Botanical workshop
UAB
Sept 11, 2006

Sample preparation of biological samples for qualitative and quantitative analysis

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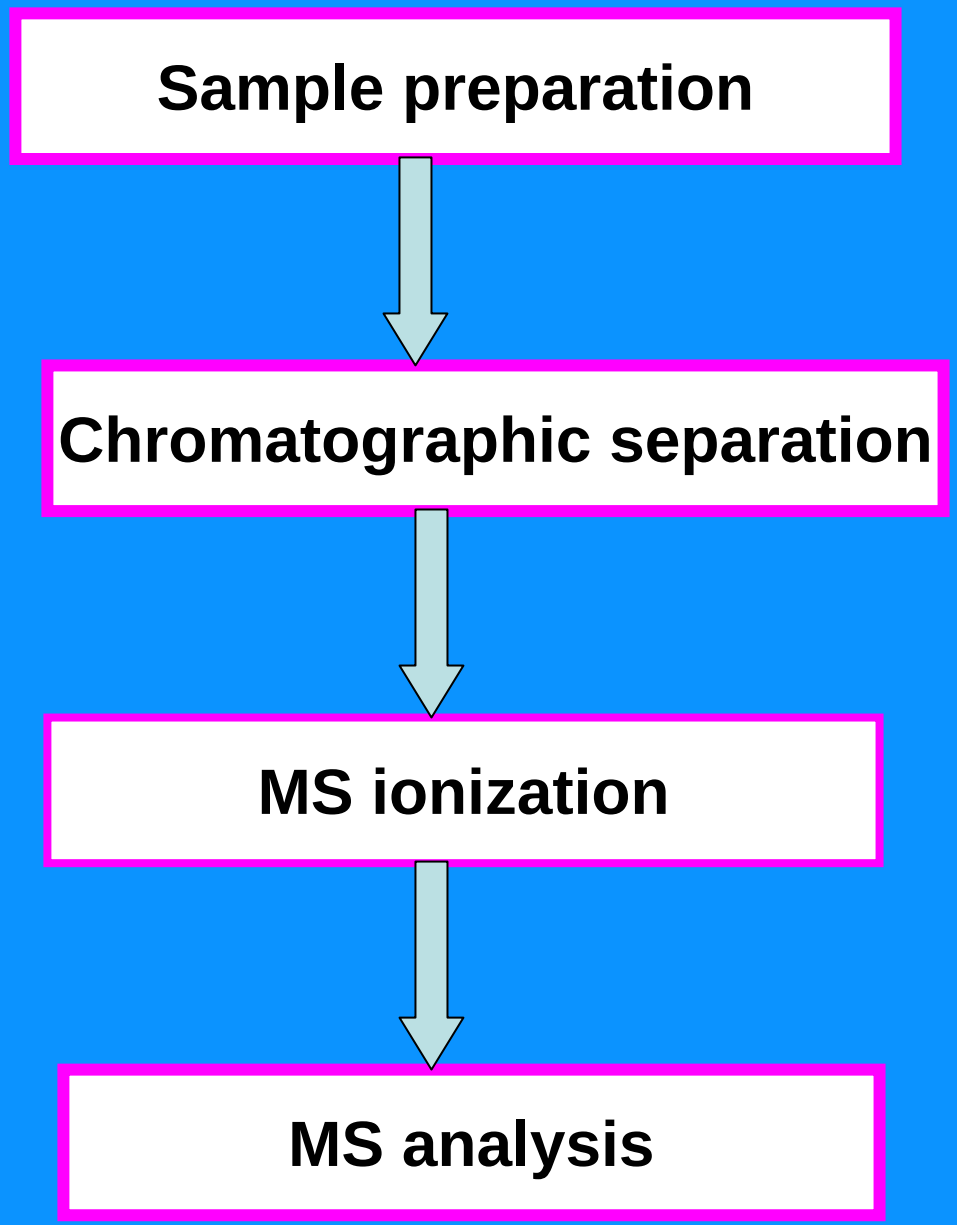
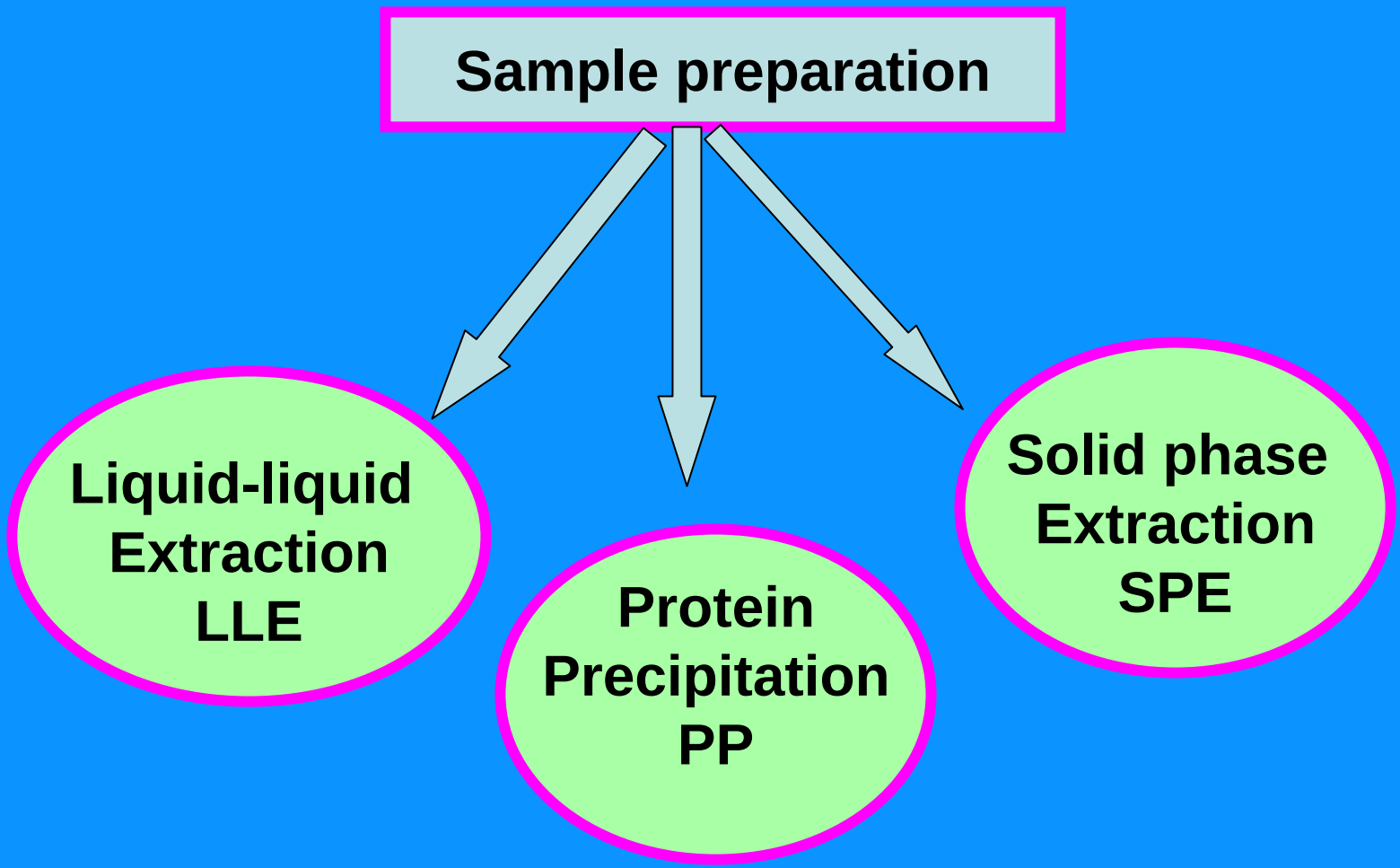
Bioanalysis Flow Chart

