

Online Resource 1 UV/Vis absorption wavelengths and absorptivity coefficients used for folate vitamer stock analysis (18)

Folate forms	Stock pH	λ max (nm)	Absorptivity coefficient ($L\ mol^{-1}\ cm^{-1}$)
Folic acid (CDC only)	7.2	282	27 600
		346	7 200
5mTHF	7.2	290	31 700
5fTHF	7.2	285	37 200
THF	7.2	298	25 000
5,10-methenylTHF	0.3	288	13 500
		348	26 500
MeFox	12.2	280	19 365

Online Resource 2 Optimal fragmentation settings and MRM transitions for folates (NIST ID-LC-MS/MS Method 1)

vitamer	precursor ion, <i>m/z</i>	product ion, <i>m/z</i>	Frag ^b (V)	CE ^c (V)	¹³ C precursor ion, <i>m/z</i>	¹³ C product ion, <i>m/z</i>	Frag (V)	CE (V)
Folic acid	442.4	295.1 ^a	100	12	447.4	295.1 ^a	110	12
		176.1	100	30		176.1	110	30
		120.1	100	30		120.1	110	30
5mTHF	460.5	313.1 ^a	115	30	465.5	313.1 ^a	135	16
		194.1	115	30		194.1	135	30
		180.2	115	30		180.2	135	30
5fTHF	474.4	327.1	135	16	479.4	327.1	135	16
		299.2 ^a	135	30		299.2 ^a	135	30
		208.2	135	30		208.2	135	30
MeFox	474.4	327.1	135	24	479.4	327.1 ^a	120	24
		284.1 ^a	135	30		284.2	120	24
THF	446.4	299.1 ^a	140	20	451.4	299.1 ^a	130	30
		120.1	140	20		120.1	130	30
		166.0	140	20		166.0	130	30
5,10-methenylTHF	456.0	412.2 ^a	210	30	461.0	416.2 ^a	225	30
		410.1	210	30		414.1	225	30
		259.5	210	24		235.5	225	12

^a Quantifier transition

^b Frag: fragmentor voltage

^cCE: collision energy voltage

Online Resource 3 Optimal fragmentation settings and MRM transitions for folates (NIST ID-LC-MS/MS Method 2)

Vitamer	Precursor ion, <i>m/z</i>	Product ion, <i>m/z</i>	Dwell time (ms)	DP^a (V)	CE^b (V)	CXP^c (V)	EP^d (V)
Folic acid	442.2	295.2	110	10	18	13	10
¹³ C ₅ - Folic acid	447.2	295.2	110	10	19	13	10

^aDP: declustering potential voltage

^bCE: collision energy voltage

^cCXP: cell exit potential voltage

^dEP: entrance potential voltage

Online Resource 4 LC mobile phase gradient for ID-LC-MS/MS NIST Method 2

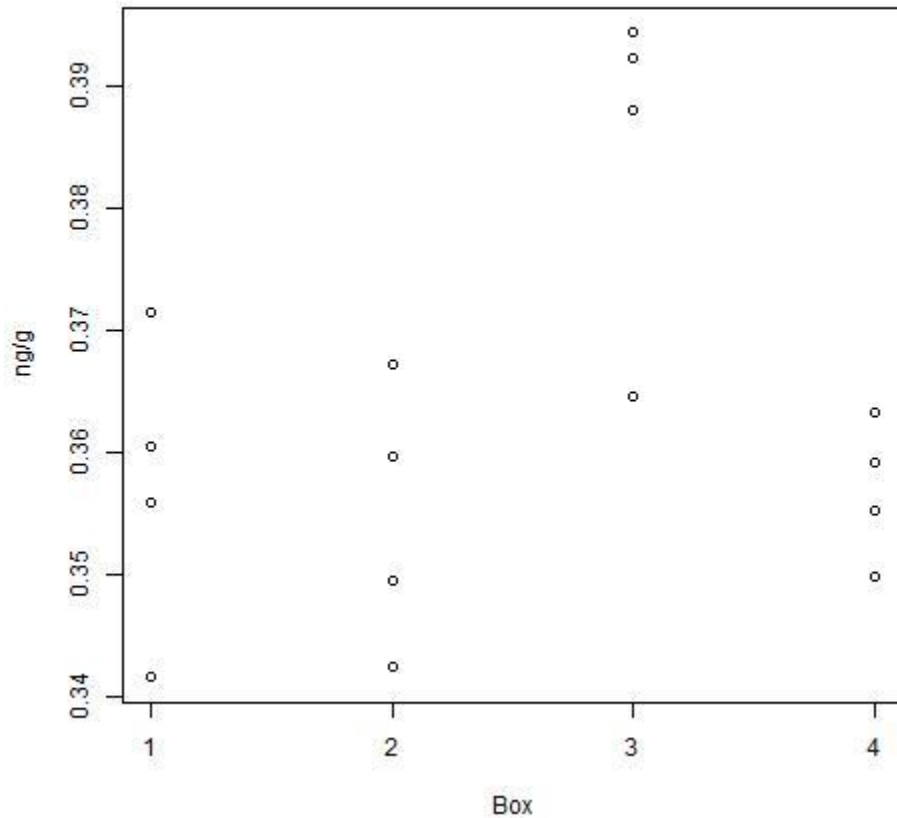
Time (min)	A % [(0.1 % formic acid in water (volume fraction)]	B % [(0.1 % formic acid in acetonitrile (volume fraction)]
0	100	0
13.5	83.8	16.2
14	20	80
17.5	20	80
19	100	0
20	100	0

