

We present two scenarios for Spectrum Deconvolution, a tool published at cceHUB for the deconvolution of LC-MS datasets. The deconvolution model was created by the Bindley Bioscience Center as part of the Purdue Discovery Pipeline. This model is now available as a cceHUB tool, and can be launched from the tool resource page or as part of the cceHUB pipeline workflow. Deconvolution is the first step in the pipeline data analysis process, and prepares input LC-MS datasets for alignment by the Peak Alignment Tool, the second step in the pipeline.

Starting up the Spectrum Deconvolution tool at cceHUB

Click on **Launch Tool**. When the tool is launched, you will see two input phases:

- 1) **LC-MS Dataset**: selection of the input dataset
- 2) **Parameters**: specification of the deconvolution parameters

LC-MS datasets can be selected from the cceHUB repository or from your own dataset collection. Datasets in the repository can be searched by **instrument** (i.e., the instrument which generated the digital representation of the sample analysis) or by **data format** (i.e., the format of the converted instrument-generated dataset). Once an instrument or data format has been selected, you can search data collections and experiments associated with the selected search criteria. When a collection is selected, the datasets belonging to the selected collection are displayed so you can identify the dataset you want to use as input. The parameters you enter will depend on the properties of your dataset, such as the desired range of retention time to use in the analysis.

The execution phase is

- 3) **Analyze**: run the deconvolution using the dataset and parameters specified.

Scenario 1: LC-MS datasets generated by the XCTPlus Ion Trap

Step 1. We will input a file from the cceHUB repository by searching for a file generated by the XCTPlus Ion Trap, from the L001 experiment. Select from the file choices as follows:

Choose input dataset from: cceHUB data repository

Choose dataset by: instrument

Which instrument: XCTPlus Ion Trap

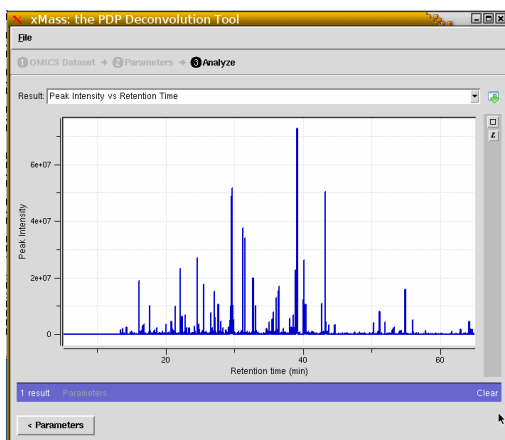
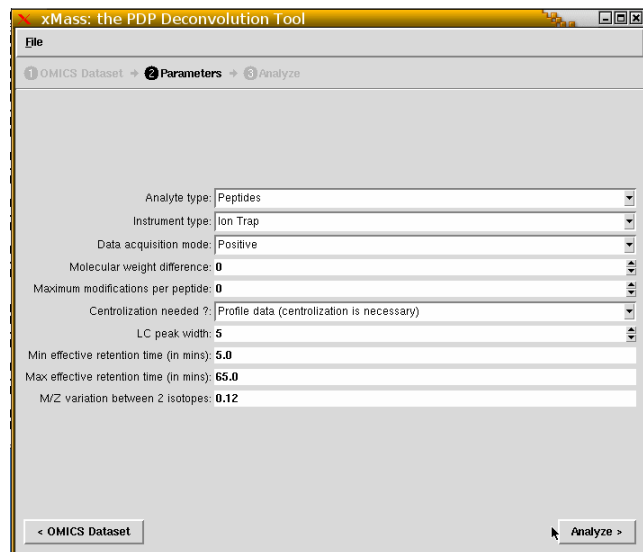
Which XCTPlus Ion Trap experiment: L001 Experiment

Select L001 Experiment file: I11.mzXML

The screenshot shows the 'xMass: the PDP Deconvolution Tool' interface. It features a menu bar with 'File' and three numbered steps: '1 OMICS Dataset', '2 Parameters', and '3 Analyze'. The 'OMICS Dataset' section has a dropdown menu for 'Choose an OMICS input dataset' set to 'cceHUB data repository'. Below this is a search section for 'Search cceHUB data repository' with a dropdown for 'Choose dataset by' set to 'instrument' and a search field containing 'XCTPlus Ion Trap'. The 'Search XCTPlus Ion Trap experiments' section has a dropdown for 'Which XCTPlus Ion Trap experiment?' set to 'L001 Experiment' and a text field for 'Select L001 Experiment file' containing 'I11.mzXML'. A 'Parameters >' button is located at the bottom right.