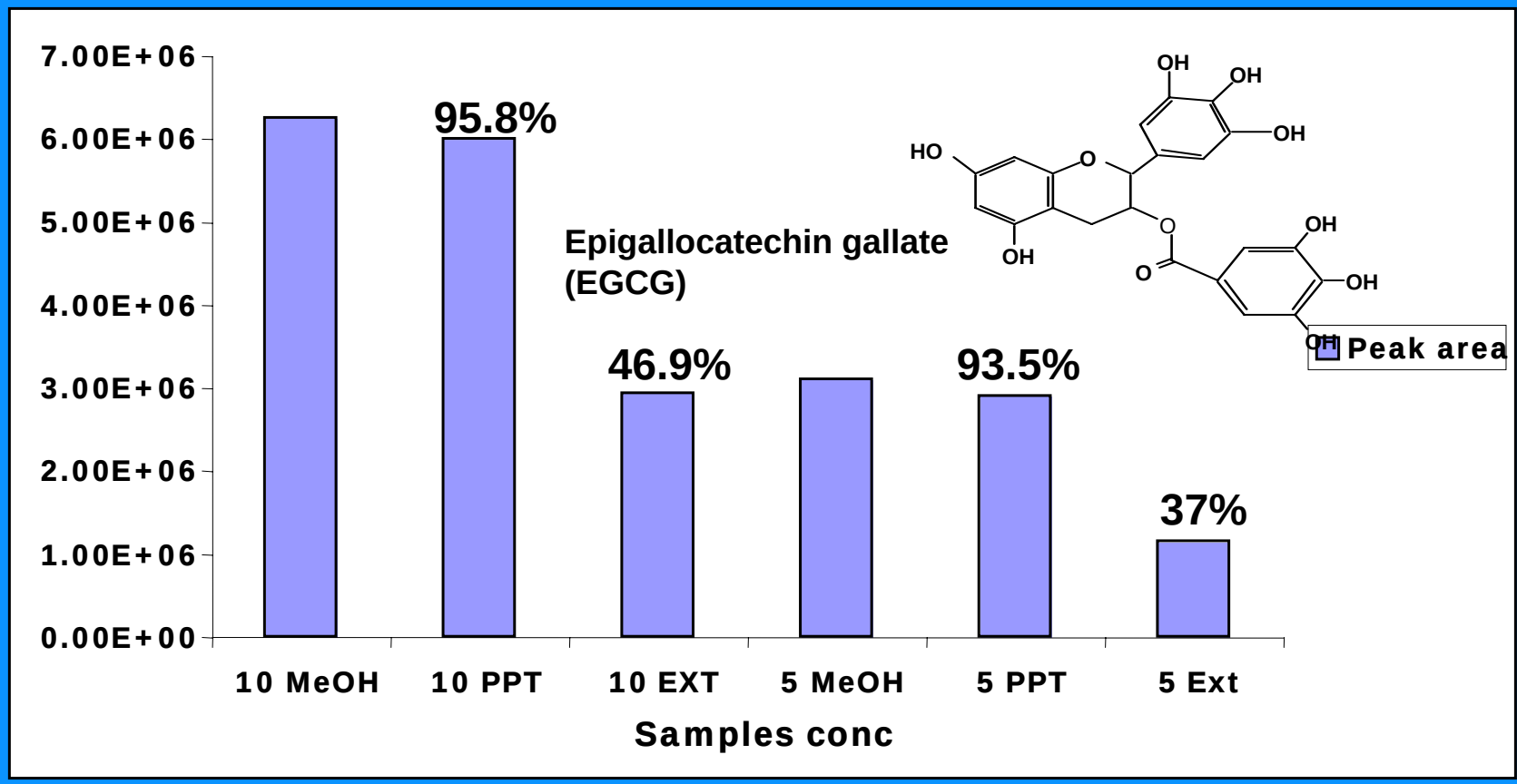


Sample preparation is a crucial step in removing the interfering compounds from biological matrix



The method of choice will be determined by the sample matrix and the concentration of compounds In samples

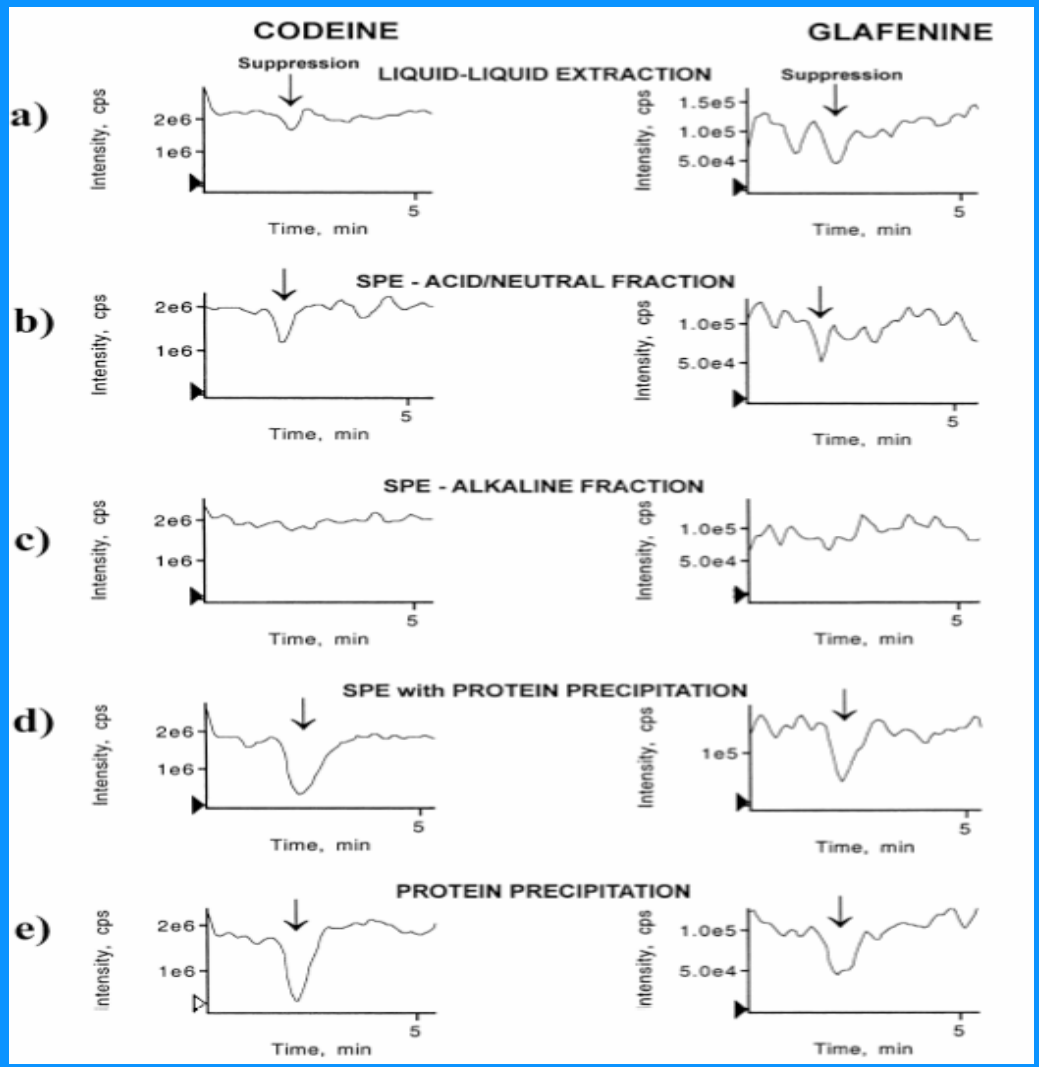
Comparison among MeOH, protein precipitation (PPT) and EtOAcextraction (EXT) method in terms of peak areas obtained from MRM experiments