Online Resource 5 LC mobile phase gradient for LC-UV purity analysis of 5mTHF

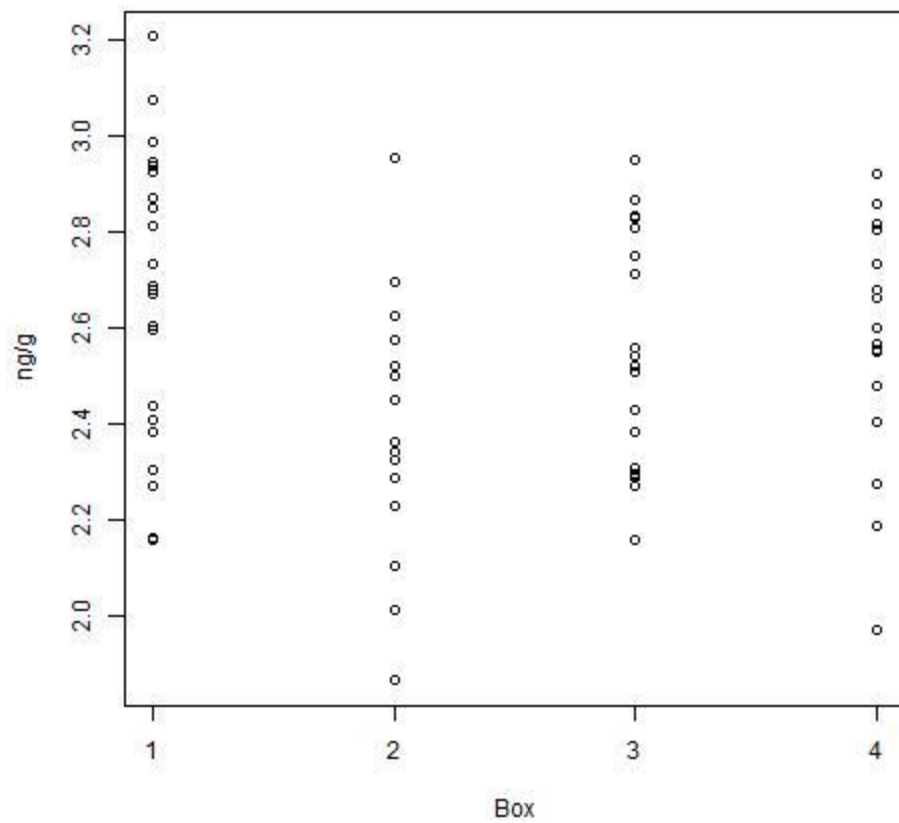
Time (min)	A % [(0.1 % formic acid in water (volume fraction)]	B % [(0.1 % formic acid in acetonitrile (volume fraction)]
0	100	0
83	50	50
84	20	80
90	20	80
91	100	0
100	100	0

Online Resource 6 Homogeneity plots (box fill order vs. folate in ng/g) for PGA (folic acid) and 5mTHF for SRM 3949 Level 1, Level 2, and Level 3

