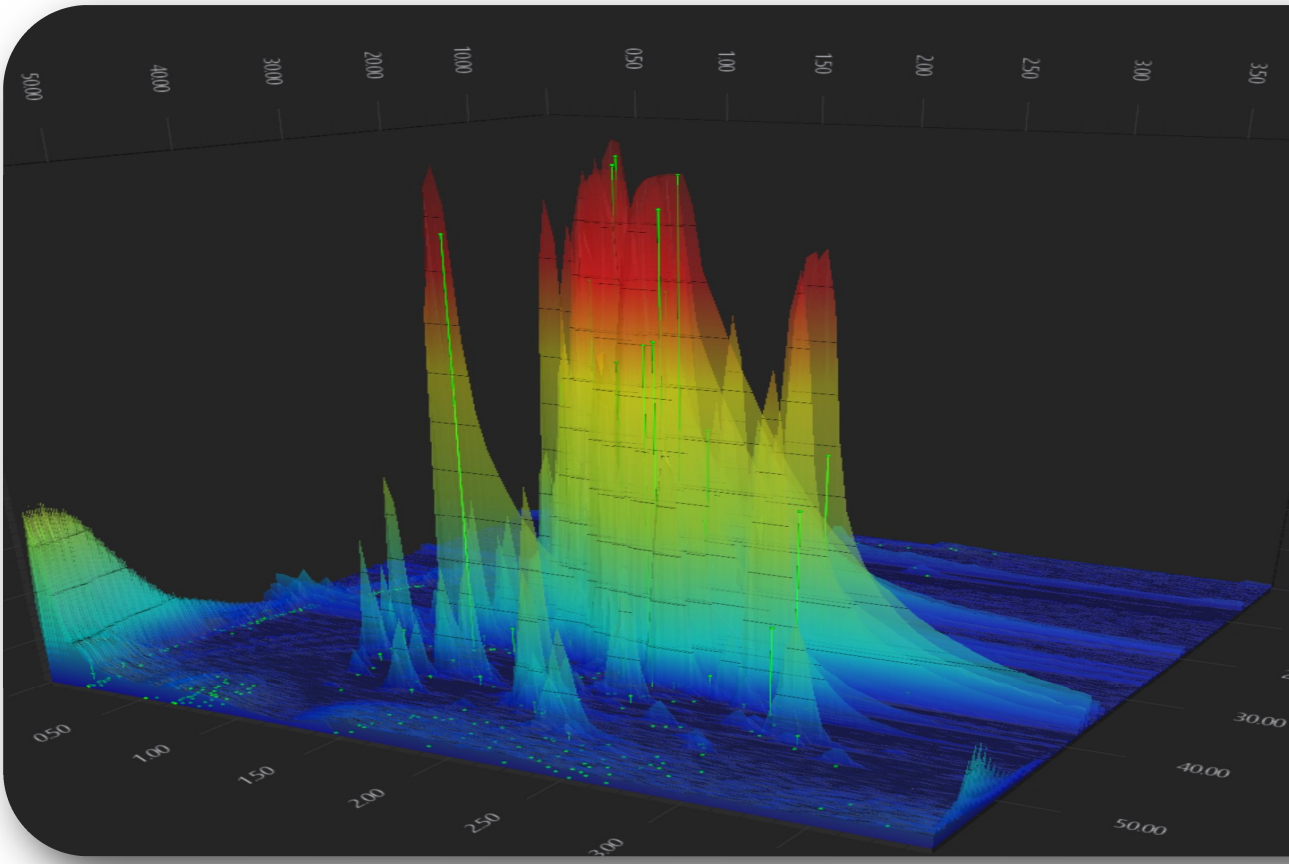
# AnalyzerPro® XD

## The Ultimate MS Tool for Data Exploration




**Deep mining software for 1D and 2D mass spectrometry**

SpectralWorks

## Introducing AnalyzerPro XD

AnalyzerPro® is a productivity software application for 1- and 2-dimensional low- and high-resolution LC-MS and GC-MS data with support for multiple vendors' data. This comprehensive post-acquisition processing utility provides optimized workflows for sample-to-sample comparison, target component analysis, and library searching. Using its proprietary algorithms to detect untargeted coeluting components, it consistently outperforms existing software packages making it the perfect tool to mine your mass spectrometry data.