PPT method appears to be superior than LLE by EtOAc for polar compounds like EGCG

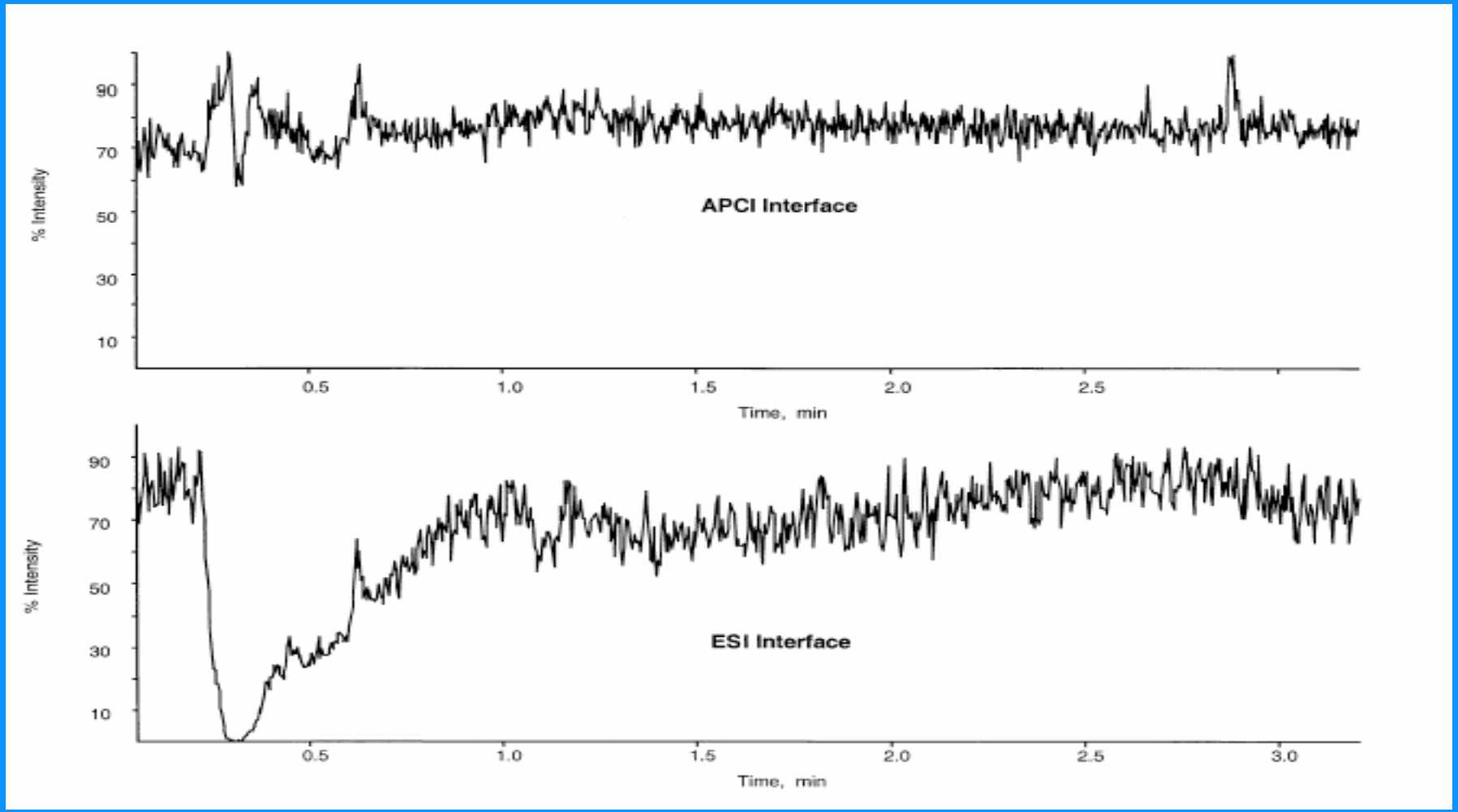
Prasain et al. (unpublished results)

