Mass Spectrometry in Bioanalysis of botanicals

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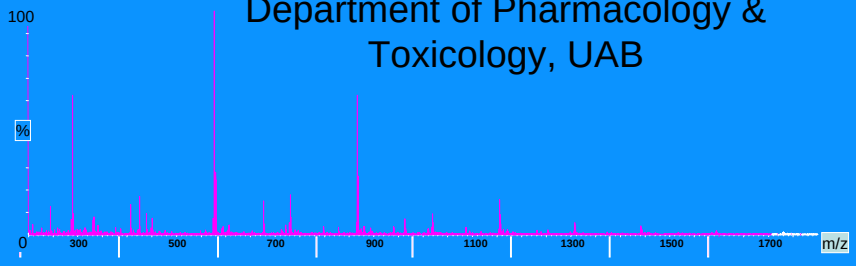
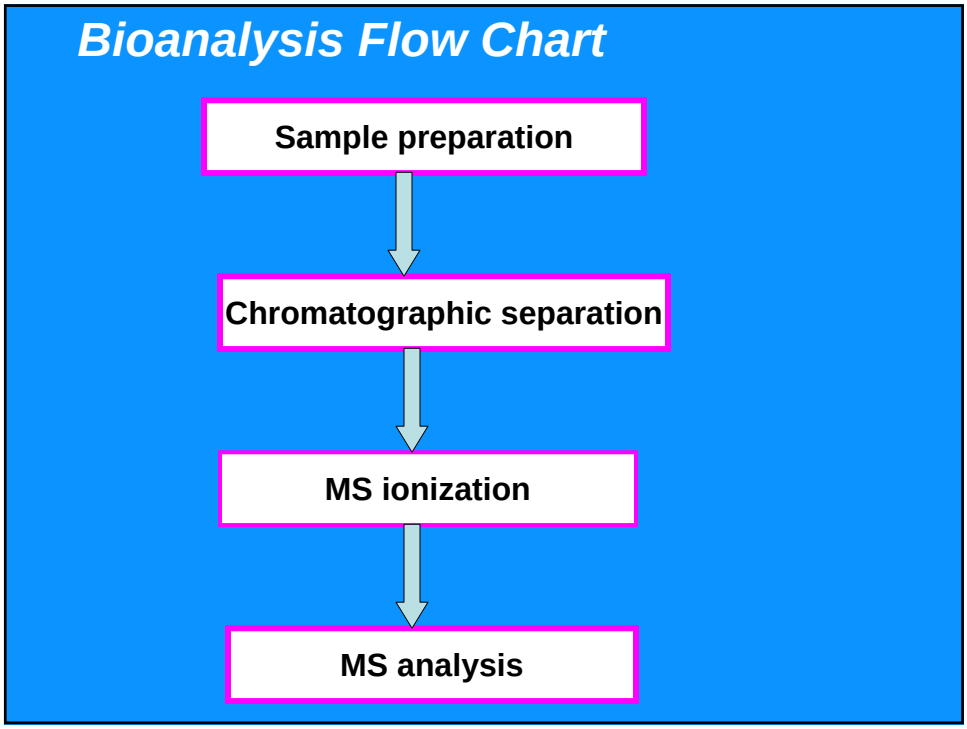


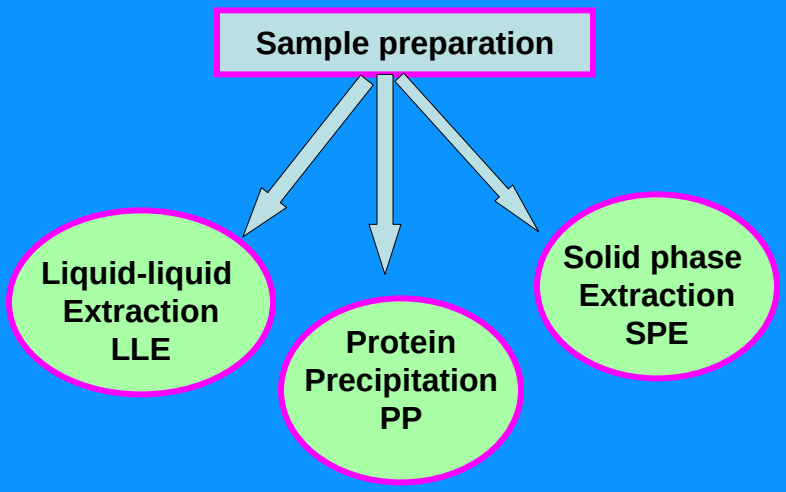
Talk Overview

- **Introduction to bioanalysis**
- **Quantitative analysis of puerarin/salvinorin A/EGCG in biological samples by LC-MS/MS**

