

Software Solutions for High Throughput Applications for Enantiomeric Excess Determination of Amino Acids Using ESI-MS

H. Fleischer¹⁾, D. Gördes²⁾, K. Thurow¹⁾

¹⁾ celisca - Center for Life Science Automation, Rostock, Germany

²⁾ Institute of Automation, University of Rostock, Germany

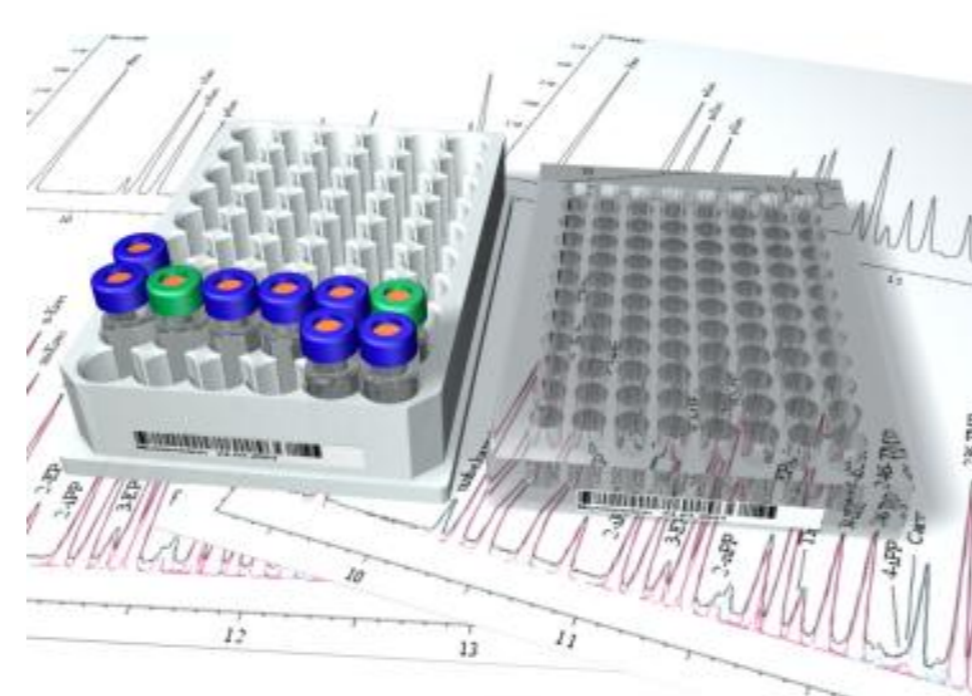
The high amount of data points generated in high throughput analytical measurements requires suitable innovative software solutions for data analysis and processing. The development of qualitative and quantitative analysis methods for chiral compounds is one of the recently important research topics since currently about 80% of all pharmaceuticals are enantiopure chiral substances. A process of entirely automated sample preparation, determination of the enantiomeric excess (ee%) using electro spray ionization mass spectrometry (ESI-MS), and data processing has been developed for various compound classes.

Enantiomeric excess determination

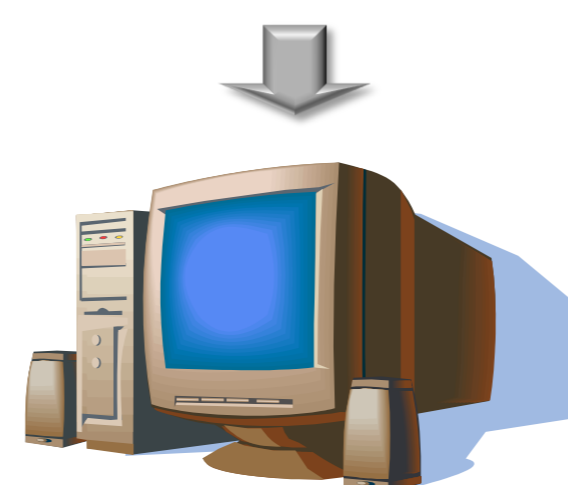
The ee% determination of enantiomers is based on the principle of parallel kinetic resolution with pseudoenantiomeric mass tagged auxiliaries. After the derivatization reaction products with two characteristic masses are resulted, which can be analyzed by ESI-MS. The ratio between the intensities of the desired masses is the base for ee% calculation.