Severe ion suppression effect for codeine and glafenin was observed with PPT and SPE-PPT



Muller et al. J. Chrom B (2002)

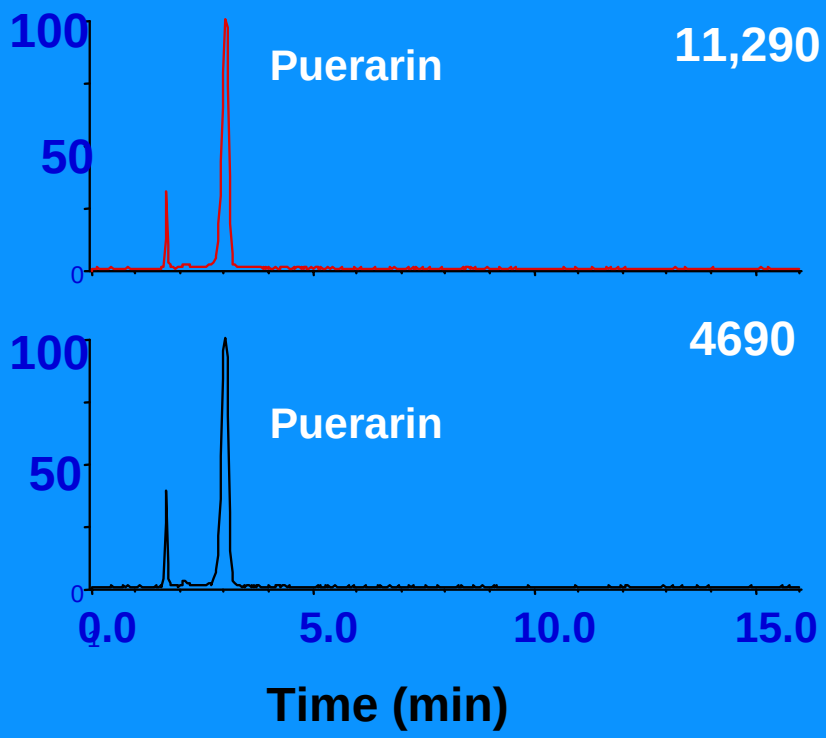
APCI is less prone to than ESI to the effects of ion suppression



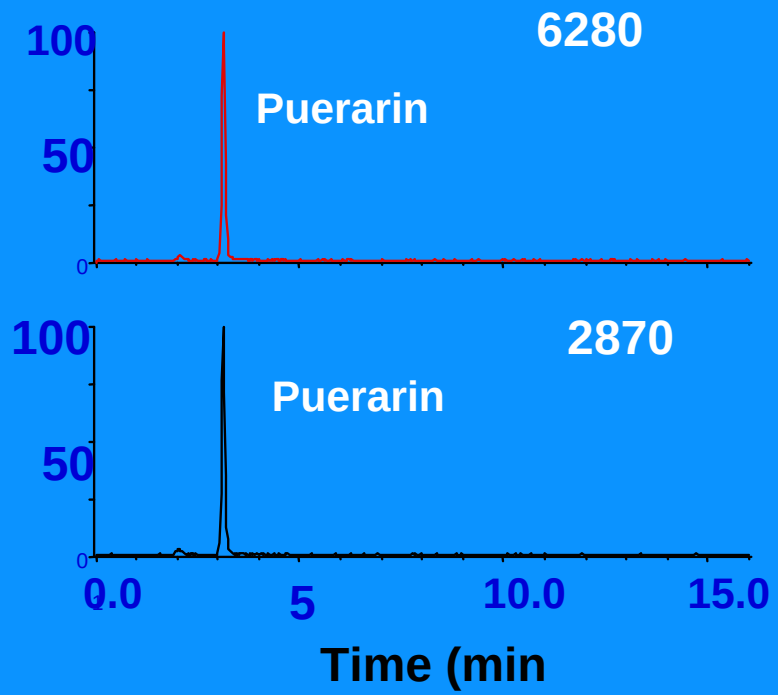
King et al. J. Am Soc Mass Spectrom 2000