Bioanalysis Flow Chart



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

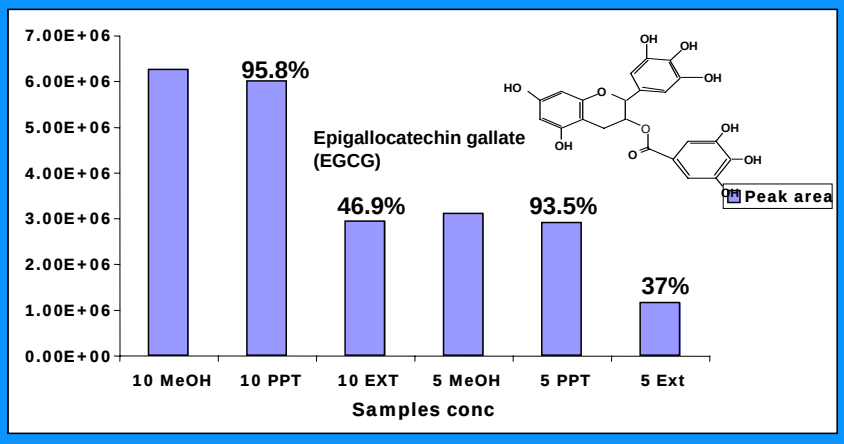


The method of choice will be determined by the sample matrix and the concentration of compounds In samples

Properties of a Good Internal Standard

- Is not found in the original sample
- The structure of the internal standard needs to be similar to the analyte.
- Provides data about your extraction process:
- a. Hydrolysis of 4-Methylumbelliferyl sulfate
- b. Hydrolysis of Phenolphthalein glucuronide
- c. Extraction Efficiency (Apigenin)

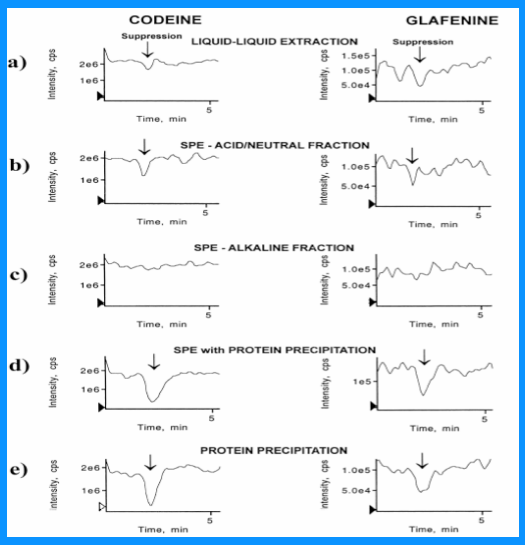
Comparison among MeOH, protein precipitation (PPT) and EtOAc extraction (EXT) method in terms of peak areas obtained from MRM experiments



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

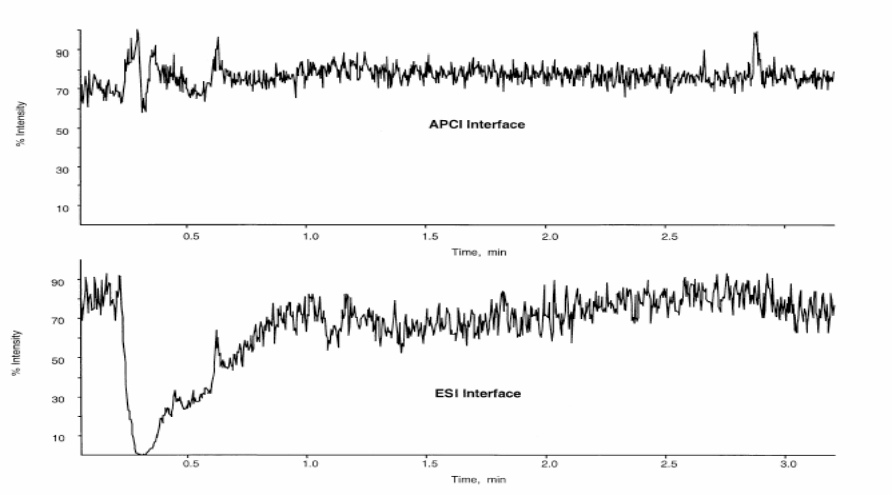
Prasain et al. (unpublished results)

