Real time monitoring of the suitability of high resolution LC-MS systems by image recognition algorithm using peaks of spiked-in internal standards

Serhiy Hnatyshyn¹, Rostyslav Hnatyshyn², Jan-Lucas Ott¹

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1 – Bristol-Myers Squibb Company

2 – Rowan University

Outline

- Introduction: Data quality in metabolomics experiments
- High resolution LC-MS workflow
- The components of data quality
- System suitability testing procedures
- Chromatographic peaks as quality indicators
- Internal standard (IS): N-Benzoyl-d5-glycine
- Visual inspection of IS peaks
- Logarithm transformation
- Data processing algorithm
- Statistics of analysis of IS peaks
- Peak parameters vs. injection order
- Peak data as 28x28 pixel image
- Convolution Neural Network (CNN)
- CNN model for peak abnormality detection
- Model prototype implementation using Jupyter Notebook (Python 3)
- CNN model applications for real-time study monitoring examples
- Lessons learned
- Conclusions and future work

Introduction

We are most concerned with the qualitative composition of a given sample, as well as its quantitative makeup.

Data quality is a cornerstone.

The continuous monitoring of system performance during high resolution LC-MS metabolomics studies is necessary not only for data quality control, but also to maintain productivity and prevent the loss of precious samples. Currently, due to practical constraints, such monitoring is not continuous. Considering the long duration of the experiment - often several days, monitoring is typically performed by introducing internal standards and then examining the peaks associated with the internal standards after data acquisition. Such an approach can overlook abnormal events during the acquisition of the data, which leads to poor data quality or in extreme cases, the need to repeat the run.

High resolution LC-MS Workflow

The Components of Data Quality

Component	Description	Frequency of usage
Analytical Instrument Qualification	Documented evidence that an instrument performs suitably for its intended purpose and that the instrument is properly maintained and calibrated.	Installation, major maintenance event, relocation
Analytical Method Validation	A collection of all necessary procedures that demonstrate that a particular method is reliable and reproducible. Special considerations are used for the measurement of analytes in a biological matrix such as blood, plasma, serum, or urine.	Method development when establishing workflow
System Suitability Testing	Daily test to verify the adequate working of the equipment used for analytical measurements.	Daily, before testing begin
Quality Control Checks	Spiked samples of know quality are used to monitor the performance of a bioanalytical method. They are allso used to assess the integrity and validity of the results of unknown samples within the same batch.	Every N (usually N=10) samples during the batch run
Internal Standards	Spiked labeled compounds to monitor indiviual sample run	Every sample

Frequency of application

rare

often

Reference : C.J. Briscoe et al. / Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 484–491

System suitability testing procedures

For long runs, it is impractical to check every sample. In practice, spot checks are utilized.

Realizing that the samples are compromised at the processing stage leads to missing data and lost opportunities.

Reference : C.J. Briscoe et al. / *Journal of Pharmaceutical and Biomedical Analysis* 44 (2007) 484–491

Chromatographic peaks as quality indicators

“The significant deviation of the peak shape from the symmetrical peak makes hardly possible the acquisition of chromatographic signal information, such as the retention time, the peak area, the peak width at half peak height, the peak overlapping, etc.”

Zs. Pápai, T.L. Pap “Analysis of peak asymmetry in chromatography” *Journal of Chromatography A*, 953 (2002) 31–38

Typical extracted ion chromatogram peak for a “well-behaved” quality control

The trade off between scan rate and mass resolution is defined in the setup of the LC-MS experiment. Both parameters influence the quality of chromatographic peaks.

UPLC provides fast chromatography improving the throughput of the analysis. A typical peak width is 4–6 seconds.

For such narrow peaks, a Q Exactive mass spectrometer operating at 70,000 resolution acquires >15 points across the peak without losing sensitivity*.

*Junhua Wang, David A. Peake, Mark Sanders, Michael Athanas, Yingying Huang
Untargeted Metabolomics Workflow Using UHPLC/Quadrupole Orbitrap Mass Spectrometer and SIEVE 2.1., Thermo Fisher Scientific Inc, San Jose, CA, USA

IS: N-Benzoyl-d5-glycine

Product code: D-5588

Chemical formula: C₉H₄D₅NO₃

CAS: 53518-98-2

CAS (unlabelled): 495-69-2

Synonyms: Hippuric Acid, Benzoylaminoacetic Acid, Bz-Gly-OH

Supplier / Manufacturer:

C/D/N Isotopes Inc.