Step 2. We will now specify the parameters for the selected dataset. Description of these parameters can be found in documentation and articles that accompany this tool. They are the default parameter values for XCT LC-MS data.

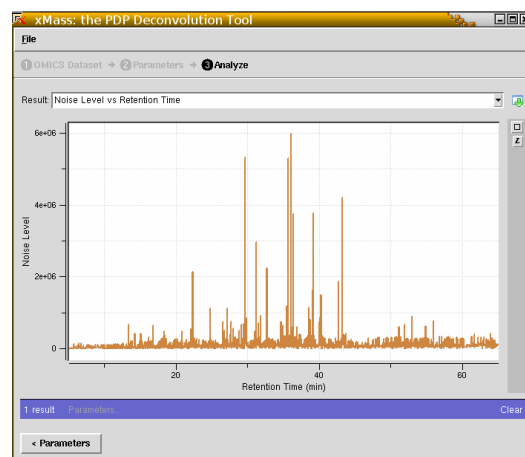
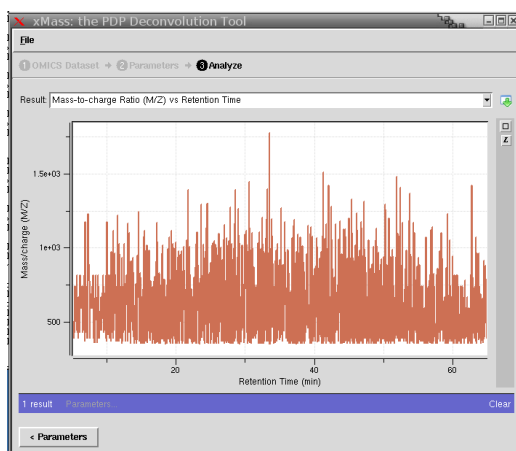
Analyte type: Peptides
 Instrument type: Ion Trap
 Data acquisition mode: Positive
 Molecular weight difference: 0
 Maximum modifications per peptide: 0
 Centralization needed? Profile data (centralization is necessary).
 LC peak width: 5
 Min effective retention time: 5.0
 Max effective retention time: 60.0
 M/Z variation between 2 isotopes: 0.12



Step 3. Click on Analyze. Trace information will be printed as xMass executes. When execution is complete, the graph to the left, Intensity vs Retention Time, is displayed .

You can zoom in to any level by creating a rectangle over the desired zoom area with your left mouse button. Return to the original display by clicking with your right mouse button in the graph area (i.e., if you zoom in 3 times, click with your right mouse button 3 times to zoom back out to the original graph).

Other graphs and data are also generated. Generated graphs and data are selected from the **Result** menu.



The graph on the left above shows M/Z vs Retention Time, the graph on the right above shows Noise Level vs Retention Time. The color of generated curves can be changed by clicking on the script **L** to the right of the graph area, second box down. Select the curve name and click repeatedly on **Recolor** in the action box below the curve name to find the color you want.

Scan, RT(min), Iso, M/Z, MW, Int, LC Noise, Charge, Score

332	5.06943	MO	391.659	390.651	13428	20705.5	1	9
332	5.06943	MO	520.423	519.415	69839	1.135081	1	9
332	5.06943	MO	594.264	593.256	50933	7.85958	8	1
332	5.06943	MO	668.11	667.102	35344	52287.1	1	9
343	5.24723	MO	505.276	504.268	7519	27.8181	22	1
343	5.24723	MO	816.014	815.006	8870	72.8733	48	1
345	5.27947	MO	430.493	429.485	5466	95.21187	7	1
345	5.27947	MO	594.295	593.287	13917	3.86702	1	9
345	5.27947	MO	668.168	667.161	21928	1.53041	4	1
348	5.32745	MO	742.073	741.066	12109	5.26512	6	1
362	5.55154	MO	505.45	504.442	6639	11.8908	27	1
366	5.61563	MO	816.045	815.038	4412	13.10830	1	9
371	5.69502	MO	446.488	445.481	14602	6.123672	1	9
372	5.71092	MO	594.341	593.333	21198	4.88224	3	1
372	5.71092	MO	742.05	741.042	16324	9.26874	9	1
376	5.77437	MO	430.4	429.392	14558	4.18394	7	1
378	5.80628	MO	391.663	390.655	2919	11.24239	3	1
381	5.85397	MO	668.161	667.153	26583	9.55042	6	1
391	6.01258	MO	430.513	429.506	14545	9.17963	9	1
392	6.0285	MO	816.057	815.05	4371	85.11423	5	1
398	6.12403	MO	676.001	674.993	4485	86.4848	32	1
406	6.25082	MO	594.398	593.39	19758	8.90099	5	1
410	6.31412	MO	668.214	667.206	25845	2.56560	4	1
413	6.36103	MO	742.075	741.067	20334	5.27409	1	9