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



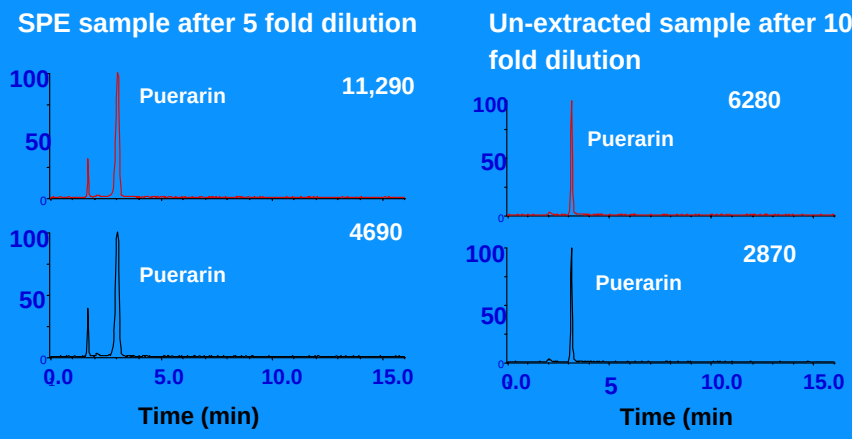
Muller et al. J. Chrom B (2002)

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



King et al. J. Am Soc Mass Spectrom 2000

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



Quantitative determination of puerarin/ salvinorin A and EGCG in rat serum by LC-MS/MS

Analytical method validation

- **SShould demonstrate specificity, linearity, accuracy, precision**
- **LLower limit of quantification**

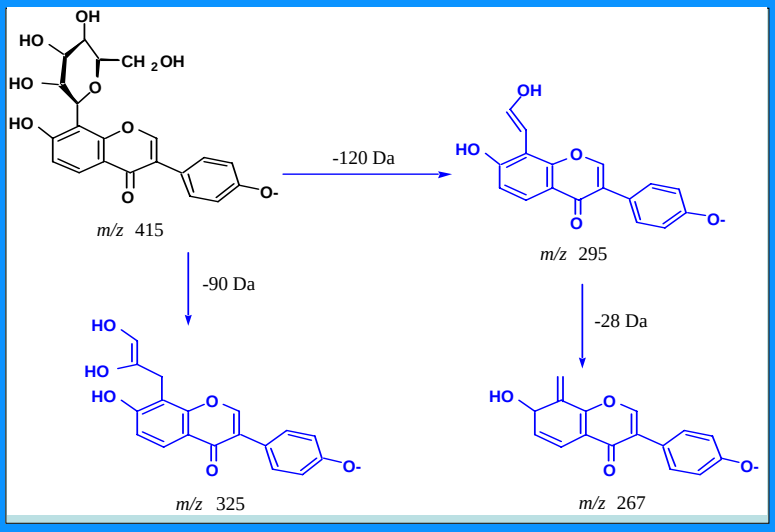
Stability (freeze/thaw)

Robustness

LC/MS/MS Method for Puerarin

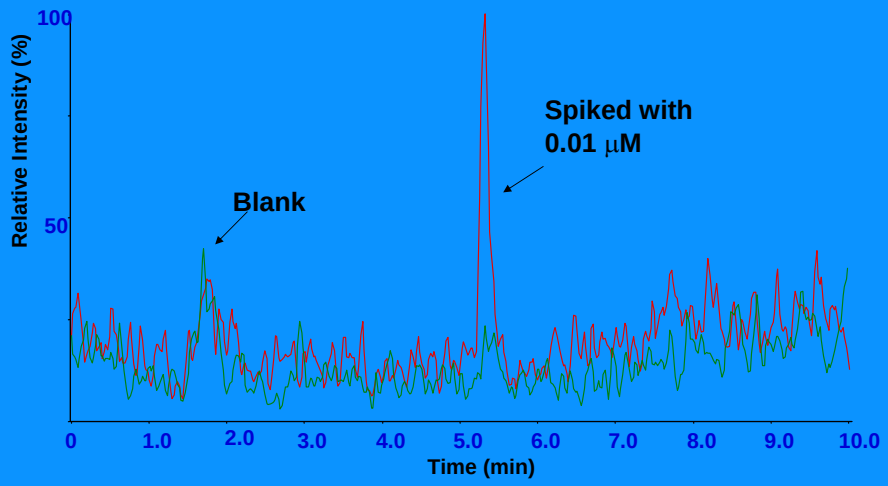
Column:	**Waters X-Terra C18 with guard, 2.1 x 100 mm, 3.5 micron**
Mobile Phase A:	**10% MeCN + 10 mM NH4OAc**
Mobile Phase B:	**70% MeCN + 10mM NH4OAc**
Gradient:	**0 minutes = 100% A**
	6 minutes = 100% B
	7 minutes = 100% A
	10 minutes = Stop
Injection Volume:	**20 ul**
Flow Rate:	**0.2 ml/min split flow**
Mass Spectrometer:	**Negative Electrospray**
Mass Transitions:	**415/267 (Puerarin)**
	415/295 (Puerarin)
	269/149 (apigenin, IS)