88 Leacock Street

Pointe-Claire (Québec) H9R 1H1

Phone: 514-697-6254

Toll-Free (Canada & USA): 1-800-565-4696

Fax: 514-697-6148

Website: www.cdnisotopes.com

d5-Hippuric Acid

IS = internal standard

Visual inspection of IS peaks

Exponentially Modified Gaussian (EMG):

$$y = \frac{ac\sqrt{2\pi}}{2d} \exp\left(\frac{c^2}{2d^2} - \frac{x-b}{d}\right) \left[\frac{d}{|d|} + \operatorname{erf}\left(\frac{x-b}{\sqrt{2}c} - \frac{c}{\sqrt{2}d}\right)\right]$$

The exponentially modified Gaussian is a convolution of Gaussian and exponential probability density. a = Gaussian height, b = location parameter (Gaussian center), c = Gaussian width, d = distortion parameter (a.k.a. modification factor or time constant).

Delley, R. (1985). "Series for the Exponentially Modified Gaussian Peak Shape." Anal. Chem. **57**: 388.

Extracted ion chromatograms peaks for a d5-hippuric acid molecular ion.

A. EMG model; **B.** Successful suitability test; **C.** Failed suitability test (peak is tailing); **D.** Failed suitability test (peak is splitting);

Logarithm transformation

Logarithm transformed extracted ion chromatograms peaks for d5-HA molecular ion:
A. EMG model; **B.** Successful suitability test; **C.** Failed suitability test (peak is tailing); **D.** Failed suitability test (peak is splitting);

Data processing algorithm

Analyzed IS peaks from
Number of projects = 598
Number of Injections = 58,718

Extracted ion chromatograms for
d5-Hippuric acid for various
generations of Thermo-Fisher
instruments

Statistics of analysis of IS peaks

Peak Parameter	Factors	Average Value*	STDev	Units
Position	analyte properties, gradient program			
start	void volume,	3.54	0.03	minutes
end		3.83	0.06	minutes
apex		3.59	0.04	minutes
Width	analyte properties, system settings,			
1/2 height	column pluming, gradient program,	0.1	0.02	minutes
1/20 height	analysis duration	0.3	0.06	minutes
Half-width				
left		0.05	0.02	minutes
right		0.24	0.07	minutes
Tailing	stationary phase condition	1.9	0.5	
Height	instrument tune, detector settings	3.50E+07	1.30E+07	counts
Area	integration procedure	1.05E+08	3.50E+06	counts/min

*The average values were calculated for a single multi-day experiment with ~1000 injections using standard metabolomics protocols and instrument tuning procedures.

Peak parameters vs. injection order

Traditional univariate statistical outlier calculations may be not sufficient to detect early signs of abnormalities in peak parameters.

Peak data to 28x28 pixel image

The peak-to-image algorithm includes a logarithmic transformation to take into account a difference of six orders of magnitude between the signal and the background.

A – suitable
B – abnormal

- ~60,000 data files were processed
- Manual data review found less than 800 cases of abnormal peaks.
- Two sets of input were created:
  - **TrainingSet_B1000.CSV**; 500 suitable, 500 abnormal peaks
  - **TestSet_B300.csv**; 150 peak images of each case

Convolution Neural Network (CNN)

- Convolutional Neural Networks (CNN) excel at detecting shapes, making it suitable for detecting abnormal peaks
- Unlike fully-connected neural networks, CNNs like this one begin with convolutional and pooling (or sub-sampling) layers
  - ✓ This makes the CNN more robust to transformations in input, such as slight retention time shifts in peak centroids, and serves as an inbuilt feature extractor
  - ✓ This also keeps the total number of trainable free parameters low, even as the input image scales up
- Feature maps synthesized by the convolutional layers are fed into fully connected layers like traditional neural networks, which finally output to a binary sigmoid neuron for classification

CNN model for peak abnormality detection

We implemented our CNN model in Python 3.7, using the Keras library and numPy.

Model prototype implementation using Jupyter Notebook

jupyter binary_classifier Last Checkpoint: 01/14/2020 (autosaved) Logout

File Edit View Insert Cell Kernel Help Not Trusted Python 3

```
In [1]: import random
import os
import numpy as np
import pandas as pd
from keras.models import Sequential
from keras.layers import Dense
from keras.layers import Dropout
from keras.layers import Flatten
from keras.layers.convolutional import Convolution2D
from keras.layers.convolutional import MaxPooling2D
from sklearn.preprocessing import LabelEncoder, OneHotEncoder
from keras import backend as K
from keras.layers import Conv2D
from sklearn import preprocessing
from PIL import Image
import matplotlib.pyplot as plt
import tensorflow as tf
from keras.models import load_model

Using TensorFlow backend.
```