Find:

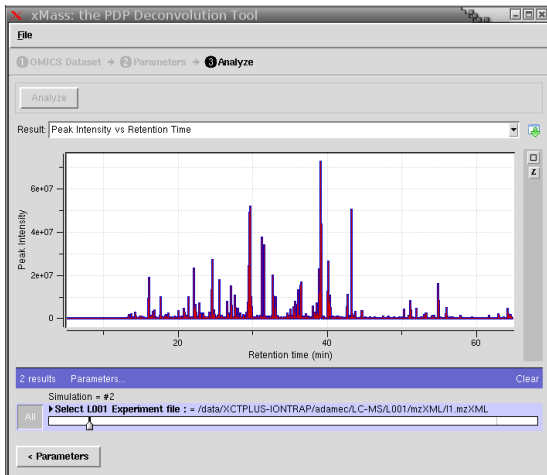
1 result Parameters...

The output trace (PAR file) and output DLT file can be viewed by selecting them from the **Result** menu. You can download the DLT file to your cceHUB directory by choosing Download from the **Result** menu while the DLT file is displayed. Likewise, any graph or data can be downloaded by selecting Download while the graph or data is displayed.

Step 4. We can now select another LC-MS file from the L001 experiment datasets generated by the XCTPlus Ion Trap. Return to the OMIC Dataset selection screen and choose

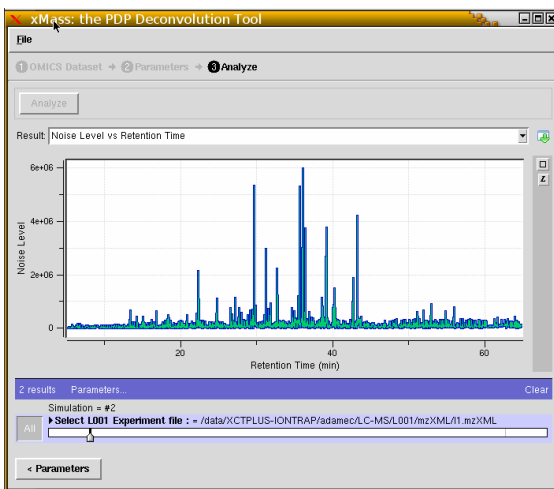
Select L001 Experiment file: [I1.mzXML](#)

The parameters will remain the same, so you can skip the parameter specification screen and immediately click on Analyze. When the deconvolution of I1.mzXML is complete, the Peak Intensity vs Retention Time graph is displayed.



Step 5. Click on the **All** button, which can be found on the left below the graph display area. A new graph will be generated which shows the peak intensity for both datasets: I11.mzXML (from the first deconvolution) and I1.mzXML (from the second deconvolution). In this way, the intensity values across the retention time range for both samples can be compared.

Note that in the graph to the left, a color change for the I1.mzXML intensity curve was made.



You can bring either of the intensity curves to the “top” by clicking on one of the two the lines in the bar to the right of **All**. Immediately above the **All** bar, the name of the dataset raised to the “top” is listed.

Comparisons can be done for any of the generated graphs, and for any number of deconvolution runs. Each new run will add a line in the bar to the right of **All**.

To the left is a comparison graph of Noise Level vs Retention Time for the two deconvolution results.