Possible product ions of puerarin in ESI-MS/MS negative ion mode



Prasain et al. J Agric Food Chem. 51, 4213, 2003.

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

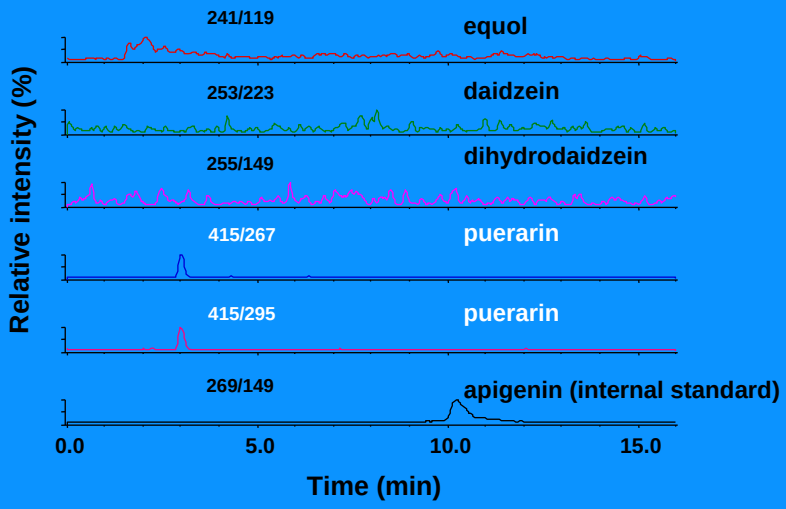
uM	calculated uM beginning of run	calculated uM end of run	mean calculated uM	mean % accuracy
0.01	0.00971	0.00971	0.00971	97.1
0.05	0.0629	0.0449	0.0539	107.8
0.1	0.0957	0.0821	0.0889	88.9
0.5	0.563	0.534	0.5485	109.7
1	0.876	0.994	0.935	93.5

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

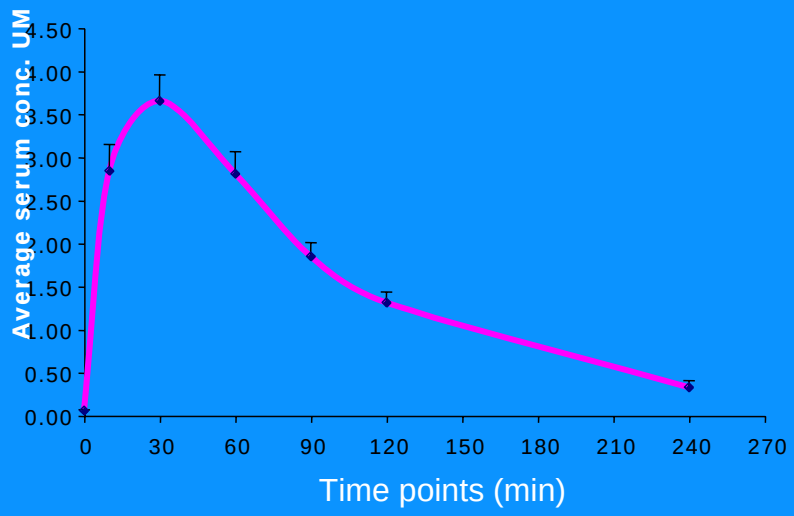
	calculated concentration stats		calculated concentration stat
	2.0 uM		**0.2 uM**
2.0 QC1	1.76	0.2 QC1	0.199
2.0 QC2	1.92	0.2 QC2	0.186
2.0 QC3	1.76	0.2 QC3	0.205
2.0 QC4	2.03	0.2 QC4	0.202
2.0 QC mean=	1.868	0.2 QC mean=	0.198
2.0 QC st dev=	0.132	0.2 QC st dev=	0.008
% C.V. =	7.1	% C.V. =	4.2
2.0 Re1	2.16	0.2 Re1	0.215
2.0 Re2	1.88	0.2 Re2	0.211
2.0 Re3	2.54	0.2 Re3	0.228
2.0 Re4	1.87	0.2 Re4	0.202
2.0 Re mean=	2.113	0.2 Re mean=	0.214
2.0 Re st dev=	0.315	0.2 Re st dev=	0.011
% C.V. =	14.9	% C.V. =	5.0
	% recovery		% recovery
	2.0 uM		**0.2 uM**
mean % recovery =	90.4	mean % recovery =	92.7
st dev % recovery =	18.187	st dev % recovery =	5.221
% C.V. =	20.1	% C.V. =	5.6

