms_method\lcms_esi_pos_wide.m<br>ms_method\lcms_esi_pos_wide.m |            |
| iene<br>Sa        | ral  <br>ample<br>ample        | Methods<br>e descrip<br>e Identifie | Details   1<br>tion<br>ar: [calibre | Calibra      | ation ]                 |             |   |            | (max. 30 lettere)   |   |   |            |
| Au<br>Via<br>Po   | utosa<br>al<br>sitior<br>esult | mpler Pa                            | rameters                            | {Mai<br>e.g. |                         |             |   | Nu<br>Inje | mber of 1   | 1): 5 Prerun (mir<br>1): 0                      | ų [0  |            |

11.Click Save as and rename this sample table.

12.Select top line of the sample table, then click Acquisition.



13.If Pump is not ready(Yellow), Click right mouse button then select"pump on"

| P                | 'ump On<br>'ump Off  |
|------------------|--|
| P<br>S<br>R<br>D | ump Settings<br>iolvent Bottle Fillings<br>testore Initial Conditions<br>Diagnostics |
|                  |  |

Reset Communication...

About Agilent Pump

If Gilson215 is not recognized, Click right mouse button then select"initialize"

| Select Tray Config<br>Show Vial Contents<br>Show Tray         |
|---|
| Stop Recover<br>Stop Procedure<br>Prime Syringe<br>Initialize |
| Add. Settings   |
| About Gilson 215<br>Show Logfile                              |

## 14.Click Start to start sequence.

| HyStar (0)       | Pump: ready |            | Gilson 215          |                      | Col. 2          |                      |           |                   |
|------------------|-------------|------------|---------------------|----------------------|-----------------|----------------------|-----------|-------------------|
| ready            | Flow 0.20   | Press 11.3 | Ready               |                      | <b></b> n       |                      |           |                   |
|                  | Α%          | В%         | Vial/Inj 1          | 171                  | · ·             | MS (micrC            | TOF serie | es)               |
| Time 0.0 1.0     | 50.0        | 50.0       | Inj.Vol 5.0         | )                    | 29.9°C          | ор                   | erate     |                   |
| 🕒 Sample Table   | mple 🚱 Rela | bad Line:  | Vial Position:<br>1 | Samp<br>calibr       | le ID:<br>ation |                      |           | LC Mi<br>Ic_20    |
| Start Stop Start | Stop A      | bort Pause | Cont. Zoom          | j <u>⊾</u><br>Cursor | Stretch         | <mark>ж</mark><br>PP | <b>X</b>  | <u>ал</u><br>µTOF |

15.Click Start sequence

| Start A | utosampler              |   | ×     |
|---------|-------------------------|---|-------|
|         | Start Autosampler. To s | tart the run, click one of the large buttons                              |       |
|         | Start sequence          | (will start at line 1 vial 1 of Sample Table,<br>number of injections: 3) |       |
|         | Start one acquisition   | (line 1 vial 1 of Sample Table will be used)                              |       |
| 0       | 👯 Shutdown Settings     |   | tions |

16.To stop the sequence, click Stop



Stop

18.To abort LC run, click Abort



19. To finish the acquisition, close Hystar then turn on each HPLC modules.



MS 解析マニュアル

~共通操作~

- 1. DataAnalysis を開く。
- 2. メニュータブの[File]→[Open]もしくは下の Open マークをクリックして標準サンプルまたは解析したいデータを開く。
- 3. ファイルを開くと下のような画面になる。



ゆ を押し、この曲線のなるべく高さが高いところで左クリックすると、その時間に測定されていたスペクトルが 表示されるので、スペクトルの上で右クリック→Copy to Compound Mass Spectra を選択する。すると下二段の上 にスペクトルがコピーされる。