Data Input

```
In [2]: # Load in our test + training data (OS agnostic)
testfilepath = os.getcwd() + os.path.sep + 'Data' + os.path.sep + 'TestSet_B300.csv'
trainfilepath = os.getcwd() + os.path.sep + 'Data' + os.path.sep + 'TrainingSet_B1000.CSV'

test = pd.read_csv(testfilepath, encoding='unicode_escape').values
train = pd.read_csv(trainfilepath, encoding='unicode_escape').values

In [3]: # Reshape and normalize training data
trainX = train[:, 1:].reshape(train.shape[0], 1, 28, 28).astype('float32')
y_train = train[:, 0]

# Reshape and normalize test data
testX = test[:, 1:].reshape(test.shape[0], 1, 28, 28).astype('float32')
ytest = test[:, 0]
```

Dbinary_classifier+++Jupyter+Notebook%26body%3Dhttp%3A%2F%2Flocalhost%3A8888%2Fnotebooks%2Fbinary_classifier.ipynb

<https://jupyter.org/>

Prototype implementation using Jupyter Notebook (continue)

Prepare labels

```
In [4]: # Prepare the labels for our data
lb = preprocessing.LabelEncoder()
lb.fit(y_train)
```

```
y_train = lb.transform(y_train)
lb.fit(ytest)
y_test = lb.transform(ytest)
```

```
In [5]: # Example Peak
```

```
In [6]: # Let's take a look at one of the peaks in our training set
w, h = 512, 512
plt.imshow(trainX[random.randint(0, len(trainX)-1)].squeeze(), cmap='binary')
```

```
Out[6]: <matplotlib.image.AxesImage at 0x17906c6abe0>
```

Image of suitable peak

Prototype implementation using Jupyter Notebook (continue)

A model based on the LeNet-5 Network [1]

```
In [7]: # binary model
model = Sequential()
K.set_image_data_format('channels_first') # corresponds to K.set_image_dim_ordering('th')
model.add(Conv2D(30, (5, 5), input_shape=(1, 28, 28), activation='relu', border_mode='valid'))
model.add(MaxPooling2D(pool_size=(2, 2)))
model.add(Conv2D(15, (3, 3), activation='relu'))
model.add(MaxPooling2D(pool_size=(2, 2)))
model.add(Dropout(0.2))
model.add(Flatten())
model.add(Dense(128, activation='relu'))
model.add(Dense(50, activation='relu'))
model.add(Dense(1, activation='sigmoid'))
```

```
In [8]: # Compile our model
model.compile(loss='binary_crossentropy', optimizer='adam', metrics=['accuracy'])
```

```
In [9]: # Examine the model's parameters
model.summary()
```

Model: "sequential_1"

Layer (type)	Output Shape	Param #
conv2d_1 (Conv2D)	(None, 30, 24, 24)	780
max_pooling2d_1 (MaxPooling2D)	(None, 30, 12, 12)	0
conv2d_2 (Conv2D)	(None, 15, 10, 10)	4065
max_pooling2d_2 (MaxPooling2D)	(None, 15, 5, 5)	0
dropout_1 (Dropout)	(None, 15, 5, 5)	0
flatten_1 (Flatten)	(None, 375)	0
dense_1 (Dense)	(None, 128)	48128
dense_2 (Dense)	(None, 50)	6450
dense_3 (Dense)	(None, 1)	51
Total params: 59,474		
Trainable params: 59,474		
Non-trainable params: 0		

Train Model

```
In [10]: history = model.fit(x_train, y_train,
                             epochs=10,
                             batch_size=32, validation_data=(x_test, y_test))
```

Train on 1000 samples, validate on 200 samples

Epoch	1/100	2/100	3/100	4/100	5/100	6/100	7/100	8/100	9/100	10/100	
1/100	loss: 0.3318 - accuracy: 0.8910 - val_loss: 0.0083 - val_acc: 0.9900	loss: 0.0048 - accuracy: 1.0000 - val_loss: 0.0074e-04 - val_acc: 1.0000	loss: 0.0011 - accuracy: 1.0000 - val_loss: 1.0837e-03 - val_acc: 1.0000	loss: 0.0017 - accuracy: 1.0000 - val_loss: 2.0894e-04 - val_acc: 1.0000	loss: 0.0017 - accuracy: 1.0000 - val_loss: 4.4112e-04 - val_acc: 1.0000	loss: 0.0009e-04 - accuracy: 1.0000 - val_loss: 1.0248e-04 - val_acc: 1.0000	loss: 2.4510e-04 - accuracy: 1.0000 - val_loss: 5.1099e-04 - val_acc: 1.0000	loss: 0.0017 - accuracy: 1.0000 - val_loss: 2.0270e-04 - val_acc: 1.0000	loss: 0.0009e-04 - accuracy: 1.0000 - val_loss: 1.0510e-04 - val_acc: 1.0000	loss: 2.1823e-04 - accuracy: 1.0000 - val_loss: 1.4294e-04 - val_acc: 1.0000	loss: 1.0000e-04 - accuracy: 1.0000 - val_loss: 2.0000e-04 - val_acc: 1.0000