MRM is useful in detection of puerarin specifically in a rat serum samples treated with puerarin



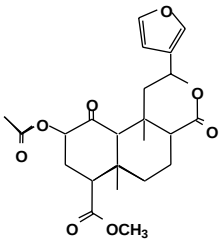
Prasain et al. J. Agric. Food Chem. 52:3708-3712; 2004

Average serum concentration of puerarin versus time after Oral administration of 50 mg/kg puerarin



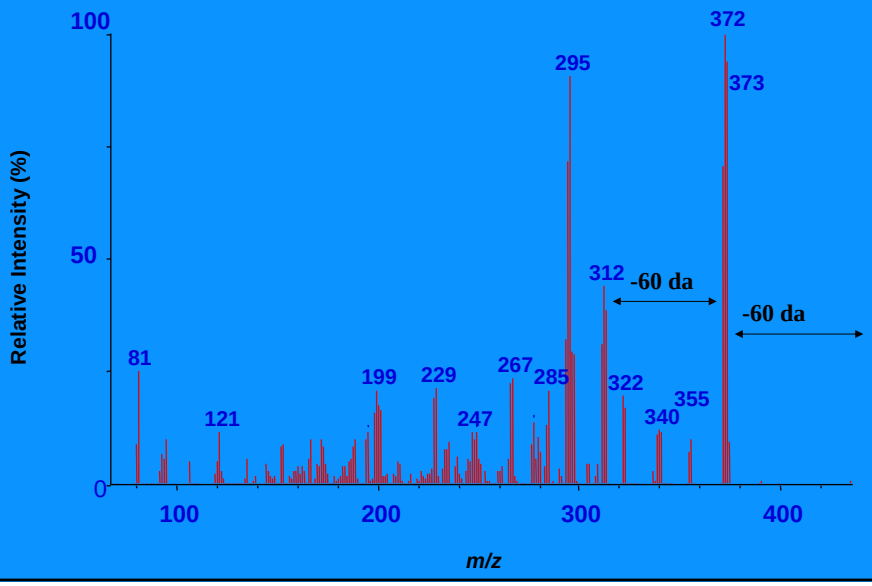
Prasain et al. (unpublished results)

Salvia divinorum - a plant of the mint family has been used as a recreational drug and salvinorin A, the presumed main active ingredient of *S. divinorum*