Model substrates

A number of various chiral compounds such as alcohols, amino alcohols, carboxylic acids, proteinogenic amino acids and natural compounds have been tested. Furthermore, new commercially available chiral auxiliaries have been applied. Calibrations and verification measurements were carried out for selected compounds from each substance class.



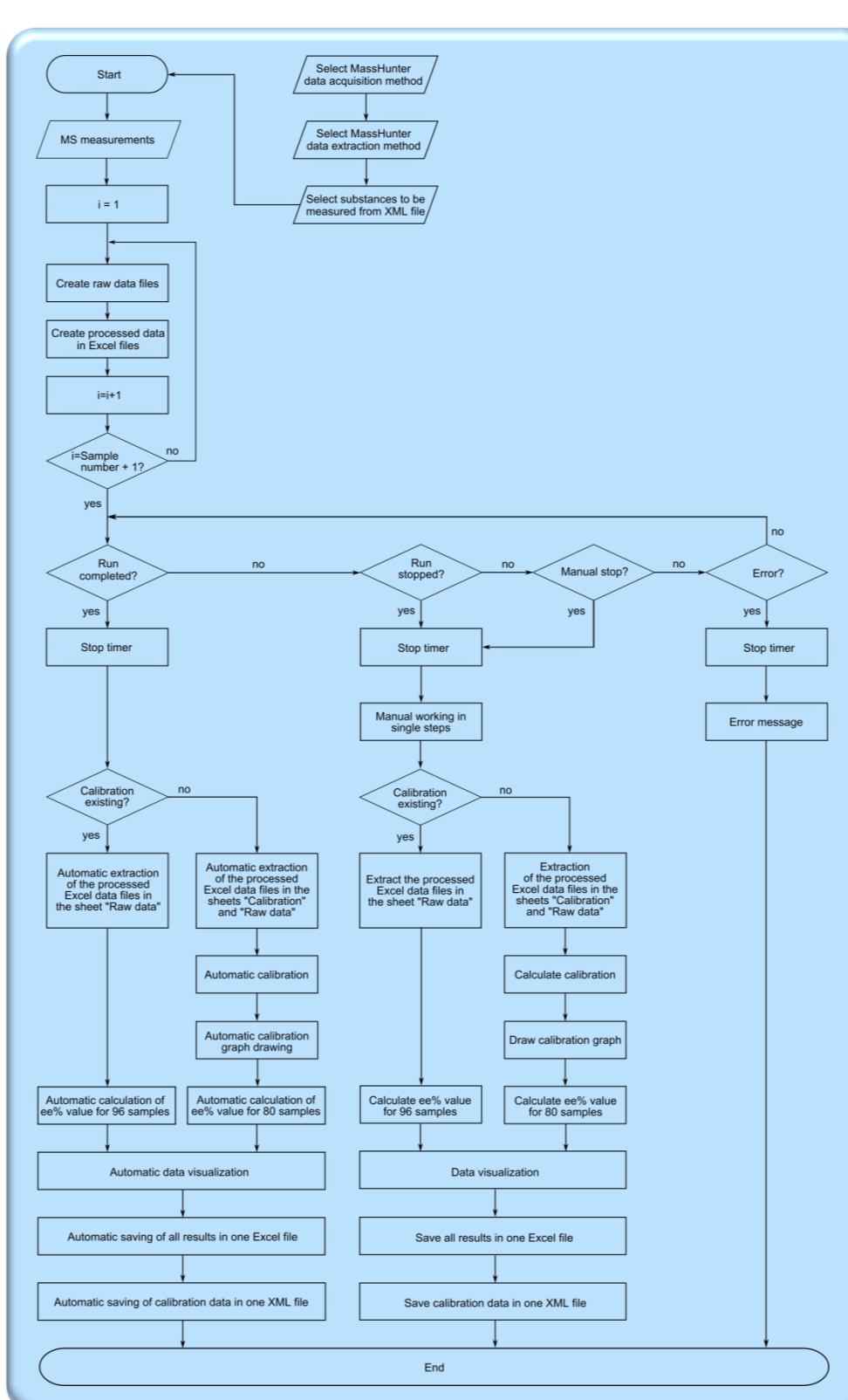
ESI-TOF MS



Software for postrun data processing and visualization

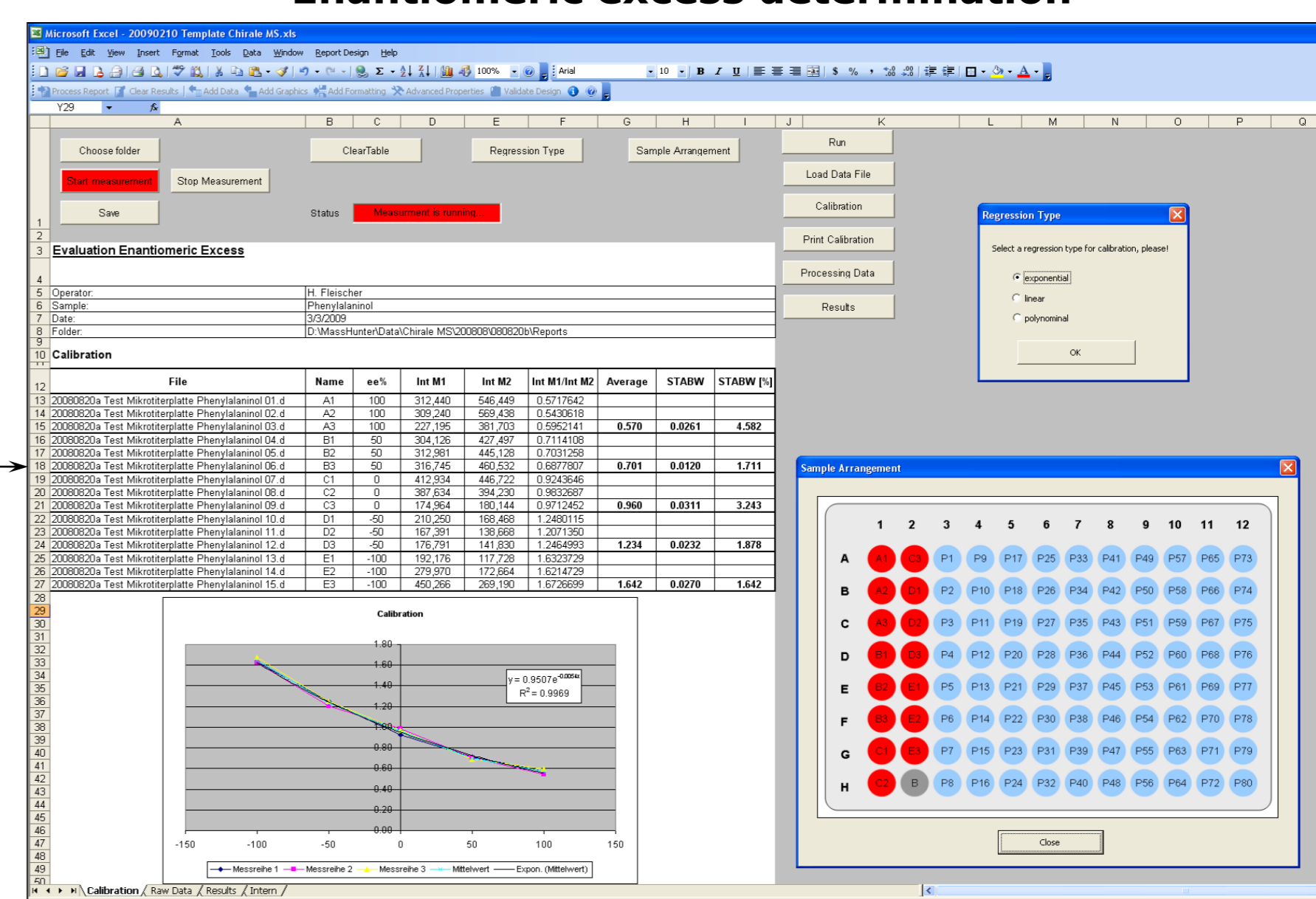
In general, commercial software packages don't provide comprehensive functions for automated data evaluation in the special field of ee% determination. For this purpose, an extension module "Chiral MS" was implemented to calculate enantiomeric excess for unknown samples and visualization of the results. The commercial software packages "Mass Hunter Data Acquisition" and "Mass Hunter Qualification" (Agilent Technologies) acquire the MS raw data, extract the desired chromatograms, integrate the peak areas of the target masses and save these values in MS Excel files. The software module "Chiral MS" uses these processed data files for creation a calibration curve. Based on this calibration the ee% value of unknown samples will be calculated. The software automatically starts after completion of the MS analysis.