Additional functionality allows the comparison of multiple samples and data sets using MatrixAnalyzer™ processing as well as statistical analysis using principal component analysis (PCA) and analysis of variance (ANOVA) to determine whether there are any significant differences. By supporting data files from multiple vendors, AnalyzerPro XD is the only data processing software you need. This reduces the requirement for training on additional software packages, increases efficiency and delivers a consistent approach to data processing and presentation of results regardless of instrument or analytical technique.

## Chromatographic Deconvolution

Data from analytical instruments needs to be processed before it can be analyzed and interpreted. The key feature in AnalyzerPro XD is its ability to chromatographically deconvolute data. Deconvolution is defined as 'reversing the effects of convolution'. For chromatographic data the biggest problem is co-elution of chromatographic peaks as many components will not be completely separated by the column. Using AnalyzerPro XD we can determine which masses belong to which of the co-eluting peaks. The deconvolution step also allows the creation of target component lists. For comprehensive data analysis, automated library building is available which includes all the components within an entire data set. This differs from a traditional target component list generated from standards in that the components do not need to be identified until later in the analysis. However, the traditional approach is also available in AnalyzerPro XD.

For example, a metabolomic study was conducted on the kidney tissue of a rat model for polycystic kidney disease. The data was processed by AnalyzerPro XD and the found components were library searched against an in-house target component library of authentic metabolite standards. Components were also searched against the NIST mass spectral database for tentative identification of the unknowns. Tryptophan, the deconvoluted peak at the retention time 35.45 minutes with a base peak of m/z 202 was only found in the polycystic kidney tissue and not the healthy age-matched and sex-matched control animal kidney tissue. As with many metabolomic studies, there may be several interpretations of this finding which in turn may generate new hypotheses to test.

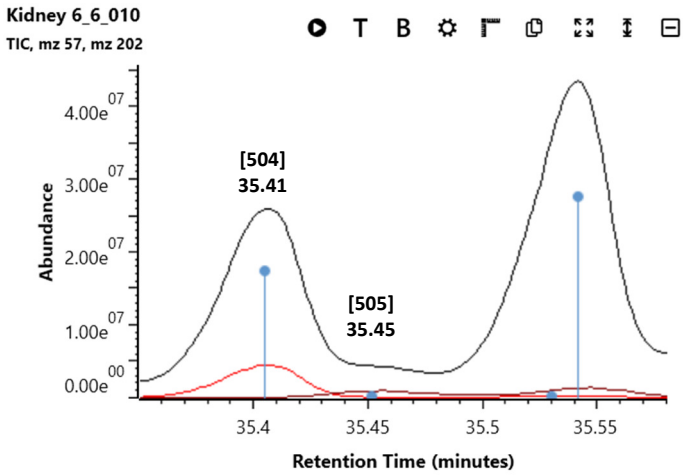



**Figure 1.** Deconvolution of Tryptophan and Docosane

Figure 1 shows that AnalyzerPro XD can automatically determine component 505 (Tryptophan) in the disease state samples in the presence of high levels of Docosane.