Scenario 2: LC-MS data generated by the MicroMass QTOF

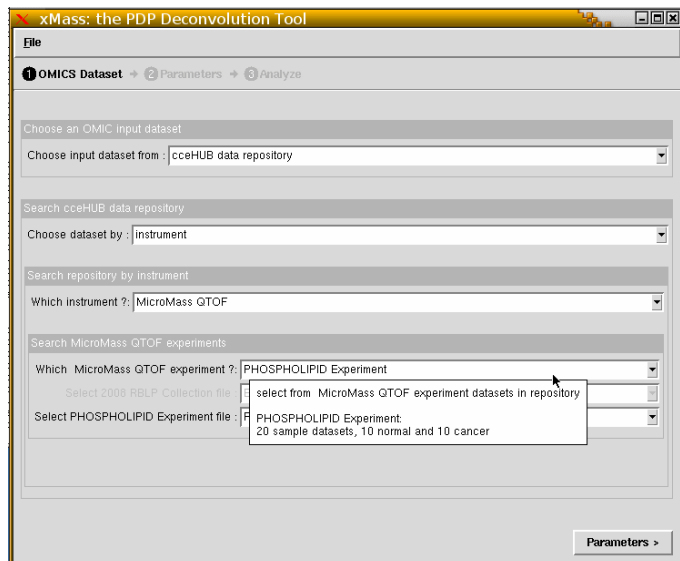
Step 1. We will again search the cceHUB repository for input, this time for a dataset generated by the MicroMass QTOF instrument. Select from the tool choices as follows:

Choose input dataset from: cceHUB data repository

Choose dataset by: instrument

Which instrument: MicroMass QTOF

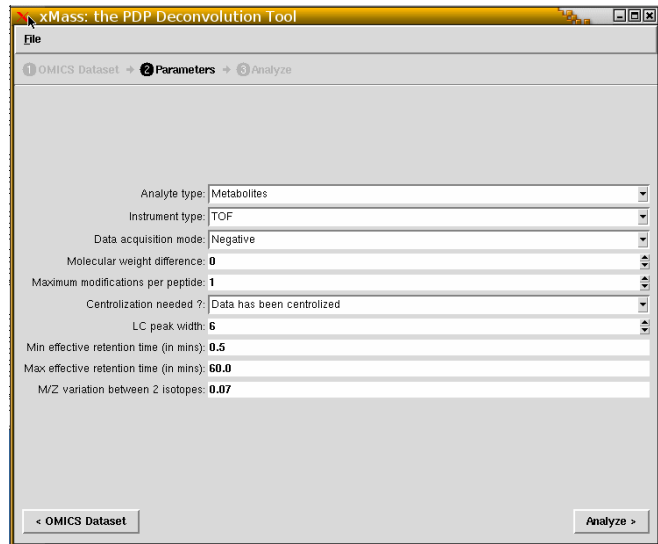
Which MicroMass QTOF experiment: PHOSPHOLIPID Experiment



Select PHOSPHOLIPID Experiment file: PHOSPHOLIPID_DENG1AFAMM01.cdf

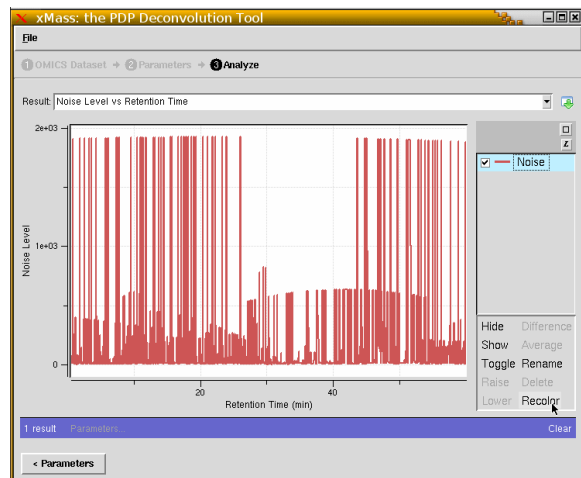
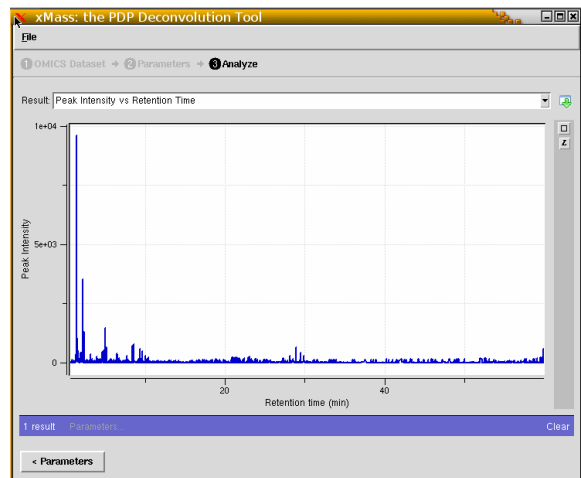
Step 2. We will now specify the parameters for the selected dataset. Description of these parameters can be found in documentation and articles that accompany this tool. These are the default parameter values for Micro Mass data files.

