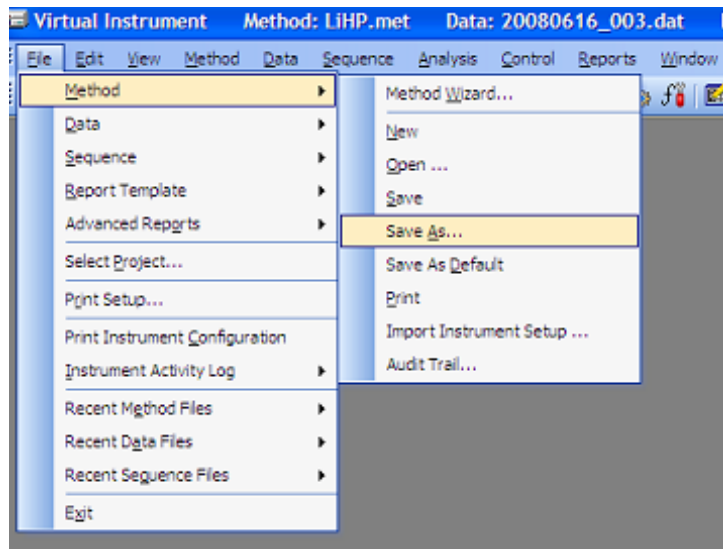


Procedure

Step 1: Create a new folder into C:\EZChrom Elite\Enterprise\Projects\Default\Data where the baseline file will be stored. This folder has to be called "Public" in order to allow the baseline filename to be modified each time a new blank run is performed.

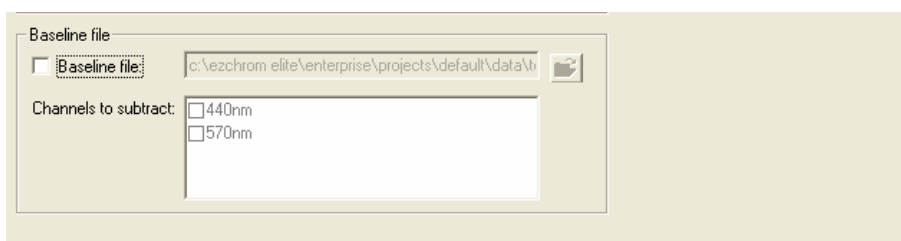
Step 2: In EZChrom Elite offline mode, save the current method used under a new name e.g. "LiHP baseline subtraction" as below.



Step 3: Setup your sample list under BIOSYS as usual. After a period of inactivity, it is recommended to start the sequence with a regeneration run. Then add two blank runs as the first will not be used. Insert your next sample as usual in the sample list

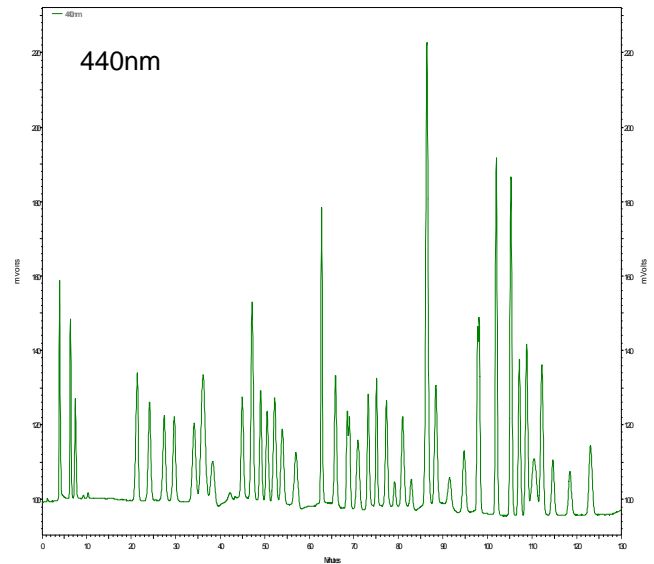
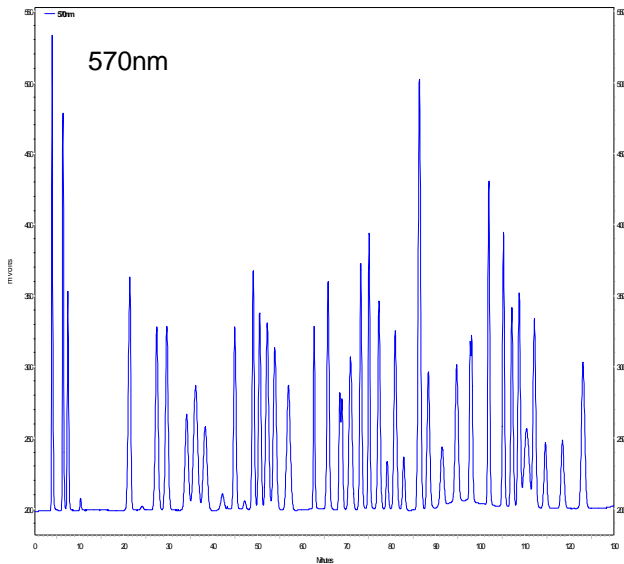
Step 4: Once your second blank run has been acquired, within Windows Explorer go to the folder previously created: C:\EZChrom Elite\Enterprise\Projects\Default\Data\Public and copy and save the blank run into it. The data file should be called something simple and memorable such as "Baseline".