Urinary metabolized may be analyzed unextracted by LC-MS/MS. However, extensive dilution is needed for quantitative analyses

SPE sample after 5 fold dilution



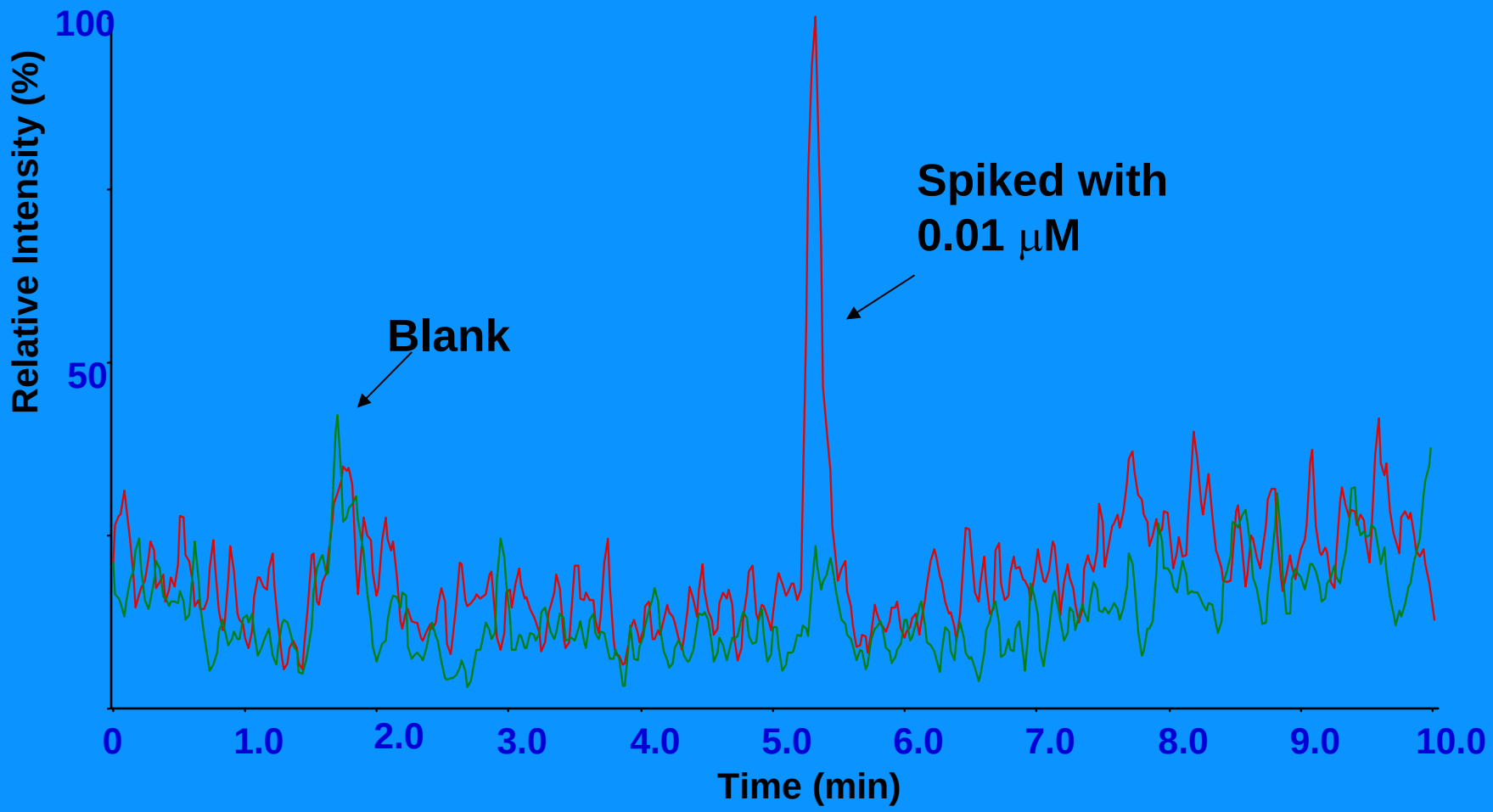
Un-extracted sample after 10 fold dilution



Analytical method validation

- **Should demonstrate specificity, linearity, accuracy, precision**
- **Lower limit of quantitation (S/N =10)**
- **Stability (freeze/thaw)**
- **Robustness**