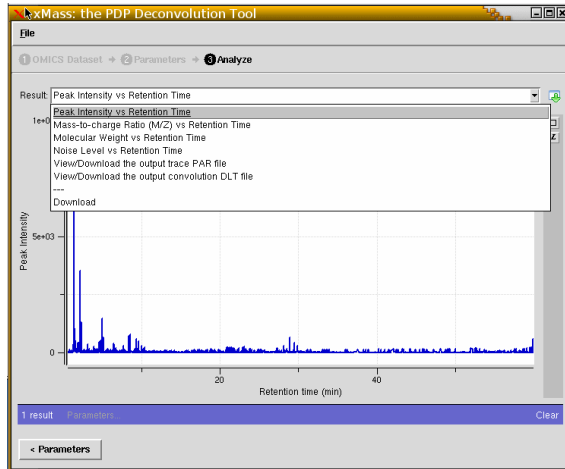
Analyte type: Metabolites
Instrument type: TOF
Data acquisition mode: Negative
Molecular weight difference: 0
Maximum modifications per peptide: 1
Centralization needed? Data has been centralized
LC peak width: 6
Min effective retention time: 0.5
Max effective retention time: 60.0
M/Z variation between 2 isotopes: 0.07



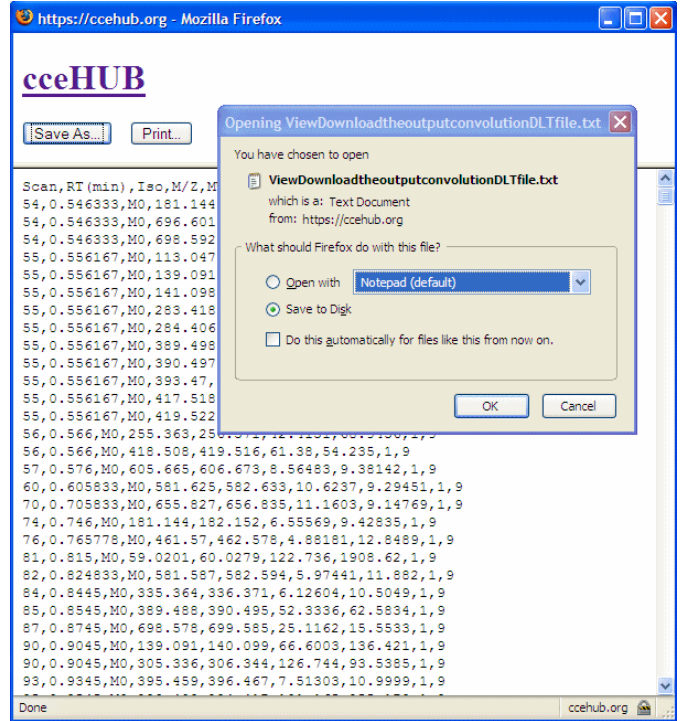
Step 3. Click on Analyze. Trace information will be printed as xMass executes. When execution is complete, the graph to the right, Intensity vs Retention Time, is displayed. Use zoom rectangles to explore the data. If you place your mouse on the curve, all the data points appear for the measured retention times. If you place your mouse on one of the points, the values for the intensity and retention time at that point will be shown.

Other graphs and data are generated by xMass. Generated graphs and data are selected from the **Result** menu. The Noise Level graph is shown on the right below.





The output trace (PAR file) and output DLT file can be viewed by selecting them from the **Result** menu. You can download the DLT file to your cceHUB directory by choosing Download from the **Result** menu while the DLT file is displayed. The DLT will be stored as a “text” format file.