| 3 NaFormate_1_01_503.d (m  | nodified) [Date Analysis micro TOF analysis.m] - Bruker Daltonics DataAnalysis   | - 0 <mark>- X</mark>    |
|----------------------------|--|-------------------------|
| Eile Edit Find MassList De | econvolute Identify Process Calibrate Annotation Method View Iools Window Help   |                         |
| NaFormate                  |  |                         |
| S . 6 . 6 .                | Bu a a Q.  |                         |
| A & A A A A                | · · · · · · · · · · · · · · · · · · ·  |                         |
| Analysis List              | Chromatogram - NaFormate_1_01_503.d: TIC +All MS   | 8                       |
| n e II na anna iorionaí    |  | Time [min]              |
|                            | \Overlaid Astacked A List /  |                         |
|                            | Mass Spectrum View - NaFormate_1_01_503.d  |                         |
|                            | ×10 <sup>4</sup> 225 9526  | +MS, 0.30.3min #(17-20) |
|                            | 1 100.0000 ↔ Auto-Scaling Alt+F9 566,8872 634,8761 702,8538<br>+ + 100 Q Zoom In F9, [Shift] 660 7/0 800 9   | 900 m/z                 |
|                            | Compound Mass Spectra - NaForm<br>Add Base Boak Chromatoram (m/z 180, 589, 181, 589)<br>Add Batracted Ion Chromatogram (m/z 180, 589, 181, 589)<br>Copy to Compound Mass Spectra and Identify<br>III. Copy To Compound Mass Spectra and Identify<br>IIII. Copy To Compound Mass Spectra and Identify<br>III. Copy To Copy To Copy To Copy To Copy To Co |                         |

## ~検量線の作成~

- 1. スペクトルをコピーしたらメニューバーから[Calibrate] $\rightarrow$ [Internal]を選択する。
- 2. 下のような画面が開く (図はギ酸ナトリウムの場合)。

| 1           | Substance   | Ref. Mass            | Cur. Mass | Res. (m/z)  | Error [          | 1           |
|-------------|---|----------------------|-----------|---|------------------|-------------|
| 1           | Na(NaCOOH)1   | 90.9766              |           |   |                  | Auto Searc  |
|             | Na(NaCOOH)2   | 158.9641             |           |   |                  |             |
|             | Na(NaCOOH)3   | 226.9515             |           |   |                  |             |
|             | Na(NaCOOH)4   | 294.9389             |           |   |                  | Hemove      |
|             | Na(NaCOOH)5   | 362.9263             |           |   |                  |             |
|             | Na(NaCOOH)6   | 430.9138             |           |   |                  | Hemove A    |
|             | Na[NaCUUH]/   | 498.9012             |           |   |                  |             |
|             | Na(NaCUUH)8   | 566.8886             |           |   |                  |             |
|             | Na(NaCUUH)9   | 534.8750             |           |   |                  |             |
|             | Na(NaCOOH)11  | 702.8635             |           |   |                  |             |
|             |   | 000 0000             |           |   |                  |             |
|             | Na(NaCOOH)12  | 906 8257             |           |   |                  |             |
| 8           | Na(NaCOOH)14  | 974.8132             |           |   |                  |             |
|             |   |                      |           | Standard  | deviation [ppm]: | 0           |
| li          | bration coefficient:                                    | *)                   |           | 11. martine 1. ma<br>tened 1. martine 1. martin |                  | 0.0001419   |
| ]<br>III    | bration coefficients<br>revious: C0 19                  | 2.854146             | - (       | 402663.24   | 12               | 1-0.0001413 |
| alii<br>P N | bration coefficients<br>'revious: CO [19<br>lew: CO [19 | 2.854146<br>2.854146 |           | 402663.24           1         402663.24           1         402663.24   | C2               | -0.0001419  |

まず、右上の[Select List]から使用した標準サンプルとメソッド(pos or neg)を[Calibration list]より選択する。その 後[Apply]→[OK]

| Calibration list   | Tunemix (pos)  |  |      |
|--|--|--|------|
| C5H1202N<br>C6H1906N:<br>C12H1906N<br>C18H1906N<br>C18H1906N<br>C30H1906N<br>C30H1906N<br>C42H1906N<br>C42H1906N | 3P3<br>(3P3F12<br>(3P3F24<br>(3P3F40<br>(3P3F48<br>(3P3F72<br>(3P3F72<br>(3P3F96 | Mase Com<br>118/0963<br>322/0481<br>622/0290<br>922/0098<br>1321/9842<br>1521/9715<br>2121/9332<br>2721/8948 | ment |
|  |  |  |      |