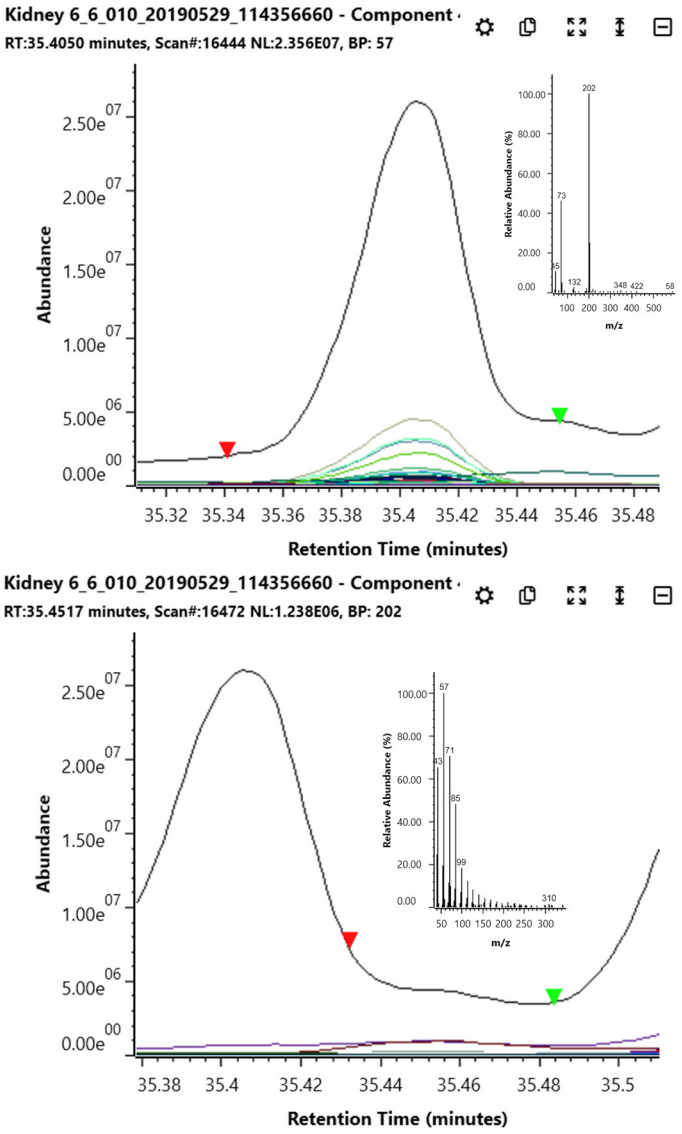



**Figure 2.** EIC and Spectra for Tryptophan and Docosane

In each case, high quality library searchable spectra were obtained for each component with no prior knowledge of the sample. Figure 2 shows the extracted ion chromatograms for each of the masses of the components and their corresponding spectrum.

## Why Multi-Dimensional?

2-dimensional chromatography is a powerful analytical tool that has evolved from technology used mainly in the R&D laboratory to a robust commercially available solution from several manufacturers. The technology continues to evolve to address challenges in the analysis of complex samples.

As the amount of data from these instruments can be overwhelming, we have developed AnalyzerPro XD as a vendor neutral 2D data processing solution for all chromatographic-MS data. Although 2D chromatography greatly increases the effective resolution and peak capacity of the separation, chromatographic deconvolution still has an important part to play in being able to effectively separate closely eluting components. Current software, which tends to deal with the data on a pixel-level, such as imaging software, no longer meets the requirements of being able to extend chemometric analysis to peak-level data.

## Target Component Analysis

Sample profiling using target component analysis is a useful tool for determining the relative amount of a target substance present in a complex mixture or to search for characteristic components of your samples. It is widely used in application areas such as environmental screening or the forensic determination of drugs of abuse. It is also important to be able to determine non-target components in areas of

product adulteration and AnalyzerPro XD can cover these workflows with ease.

In effect, 1- and 2-dimensional chromatography data are similar but the way that the chromatograms and components are constructed, using the second dimension information, is different. This means that the chromatographic deconvolution capabilities of AnalyzerPro XD can be used in both 1D and 2D situations.