Software workflow



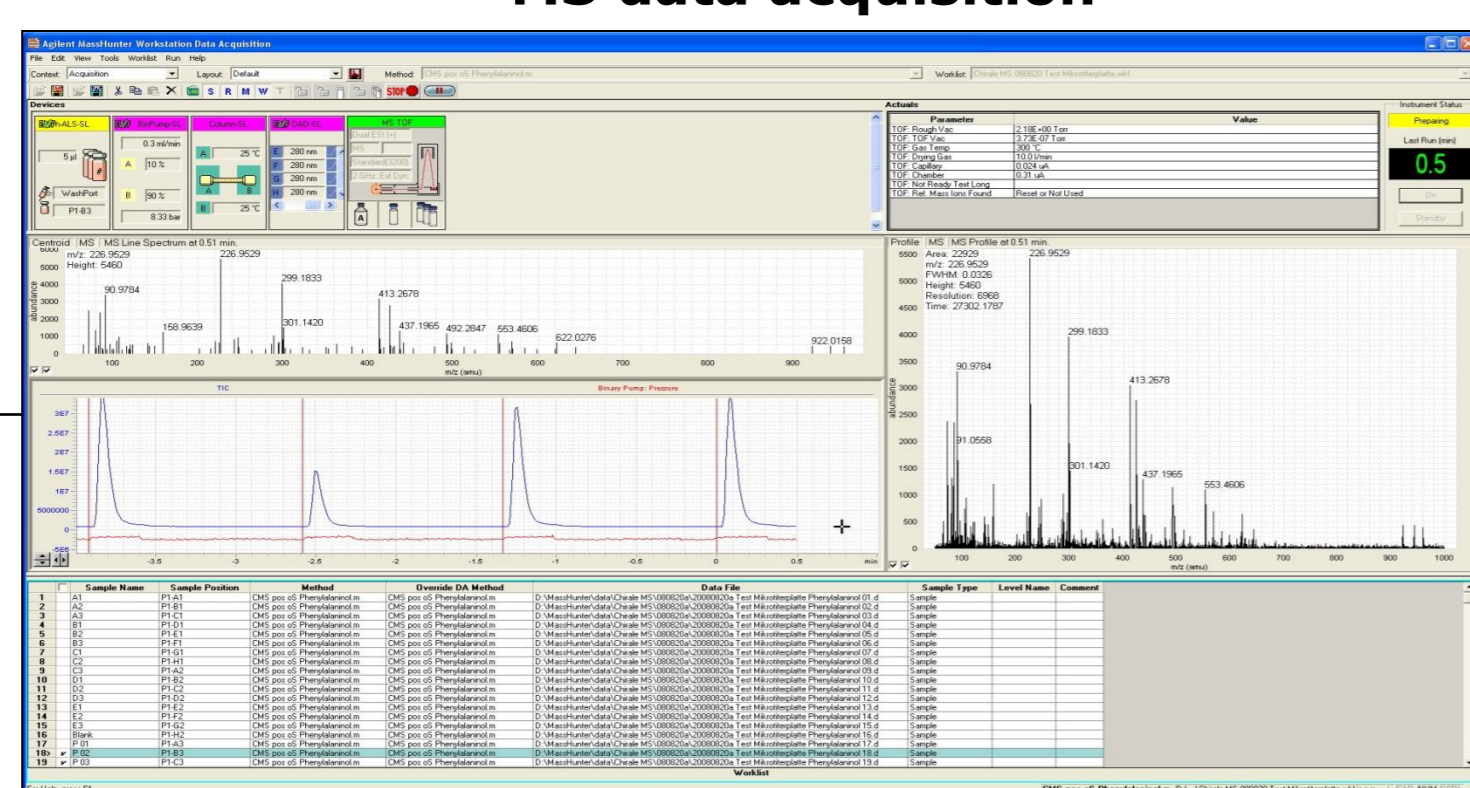
After the data acquisition an additional software module for further data evaluation in the special field of enantiomeric excess determination will be automatically started. From all processed MS Excel files the required data will be copied into a new MS Excel workbook. A calibration and the ee% values of the samples can be calculated.