Step 5: Go into the EZChrom offline window. Open the method previously created at Step 2 for baseline subtraction. Select "Method\advanced" and select the "file" tab.

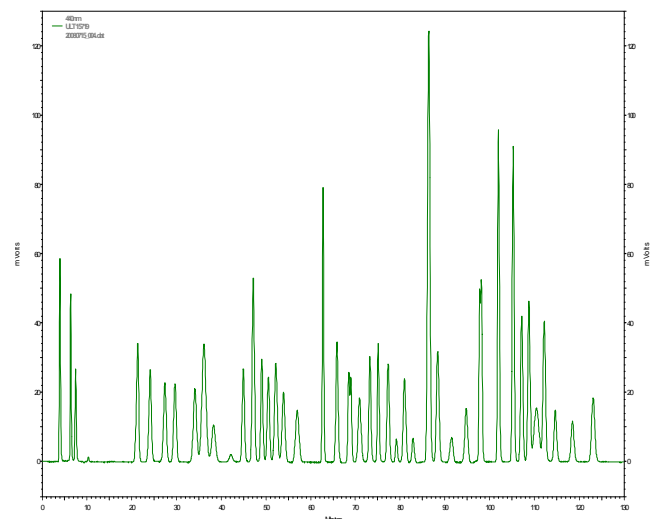
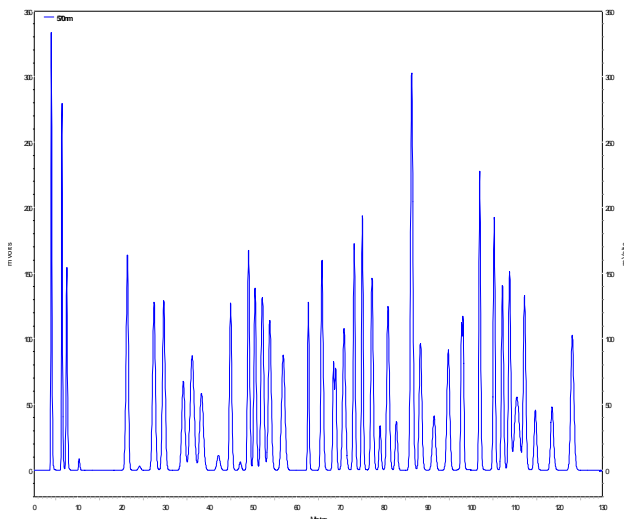


Step 6: Tick the box "baseline file" and click the browse button to select the file called "Baseline" which should now be found into the public folder. Tick the channels to subtract and save the method. Close the advanced method window.

Step 7: After a sample has been run, open it in EZChrom offline. Opening a Data file will immediately and automatically apply the subtraction procedure providing "Current" is selected in the Open Data File options box. Further processing can then proceed as normal.



Baseline subtraction applied



Note:- It is recommended that a blank chromatogram be run regularly, especially when any changes have been made to the elution program or when a new bottle of Ninhydrin is installed. No changes to the Method are required; simply delete* the old baseline file and drop the new one into the "Public" folder, renaming it as "Baseline".

[*To comply with regulatory requirements the original data is retained permanently in the usual place].

Influence of the baseline subtraction on the peak areas

The table below shows only minor variations occur between the peak areas before and after baseline subtraction for peaks in places where the baseline is already clean, e.g. for Taurine.

The variation in areas before and after subtraction for sensitive parts of the chromatogram, where buffer steps or baseline undulations are present, (e.g. Sarcosine and Ethanolamine) is significant.

A comparison with the more subjective and less accurate manual adjustment of the baseline is also shown for these two example amino-acids.

Channels	Selected amino acids	Areas (Raw Data)	Areas (manual adjustment of the baseline)	%CV	Areas after subtraction	%CV
570nm	Taurine	4768251	n/a	n/a	4767560	0.01
	Sarcosine	647896	575115	8.42	603659	5.00
	Ethanolamine	2235902	2080645	5.09	2092342	4.69
440nm	Hydroxyproline	1064214	n/a	n/a	1062329	0.13
	Proline	1934685	n/a	n/a	1946472	0.43

Please note that this operation does not modify the original data file. It is only a calculation process that can be easily reversed by re-opening the data file with any other method that has not been setup to carry out the baseline subtraction calculation.