AnalyzerPro XD provides a simple and accurate way to create your target component libraries from the components found in a sample. Retention time information as well as spectral, NIST format library match information and meta data can all be captured for each component. Additional confirmation criteria using specified ion ratios for components can also be set. Target component analysis also captures reliable component abundance information (height and area) for each sample that is processed.

Figure 3 shows found target components in green and non target components in blue. Target components that are not found are indicated by a red marker on the baseline.

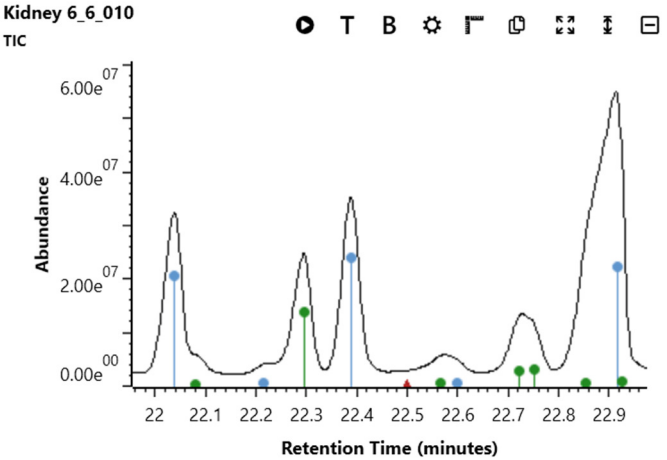



**Figure 3.** Targeted and untargeted components

Components can be visualized to allow you to see the deconvoluted chromatographic components, Figure 4 shows an example of this.

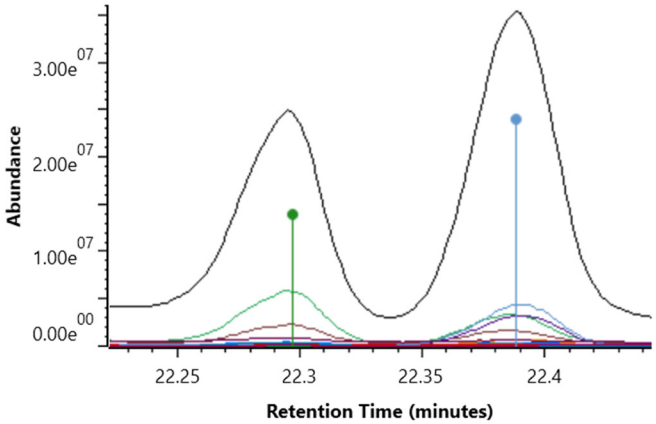



**Figure 4.** Component chromatograms

## Library Searching

Chromatographic deconvolution is carried out on the raw 1-dimensional data. The 2-dimensional components are then created from multiple 1D components. The component spectra can be searched against NIST formatted libraries on either or both component dimensions.

## 2-Dimensional Plotting

Figure 5 shows the raw TIC of a 2-dimensional separation of 2 chromatographic peak. Multiple peaks of the same compound can be associated with the modulation frequency of the acquisition. It is then possible to reconstruct a multi-dimensional plot of the separation and to assign components to that plot. Figure 6 shows the reconstructed TIC i.e. the 1-dimensional separation at the top, the TIC from the second dimension separation on the left and the 2-dimensional

contour plot at the bottom. Figure 7 shows a 3-dimensional plot of the overall separation, showing the resolved components.