Enantiomeric excess determination

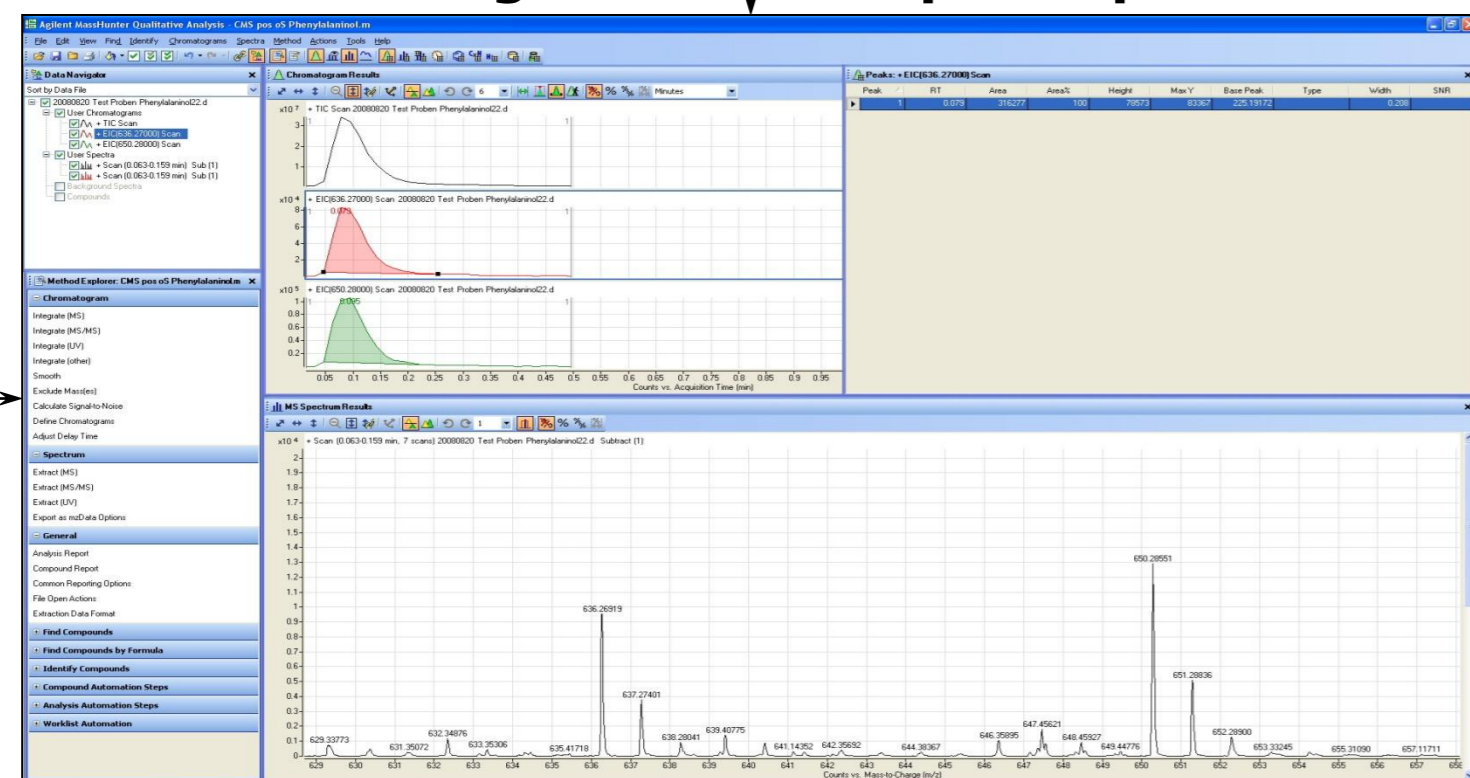


A condition precedent for the further data evaluation is a defined sample arrangement on the microtiter plate. To create a new calibration before the analysis of unknown samples, the first 15 wells must contain samples with a defined enantiomeric excess. Usually, five different ee% values with three replicates are used. The 16th well may contain a blank for control. If a calibration is saved, the microtiter plate can completely filled with samples which have an unknown ee% value. This software module allows to create various types of calibration curves such as linear, exponential and polynomial curves. The first sheet of the Excel workbook contains the data for the calibration. On the second sheet the unknown ee% values are calculated. Finally, the third sheet shows two 3D charts visualizing the results. The results of the whole microtiter plate are saved in one MS Excel workbook. Additional, the software allows a manual data manipulation for each step. Features such as pop-up-menus for easy changing properties and data paths are provided.