OK を押すと元の画面に戻るので[Auto Search]をクリック。

|                       | 1       | Ref. Mass            | Cur. Mass | Res. (m/z) |              | Error [ppm | 1           |
|-----------------------|---------|----------------------|-----------|------------|--------------|------------|-------------|
| Na(NaCOC              | IH)1    | 90.9766              |           |            |              |            | Auto Searcl |
| Ma(NaCOC              |         | 108.3641<br>220 0515 |           |            |              | _          |             |
| Na(NaCOC              | ны      | 220.3313             |           |            |              |            | Bemove      |
| NafNaCOC              | 1115    | 362 9263             |           |            |              |            |             |
| NafNaCOC              | HI6     | 430.9138             |           |            |              |            | Remove Al   |
| Na(NaCOC              | IH)7    | 498.9012             |           |            |              |            |             |
| Na(NaCOC              | JH)8    | 566.8886             |           |            |              |            |             |
| Na(NaCOC              | JH)9    | 634.8760             |           |            |              |            |             |
| Na(NaCOC              | JH)10   | 702.8635             |           |            |              |            |             |
| Na[NaCUL              | IHJ11   | 770.8509             |           |            |              |            |             |
| Na(NaCOC<br>Na(NaCOC  | 1112    | 906 9257             |           |            |              |            |             |
| Na(NaCOC              | JH)14   | 974.8132             |           |            |              |            |             |
| ibration coef         | icients |                      |           | Stan       | dard deviati | on [ppm]:  | 0           |
|                       | 0 192   | .854146              | - 1       | 402663.2   | 4            | C2         | -0.0001419  |
| Previous: C           | 0 192   | .854146              |           | 402663.2   | :4           | C2         | -0.0001419  |
| Previous: C<br>New: C | 0 1102  |                      |           |            |              |            |             |

すると、各 Error の値が表示されるので、Error の値が大きいものをクリックし、[Remove]で取り除く。(ただし、 測定するサンプルの分子量付近のピークは除かない方がよい。)各 Error の値が3未満、右に表示されている Standard deviation の値が1~2未満となるようにする。

| 2   | Substance            | Ref. Mass | Con Marcel 1 | ·          | Em              | or [ppm] |             |
|-----|----------------------|-----------|--------------|------------|-----------------|----------|-------------|
| ĺ   | Na(NaCOOH)1          | 90.9766   |              |            |                 |          | Auto Search |
|     | Na(NaCOOH)2          | 158.9641  |              |            |                 |          |             |
|     | Na[NaLUUH]3          | 226.9515  |              |            |                 |          | Domous      |
|     | Na(NaCOOR)4          | 234.3363  |              |            |                 |          | nemove      |
|     | Na(NaCOOH)S          | 430 91 38 |              |            |                 |          | Remove All  |
|     | Na(NaCOOH)7          | 498,9012  |              |            |                 |          |             |
|     | Na(NaCOOH)8          | 566.8886  |              |            |                 |          |             |
|     | Na(NaCOOH)9          | 634.8760  |              |            |                 |          |             |
|     | Na(NaCOOH)10         | 702.8635  |              |            |                 |          |             |
|     | Na(NaCOOH)11         | 770.8509  |              |            |                 |          |             |
|     | Na(NaCUUH)12         | 838.8383  |              |            |                 |          |             |
| •   | Na(NaCOOH)14         | 974.8132  |              |            |                 |          |             |
| - 6 | bration coefficients |           |              |            | ard deviation [ | nomi 0   |             |
|     |                      | 0.054140  | -            |            |                 |          | 001.410     |
| P   | revious: CU [19.     | 2.854146  | _ `          | 40266 8.24 | 4               |          | 1001419     |
| N   | lew: C0 19           | 2.854146  | С            | 402663.2   | 4               | C2 -0.0  | 001419      |
|     |                      |           |              |            |                 |          |             |
|     | -                    |           |              |            | To a            | 10       | 1           |

3. 調整が終わったら Recalibrate をクリックし、その後ファイルを上書き保存する。

~測定サンプルの解析~

1. メニューバーから[Calibrate]→[External]を選択する。

2. 下のような画面が表示されるので、[Load]をクリックし先ほど作成した検量線のデータを選択し OK をクリック する。

| External Mass Spectrum Calibration          |      | ? ×    |
|---|------|--------|
| Use calibration coefficients from analysis: |      | ок     |
|   | Load | Cancel |
|   |      | Help   |

3. メニューバー[Tools]→[Generate Moleclar Formula]をクリック。

| d <u>V</u> iew | Tools  | Window Help   |                                 |   |
|----------------|--------|---|---------------------------------|---|
|                |        | nicrOTOFcontrol<br>ibraryEditor<br>GeportDesigner<br>SutomationEngine | Ctrl+F11                        |   |
|                | GMF G  | Simulate <u>P</u> attern<br>Generate Molecular Fo                     | rmula F8                        | F |
|                |        | <br>Dustomize<br>Options<br>Colors                                    | anna an Gind a' se dd a son 250 |   |
|                | E<br>E | ProcessWithMethod<br>eprocess<br>IpdateAnalyses                       |                                 |   |