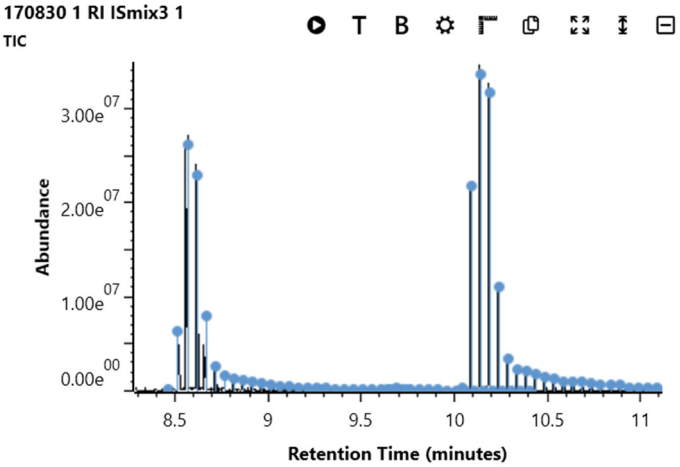



**Figure 5.** Raw TIC of a 2D separation of 2 peaks

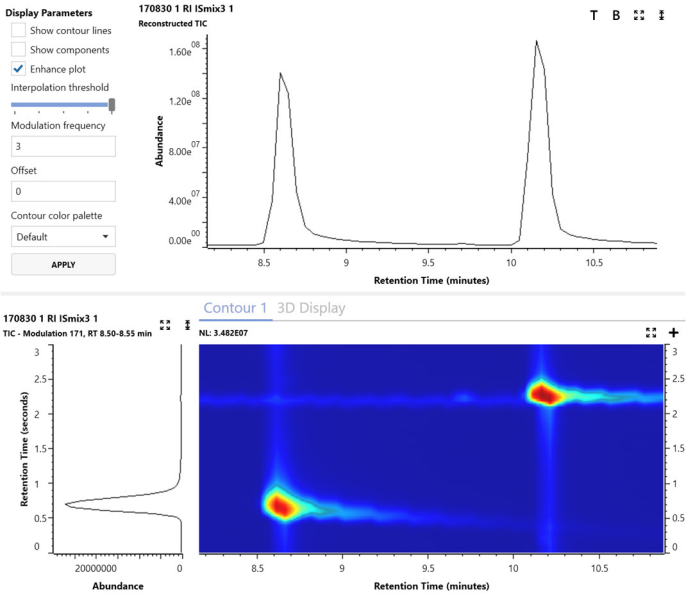



**Figure 6.** Contour plot and associated TIC for the 2 components

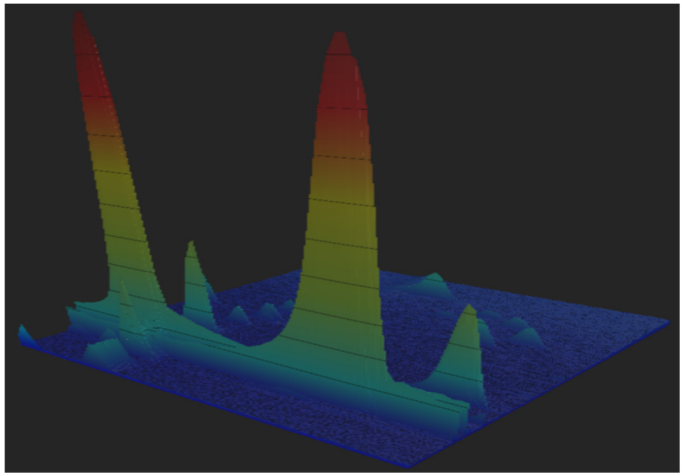


**Figure 7.** 3D plot for the 2 components

## MatrixAnalyzer & PCA

MatrixAnalyzer allows you to view the individual target component abundances in each of your processed samples. The data can be normalized to an internal standard component and to sample weight information if required. Results can be reported based on component abundance derived from height or area and from the sum of the component ions, specified component ions or base peak ion depending on specific requirements. The data can be viewed in multiple ways. A standard tabular view includes delta RT/ RIs, average values, standard deviation, a component coverage percent and p-values. Further processing and reporting of principal component analysis is also available. MatrixAnalyzer is used extensively in metabolomics but the underlying workflow can be used in many other applications where sample differences are being investigated.

Class information can be incorporated into your data processing sequence to assist with the interpretation of the PCA results and the component information including

abundance can be exported to third party software for further processing. Figure 8 shows an example of a PCA plot with 3 replicates of 4 whiskies. Clear separation of the different categories can be seen.