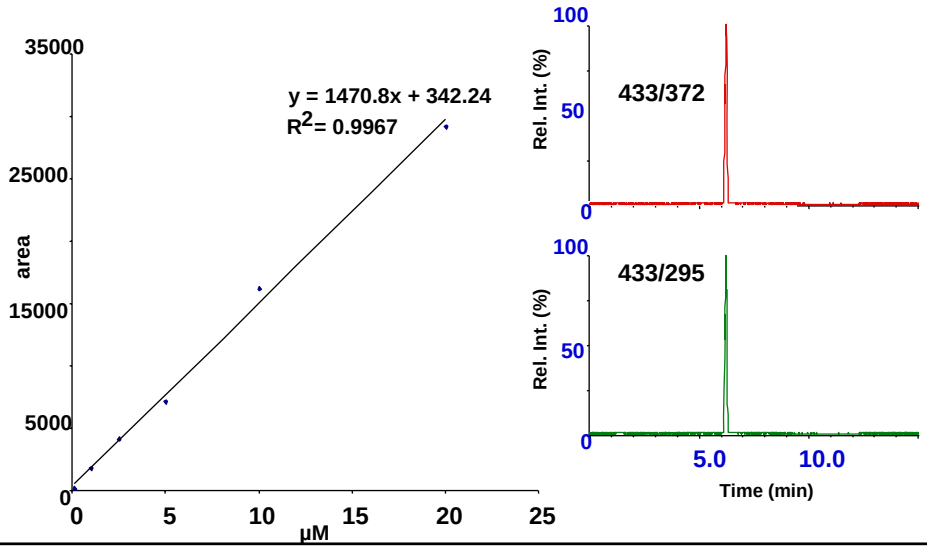


Salvinorin A

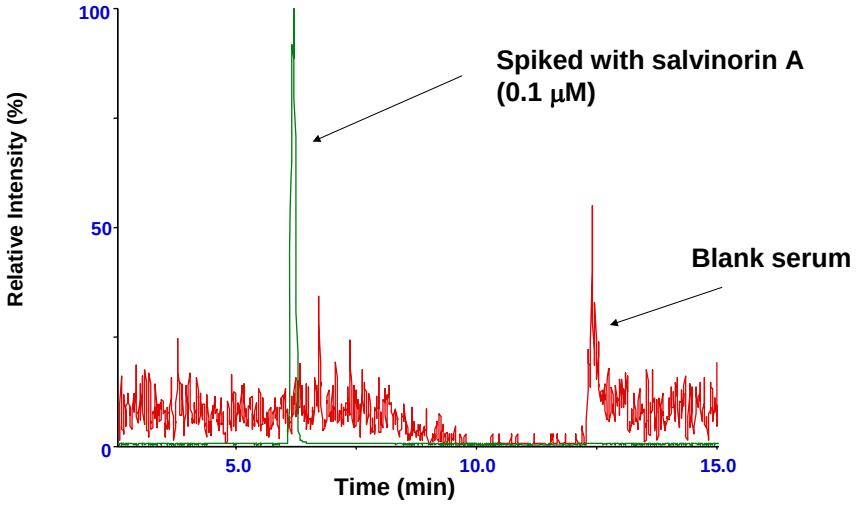
Neutral losses of CH_3COOH (2 x 60 Da) are characteristics of MS/MS fragmentation of protonated salvinorin A m/z 433



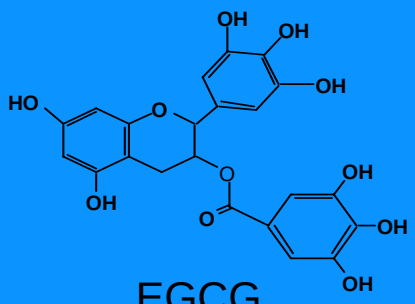
MRM chromatogram of salvinorin A and standard curve showing excellent linearity (0.1-20 μM) with a correlation coefficient >0.99



A representative chromatogram of a serum sample spiked with salvinorin A after LLE showing specificity of the LC-MS/MS method



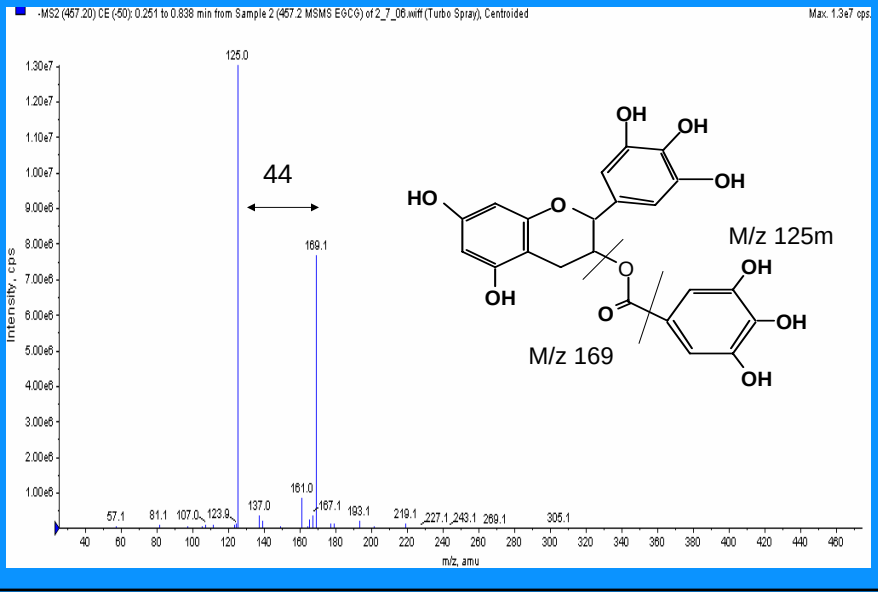
Analysis of tea catechin in biological samples