Ion chromatograms of a rat serum spiked sample (0.01 μM of puerarin) vs. blank serum



Intra-day and inter-day % accuracy and precision of Puerarin in rat serum

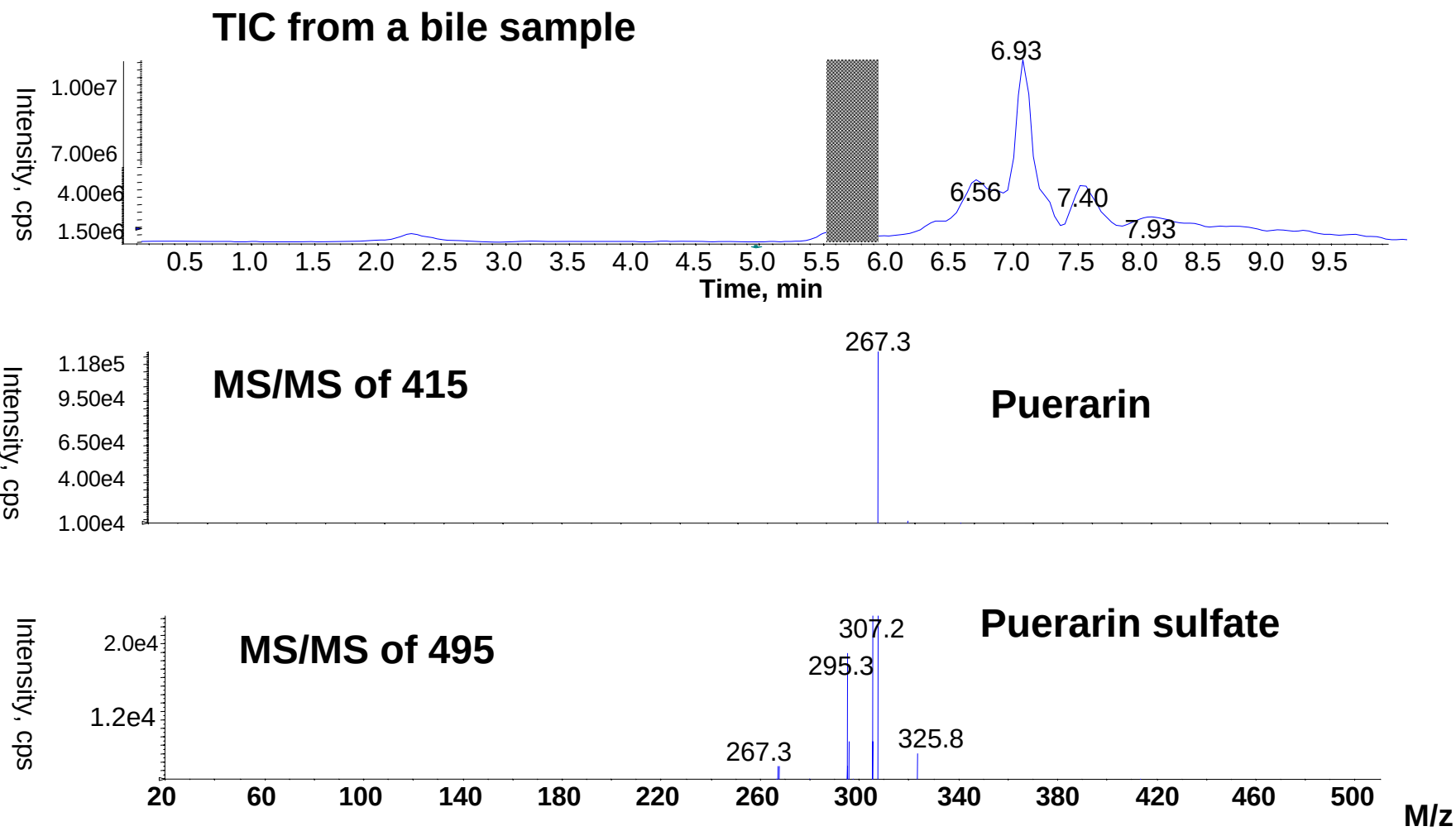
Standard Curve Linearity r = 0.990

uM	calculated uM beginning of run	calculated uM end of run	mean calculated uM	mean % accuracy
0.01	0.00971	0.00971	0.00971	97.1
0.05	0.0629	0.0449	0.0539	107.8
0.1	0.0957	0.0821	0.0889	88.9
0.5	0.563	0.534	0.5485	109.7
1	0.876	0.994	0.935	93.5