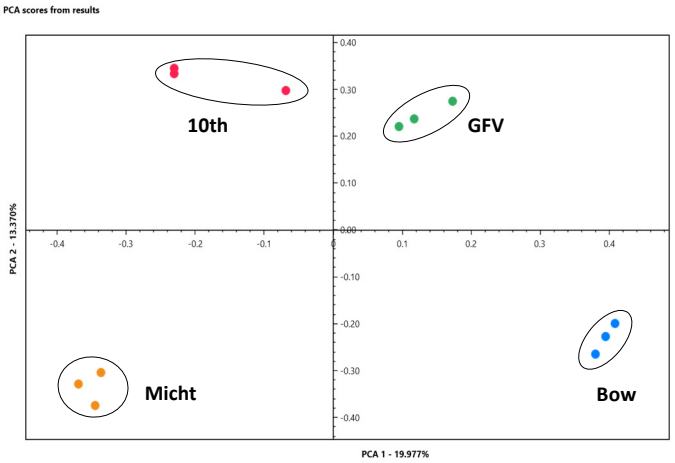



**Figure 8.** PCA of 4 whiskies

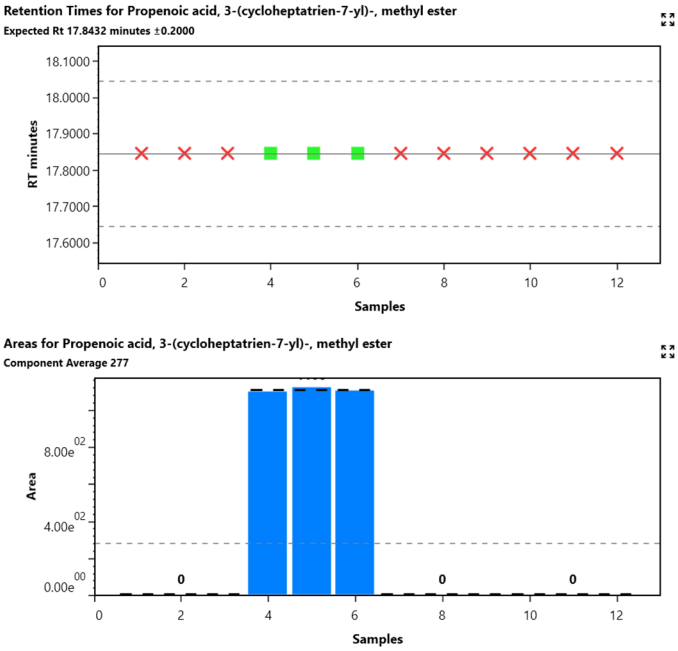



**Figure 9.** Example of RT and response tracking

PCA is used in many applications were differences between samples are being investigated. Additional visualization tools in MatrixAnalyzer allow for comparison of response and retention times for components across all samples and classes further assisting with the interpretation of the overall results, Figure 9 shows an example of this.

Volcano plots show the differences observed between two categories of sample. The changes in components common to both categories as well as the absence or presence of unique components can be seen. Statistical testing of these differences provides a measure of probability that these differences are significant or not. In this case we are looking at the comparison between the Bow and GFV samples using the relevant Volcano Plot shown in Figure 10. The legend makes it very easy to identify significant differences between categories.

## QC Correction

Metabolomic and other studies using mass spectrometry can extend over a period of time and multiple batches of samples. Quality Control samples are routinely run to assess the natural analytical variation and precision of the analytical process. There has been a demand to use this QC information to improve the overall performance of long term or multiple batch studies.

QC Correction is a function within AnalyzerPro XD to evaluate, visualize and correct for long and short term drift where necessary. The QC samples are treated as a specific class of sample where a QC curve can be calculated and then corrected to.