下のウィンドウが表示されたら必要な数値を入力する。

| Min   | C32H52                         | ) <sub>5</sub> Na |                            |          |                             |  |                    | Genera           | ate      |
|-------|--------------------------------|-------------------|----------------------------|----------|-----------------------------|--|--------------------|------------------|----------|
| Мах   |                                |                   |                            |          |                             |  | Sa                 | ave Res          | ults     |
|       | C 24-n, H                      | 42•n, N           | la 1-n, O                  | 3-n      |                             |  |                    | Help             | )<br>    |
|       | Note: for r                    | n < 200           | 10 the ele                 | ments C, | H, N, and                   | l O are conside  | ered implicitly.   |                  |          |
| Measu | red m/z 53                     | 39.369            |                            | Toler    | rance (pp                   | m] 100   | Charge             | 1                | ×        |
| #     | Mol. Formu                     | ila               |                            | a        | m/z                         | lerrl [ppm]  | err [ppm]          | mean             | n err (p |
|       |                                |                   |                            |          |                             |  |                    |                  |          |
| Au    | omatically lo                  | cate m            | onoisotop                  | ic peak  | Maximu                      | im number of f   | ormulas            | 200              |          |
| Aul   | omatically lo                  | cate m            | onoisotop                  | iic peak | Maximu<br>Minimu            | im number of f   | ormulas            | 200              |          |
| Aul   | omatically lo<br>ack rings plu | cate m<br>is doub | onoisotop<br>le bonds<br>] | ic peak  | Maximu<br>Minimu<br>Electro | im number of finance of finance of finance of finance of the finan | ormulas<br>Maximum | 200<br>0<br>both |          |

Min: 測定したサンプルの分子式とその分子に付くイオン(上図の場合では Na+)を合わせたものを入力 Tolerance: 100 や 1000 など(適当でよいが、あまり小さいと計算値のピークが表示されない場合がある) Measured m/z: 入力欄をクリックした後スペクトルに戻るとカーソルの右に"GMF"と表示されるので、拾いたいピ ークの上でクリックすると自動で数値が入る。

この3つを入力したら Generate を押すと、Error の値が近い分子式と分子量が表示される。右下の Show Pattern を押すと計算値のピークが表示される。

| Max                 | C <sub>32</sub> H <sub>52</sub> O <sub>5</sub> Na                         |              |                                |                  |                               | Gen<br>Save F    | ierate<br>Results |
|---------------------|---|--------------|--------------------------------|------------------|-------------------------------|------------------|-------------------|
|                     | C 32·n, H 52·n, Na  | н            | elp                            |                  |                               |                  |                   |
|                     | Note: for m < 2000  | the elements | C, H, N, and                   | 10 are con       | sidered implicitly.           |                  |                   |
| Meas                | ured m/z 539.369  | Т            | olerance (pp                   | m] 100           | Charge                        | 1                | l la              |
| #                   | Mol. Formula  | m/z          | lerri (ppm)                    | err (ppm)        | mean err (ppm)                | rdb              | N rule            |
| 1                   | C 32 H 52 Na 1 O 5  | 539.3707     | 3.169                          | 3.169            | 2.552                         | 6.5              | ok.               |
|                     |   |              |                                |                  |                               |                  |                   |
| 4                   | m<br>tomaticalir locate more  |              | ak Mavine                      | m numbar         | of formulae                   | 20               | •                 |
| - Au                | m<br>itomatically locate mon  | oisotopic pe | ak Maximu                      | m number         | of formuliss                  | 20               | •                 |
| - (<br>  Aı<br>  Cł | rrr<br>Itomatically locate mon<br>heeck rings plus double                 | oisotopic pe | ak Maximu<br>Minimu            | m number<br>m 0  | of formulas<br>Maximu         | 200<br>m 0       | •                 |
| Au<br>Au<br>Apply   | m<br>Atométically locate mon<br>neck rings plus double l<br>nitrogen rule | oisotopic pe | ak Maximu<br>Minimu<br>Electro | ım number<br>m 0 | of formulas<br>Maximu<br>tion | 200<br>m 0<br>bo | ►<br>0            |

(ACS では分子量の誤差を 5ppm(0.0005%)以内にするようにと記載がある。すなわち許容される誤差 E≦M(分子量)/1000000×5 となる。)

\*毎年最初の JOC に Guidelines for Authors としてその年の基準が記載されるの で確認すること。2006 年が最も厳しく、当研究室ではこの条件を採用している。