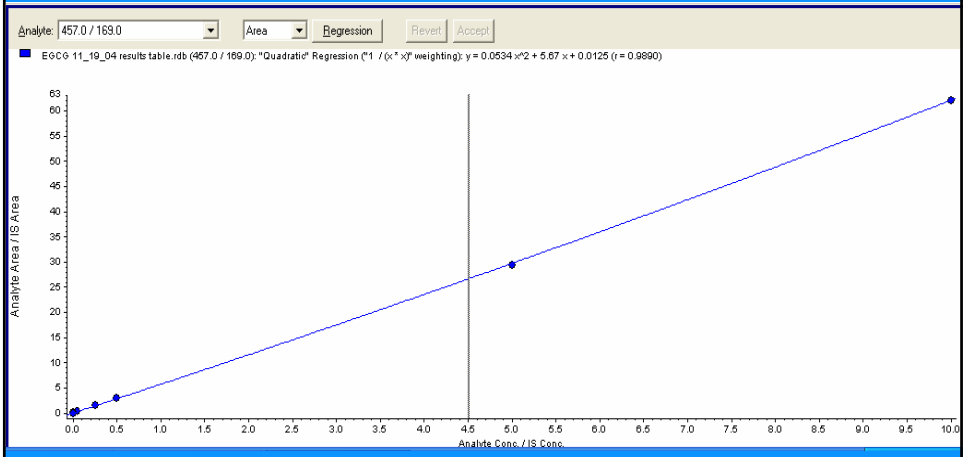
EGCG

$[M-H]^-$ *m/z* 457

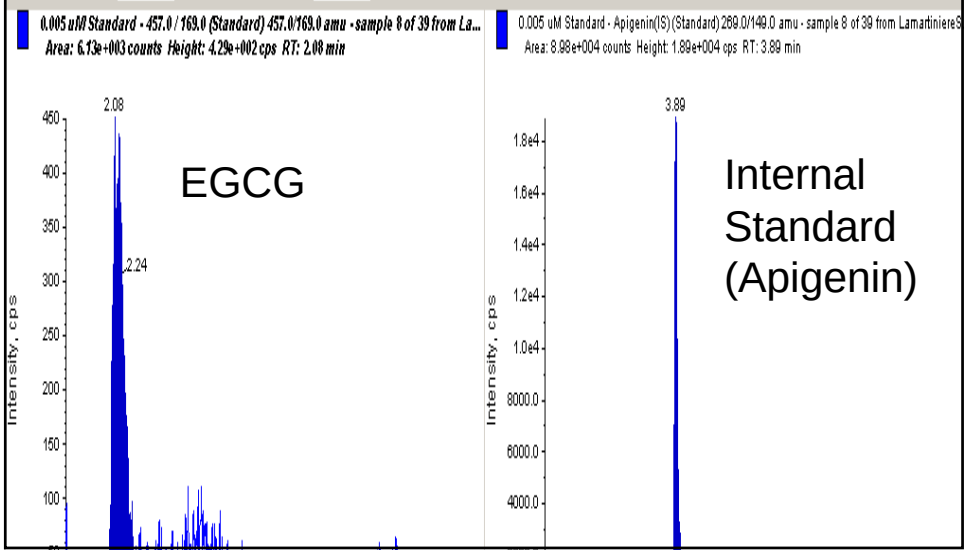
Product ion spectrum of the ion m/z 457



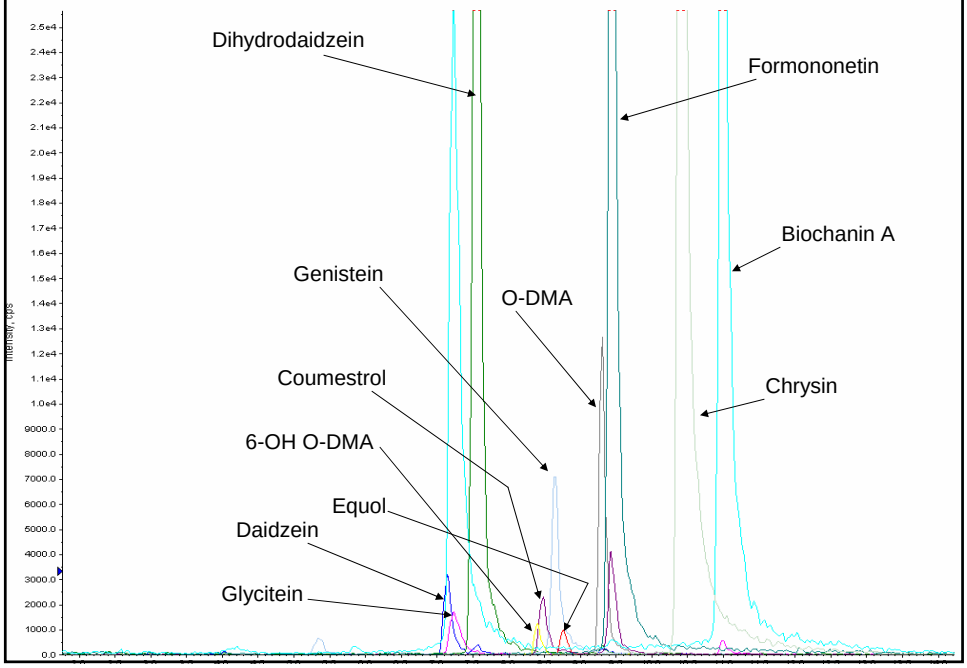
Calibration curve for EGCG (1-10,000 nM) after extracting from rat serum

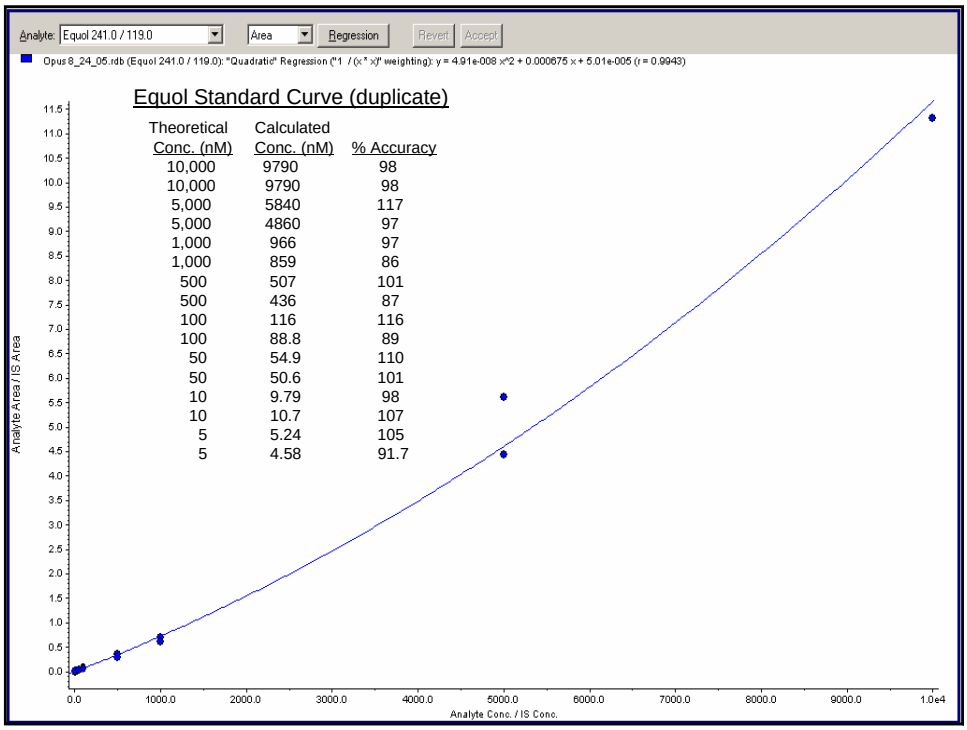


Typical chromatogram of a serum sample spiked with 5 nM after protein precipitation



Quantification of Isoflavones





Equol Standard Curve (duplicate)

Theoretical Conc. (nM)	Calculated Conc. (nM)	% Accuracy
10,000	9790	98
10,000	9790	98
5,000	5840	117
5,000	4860	97
1,000	966	97
1,000	859	86
500	507	101
500	436	87
100	116	116
100	88.8	89
50	54.9	110
50	50.6	101
10	9.79	98
10	10.7	107
5	5.24	105
5	4.58	91.7