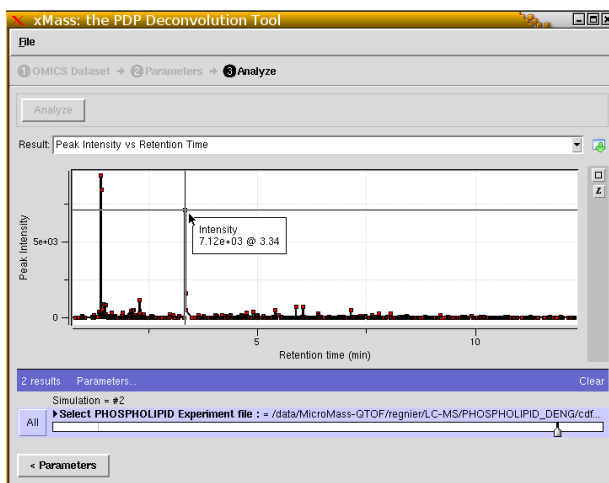


Likewise, any graph or data can be downloaded by selected Download while the graph or data is displayed.

Step 4. We can now select another LC-MS file from the PHOSPHOLIPID experiment datasets generated by the MicroMass QTOF. Return to the OMIC Dataset selection screen and choose a control dataset:

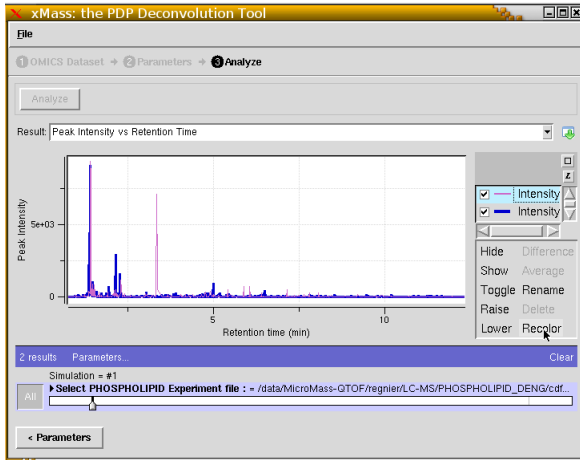
Select PHOSPHOLIPID Experiment file: PHOSPHOLIPID_CONTROL1AFAMM01.cdf

The parameters will remain the same, so you can skip the parameter specification screen and immediately click on Analyze. When the deconvolution of PHOSPHOLIPID_CONTROL1AFAMM01.cdf is complete, the Peak Intensity vs Retention Time graph is displayed.



You can use your left mouse button to create rectangles to zoom in and your right mouse button to zoom out.

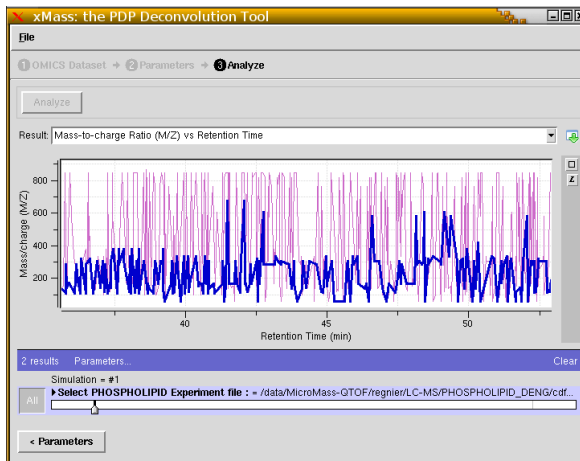
If you position the mouse button over the curve, the results data points used to draw the curve will be displayed, and if you position your mouse over a specific point, the values for intensity and retention time at that point are displayed.



Step 5. Click on the **All** button to display the peak intensity for both datasets:

PHOSPHOLIPID_DENG1AFAMM01.cdf (from the first deconvolution) and PHOSPHOLIPID_CONTROL1AFAMM01.cdf (from the second deconvolution). In the graph to the left, we compare the intensity values of a cancer sample and a control sample.

Note that a color change was made to more easily compare the intensity curves.



Comparisons can be done for any of the generated graphs, and for any number of deconvolution runs. Each new run will add a line in the bar to the right of **All**.

The graph to the left compares the M/Z vs Retention Time graphs for the cancer and control samples. The graph has been zoomed in and a color change was made.