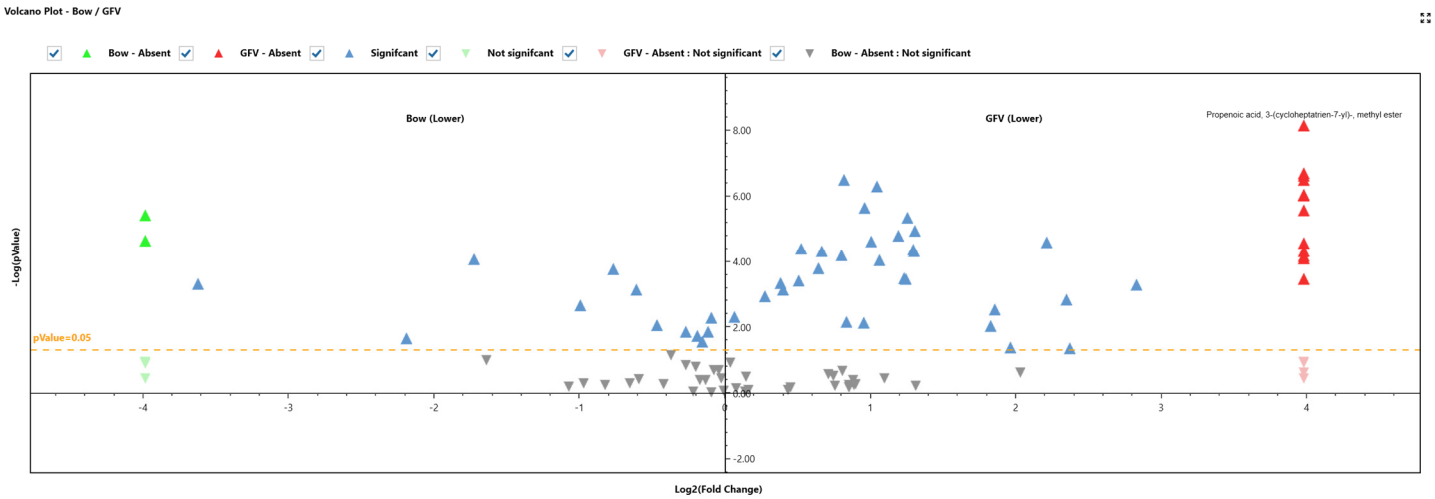



**Figure 10.** Fold Change Volcano Plot for 'Bow' versus 'GFV'.

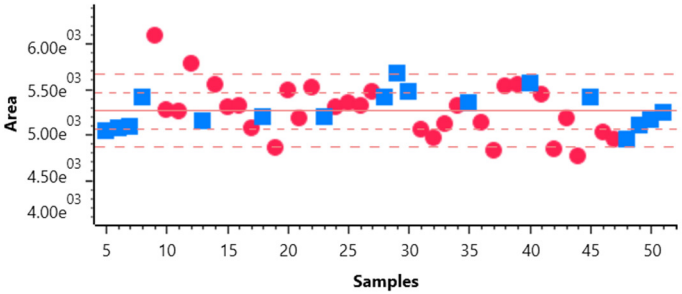



**Figure 11.** QC sample scatter plot before correction

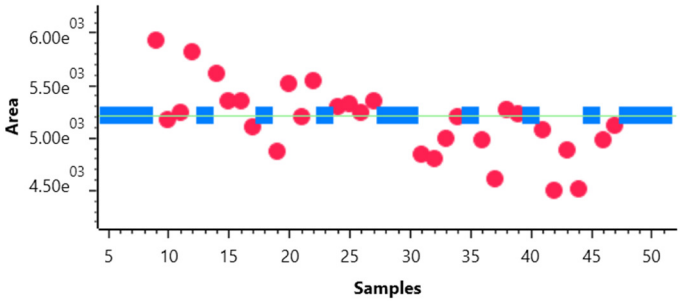



**Figure 12.** QC sample scatter plot after correction

Figure 11 shows the QC Sample scatter plot of a set of samples where the QC samples are highlighted in blue. Using the QC correction functionality effectively reduces the variance of the reported QC samples and adjusts the test sample results accordingly. Figure 12 shows the overall effect, where the variance of the test samples is also reduced. Figure 13 shows the PCA before and after QC Correction.