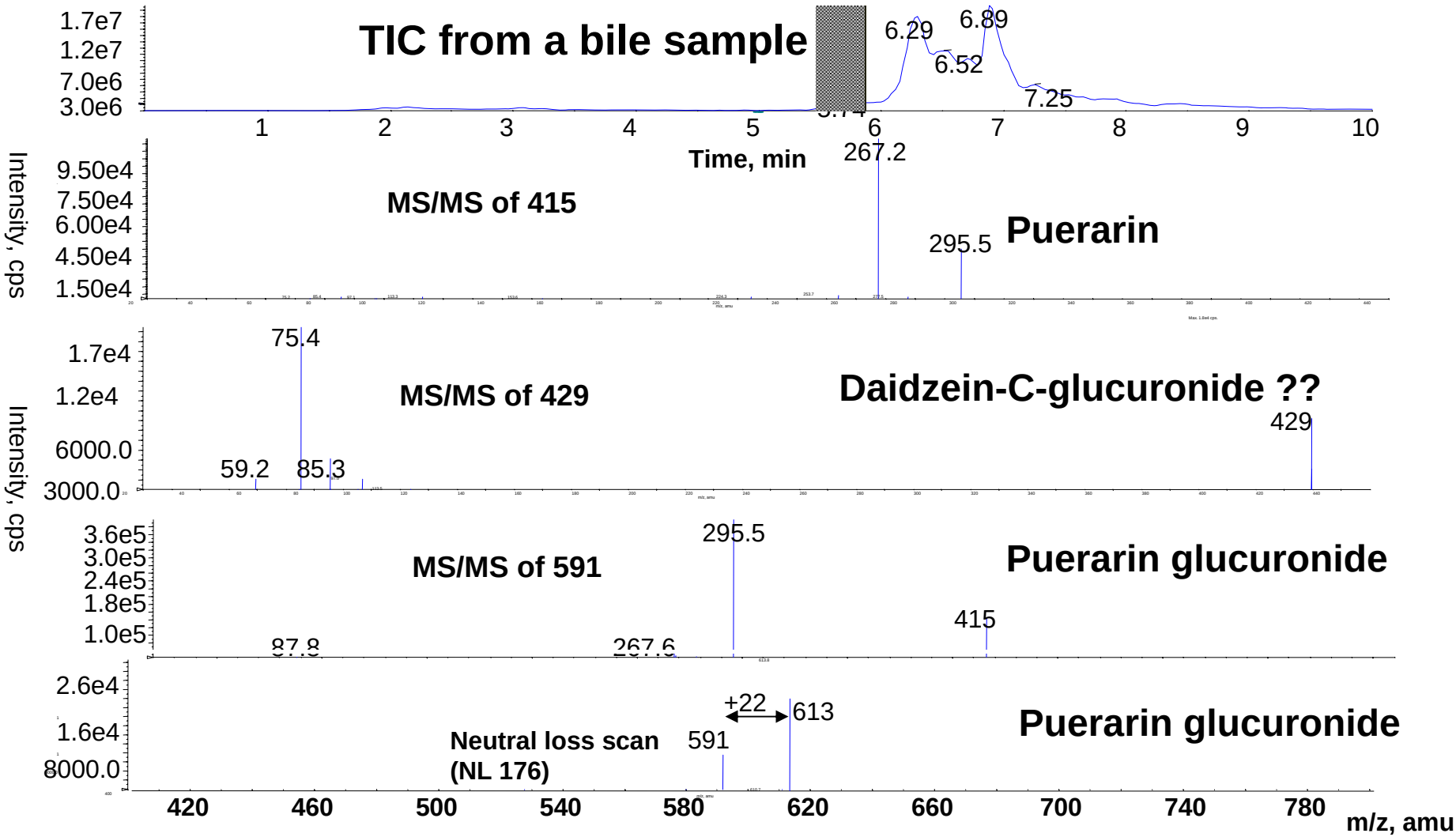
Standard Curve Linearity r = 0.990

uM	calculated uM beginning of run	calculated uM end of run	mean calculated uM	mean % accuracy
0.01	0.0098	0.0101	0.00995	99.5
0.05	0.056	0.0494	0.0527	105.4
0.1	0.0952	0.082	0.0886	88.6
0.5	0.567	0.556	0.5615	112.3
1	0.92	0.943	0.9315	93.15

“Dilute and shoot” can be used for bile samples



Several experiments can be performed in a single run to identify Metabolites in a biological sample using API-4000



Conclusions

- **To support pharmacokinetic and drug metabolism studies, LC-MS/MS plays more and more an essential role for the quantitation of drugs and their metabolites in biological matrices.**
- **To speed-up method development and validation, generic approaches with the direct injection of biological fluids is highly desirable.**
- **Improvement of mass spectrometers performance, and in particular QTRAP has tremendous impact on metabolite identification.**