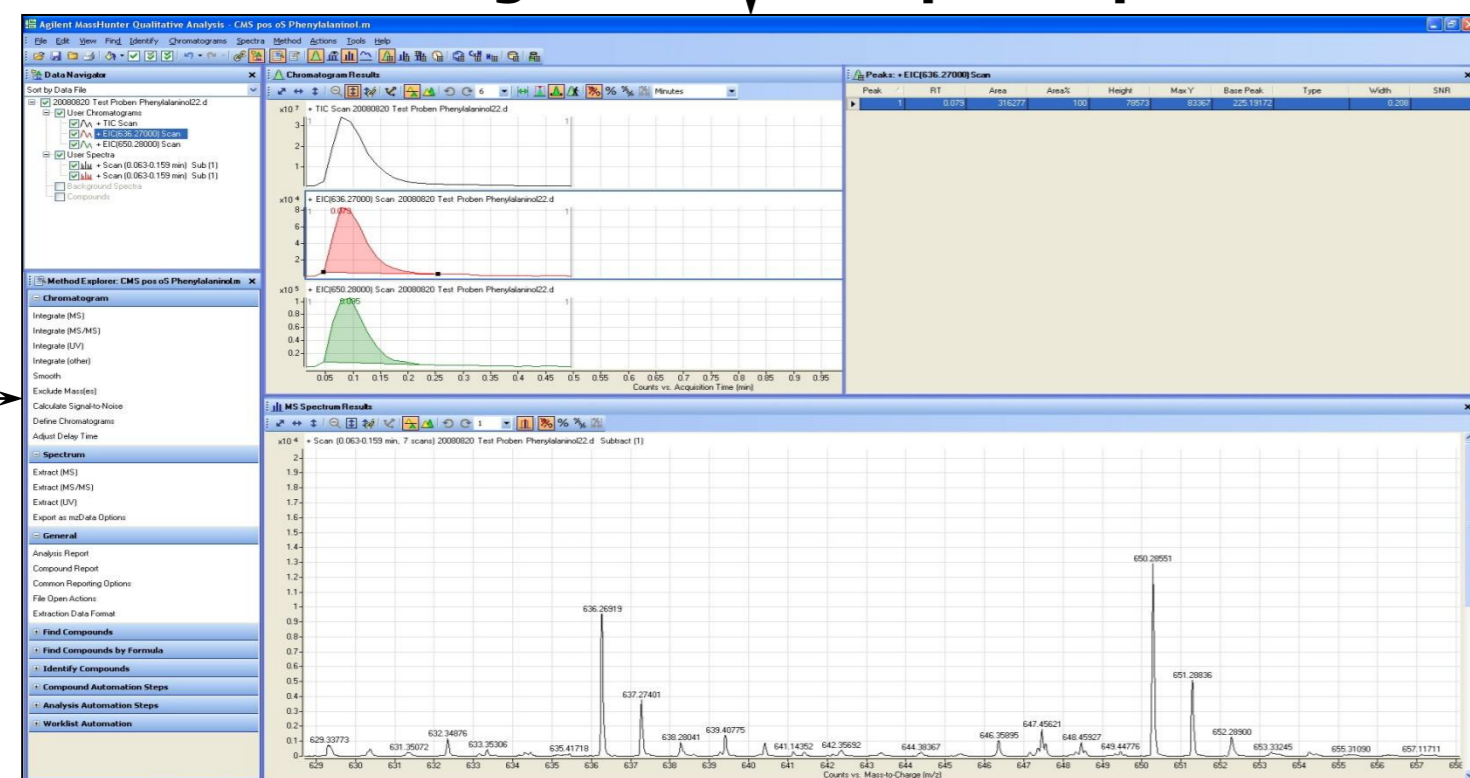
MS data acquisition



Extraction of chromatograms



Integration of the required peak areas



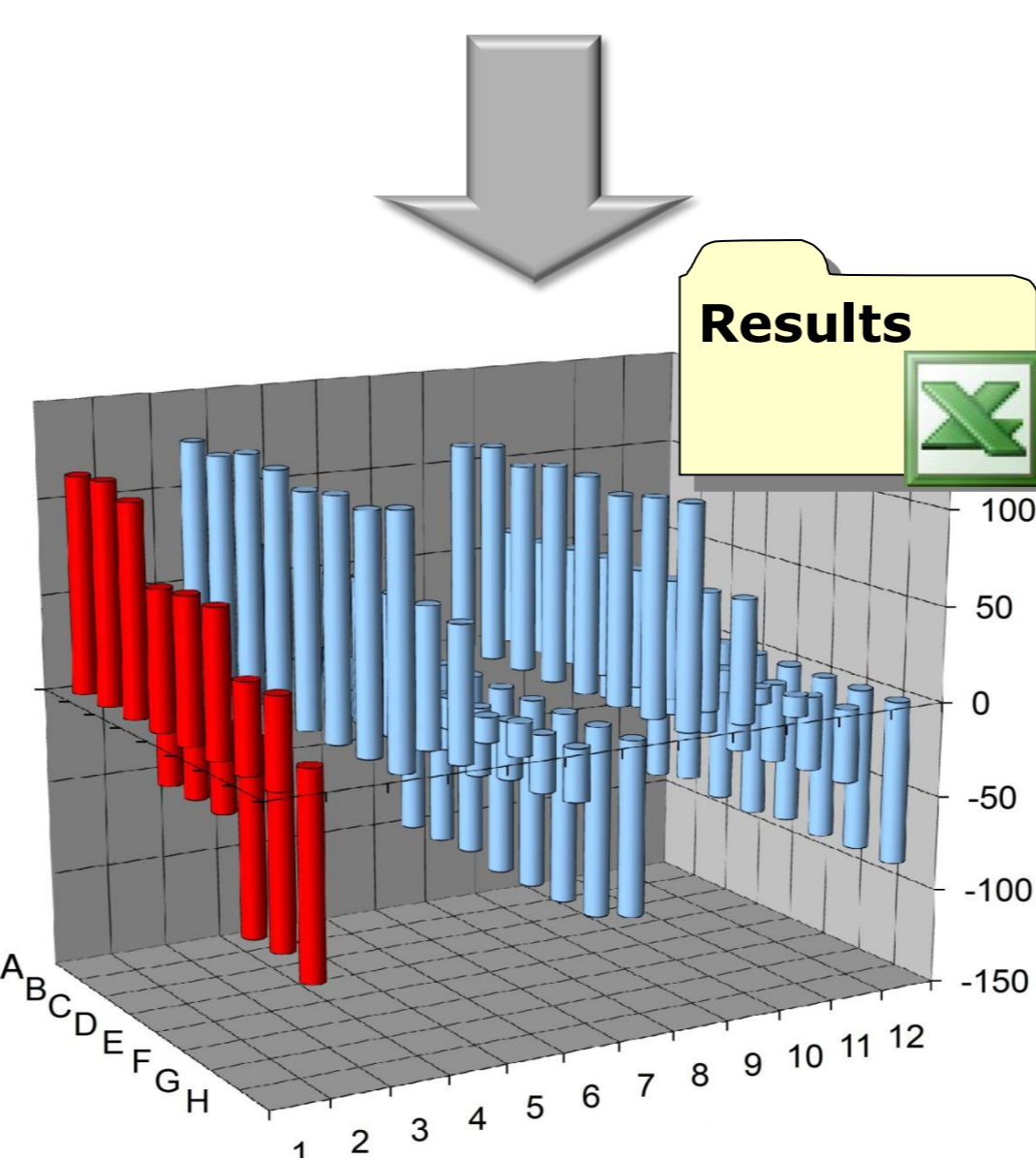
Synchronous to the raw data acquisition, for each sample a processed data file will be created containing the values of the integrated peak areas for every required mass.

Outlook

The first versions of "Chiral MS" are based on MS Excel VBA. However, there are a lot of advantages for implementing this software module with VB.NET. A standalone software module without binding to a host application such as MS Excel can be implemented. Therefore, the existing module will be converted. Furthermore, new features, especially for method development for investigation of new compound classes, will be included. For that purpose further functions for various verification measurements as well as user friendly selection of required compounds and their masses are intended.

Summary

The software solution presented is an extensional module in addition to commercial software. It allows data processing and evaluation in the special field of enantiomeric excess determination in the scope of high throughput applications. The analysis method with the associated software solution is characterized by short timeframes in analysis and data evaluation. A maximum of automation from sample preparation up to the data evaluation and visualization will be provided.



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