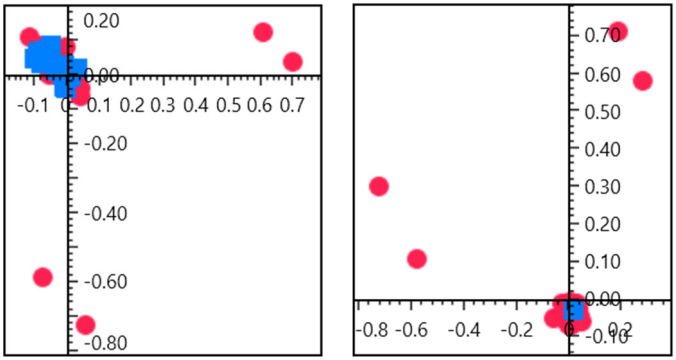


**Figure 13.** PCA plot before and after QC correction

## Applications

The use of 2-dimensional chromatography is used wherever complex samples are encountered. However, even with relatively simple samples, the additional chromatographic resolution can be a powerful asset. Typical applications are in metabolomics, proteomics, product authentication and product adulteration. Environmental and forensic applications are also becoming more prevalent.

## Conclusion

AnalyzerPro XD is the complete solution for analyzing your complex data files regardless of the instrument it was acquired on or the analytical technique used. From gaining biological insight into your data through to screening pesticides, AnalyzerPro XD is the perfect software tool for your workflow to ensure quality results are delivered as quickly as possible.

## About SpectralWorks Limited

SpectralWorks Limited is a leading UK based software development company.

We are dedicated to providing innovative solutions targeted for markets within the life sciences industry and have strong working relationships with the major instrument manufacturers. Coupled with our collaborations in academia and industry, we believe we have the right balance between scientific and software development expertise to provide the best scientific solutions for the end user.

Our vision at SpectralWorks is to improve the way software is integrated within the laboratory environment by providing the correct solutions to increase productivity and reduce overheads. We achieve this by maintaining focus on the end user, listening to their requirements and ensuring that they have the right tools to handle their day to day tasks.

In addition to the wide range of mass spectrometry software products, we provide highly respected consultancy services. These services are able to cover the complete software development cycle or specific steps within a project life cycle. These services are frequently utilized by instrument vendors and end users that demand the best for their laboratories.

We are a privately owned company, incorporated in the United Kingdom in January 2004 and are based in Runcorn, United Kingdom. Our offices are located on a 60 acre business park 25 miles from Manchester International Airport and 10 miles from Liverpool's John Lennon Airport.

Our facilities provide us with access to MS and other instrumentation as well as ensuring that we have the best infrastructure to develop and test software.

As well as direct sales, we have partner resellers. SpectralWorks offers Original Equipment Manufacturers (OEM) the ability to distribute our products and algorithms using their own branding. We also provide custom development for individual companies looking to automate and unify the software used within their laboratories using our extensive industry experience.

Our customer base is varied but predominately based within Australasia, Europe and North America. It encompasses academia, hospitals, government and industry.

Support is available via telephone, email or our web based support site. We always reply to communication as soon as possible and endeavour to reply within 24 hours with a statement of the problem and an estimated date for a solution.

SpectralWorks is certified by Approachable Certification in Europe and ISOQAR Inc. in the USA to ISO 9001:2015. ISO 9001 is a Quality Management System certification which requires companies to have implemented robust systems across all areas of the business: facilities, people, training, services and equipment. We are also proud to be a Microsoft Partner.

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