Training takes ~15 seconds (Model trained on CPU; Intel I5-8350; 8Gb RAM)

Prototype implementation using Jupyter Notebook (continue)

Model performance evaluation

```
In [11]: batch_size= 25
         (eval_loss, eval_accuracy) = model.evaluate(testX, y_test, batch_size=batch_size, verbose=1)

         print("[INFO] accuracy: {:.2f}%".format(eval_accuracy * 100))
         print("[INFO] Loss: {}".format(eval_loss))

300/300 [=====] - 0s 222us/step
[INFO] accuracy: 100.00%
[INFO] Loss: 6.15655751228239e-05
```

```
In [61]: acc = history.history['accuracy']
         loss = history.history['loss']
         val_acc = history.history['val_accuracy']
         val_loss = history.history['val_loss']
         epochs = range(len(acc))
         plt.plot(epochs, acc, 'r', label='Training acc')
         plt.plot(epochs, val_acc, 'b', label='Validation acc')
         plt.title('Training and validation accuracy')
         plt.ylabel('Accuracy')
         plt.xlabel('Epoch')
         plt.legend()
         plt.figure()
         plt.plot(epochs, loss, 'r', label='Training loss')
         plt.plot(epochs, val_loss, 'b', label='Validation loss')
         plt.title('Training and validation loss')
         plt.ylabel('Loss')
         plt.xlabel('Epoch')
         plt.legend()
         plt.show()
```

Prototype implementation using Jupyter Notebook (continue)

```
# Randomly select a peak from the set
i=random.randint(0,len(testX)-1)
new_sample = np.array([testX[i]], dtype=float)

# Run a prediction
y = list(model.predict(new_sample,verbose=0))

# Display prediction
print ("Predicted Label %s, Actual Label: %d" % (str(y), ytest[i]))
display(i)
```

Predicted Label [array([1.], dtype=float32)], Actual Label: 1

Model correctly recognizes abnormal peak.

Continuous injection quality monitoring

Example #1 experiment monitoring with CNN (monitoring run was performed post data collection)

Project: metabolomics analysis of human serum samples from 400 patients comprised of healthy volunteers and patients diagnosed with nonalcoholic steatohepatitis (NASH). A total of five 96- well plates including interpolate quality controls and blanks were used. Analysis took ~5 days per polarity.

Example #2 experiment monitoring with CNN

**Restarted system.
After cleaning,
recalibrating
and tuning
system with
new model**

Example #3 experiment monitoring with CNN

Restarted system again. Cleaning, recalibrating and tuning system with new model

Lessons learned

- Maintaining the data archives of an analysis, including its raw-files, should become standard operating procedure. Historical data is used for data mining, the optimization of models and the testing of new ideas. Pattern recognition algorithms need data from both successful and failed experiments.
- CNN-based models are excellent tools for detecting patterns in data, as well as abnormal cases.
- A major obstacle with the creation of a CNN-based model was the need for a large training set. In our experience, cases of failed runs were rare.
- Our HR-LC-MS monitoring application enables the automated review of all built-in experiment quality controls during the experiment which can potentially save precious samples

Conclusions & Future Work

Current Solution

- Implements a non-intrusive, non-disruptive, machine-learning-based continuous system suitability monitoring algorithm
- Able to detect abnormalities ~20 injections (300 minutes) before the system shuts down e.g. due to excessive back pressure

Further Investigation

- Change data retention policy, data from “failed” runs are often not saved, but needed for training accurate models
- Fine-tune peak classification, expand with known shapes to improve troubleshooting of instrument or sample based issues
- Test higher image resolutions, network configurations, etc.

Future Expansions

Further work directions include an investigation into the possibility of monitoring a mixture of stable label standards, which would increase the resolution of the data and allow us to test the stability of the model.

- Extend system to work with a variety of LC-MS systems
- Extend system with multiple stable label standards
- Extend for LC-MS/MS experiments
- Implement cloud-based solutions

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Discover the right molecule, enable the clinical plan

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