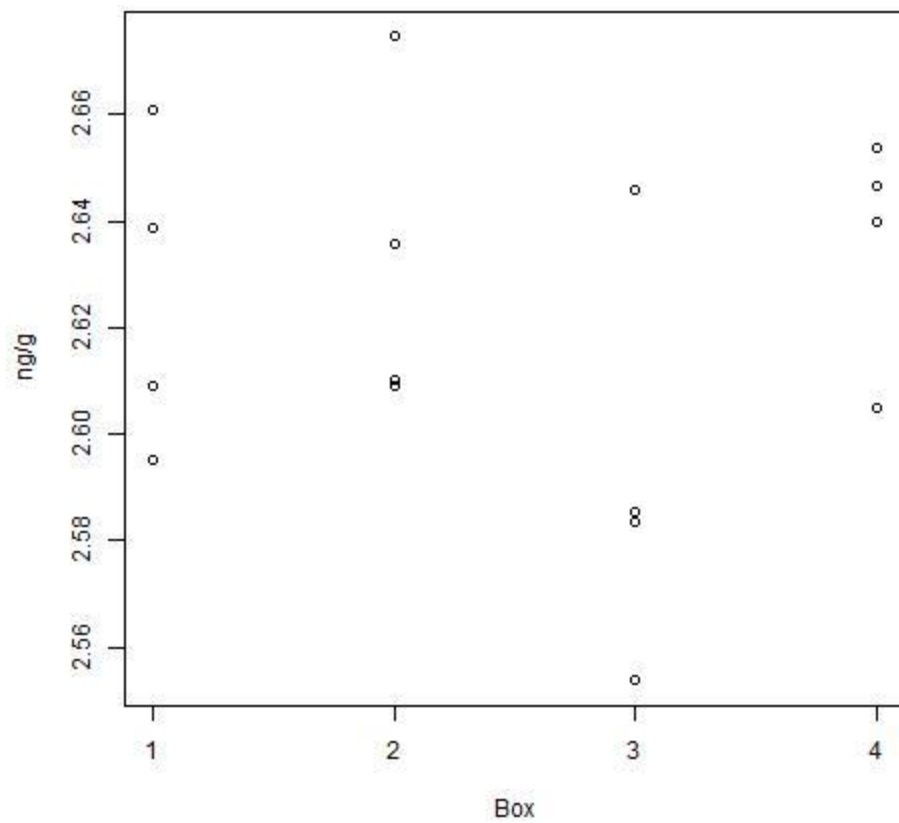
PGA-Level.1 : NIST LC-MS/MS (2)



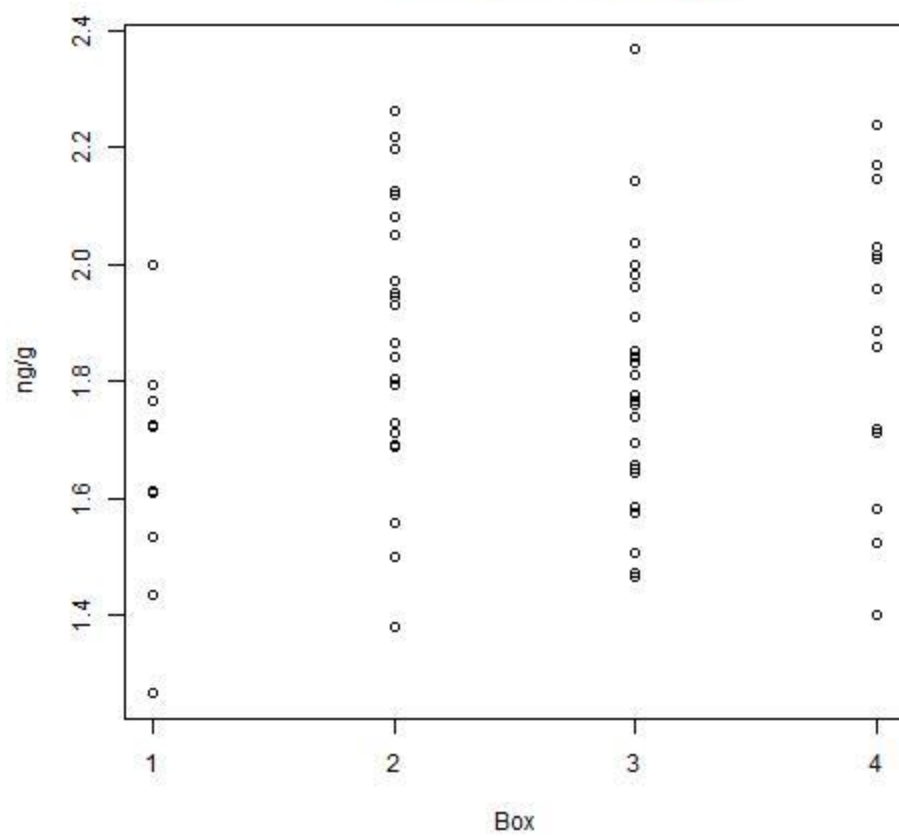
PGA-Level.2 : NIST LC-MS/MS (1)



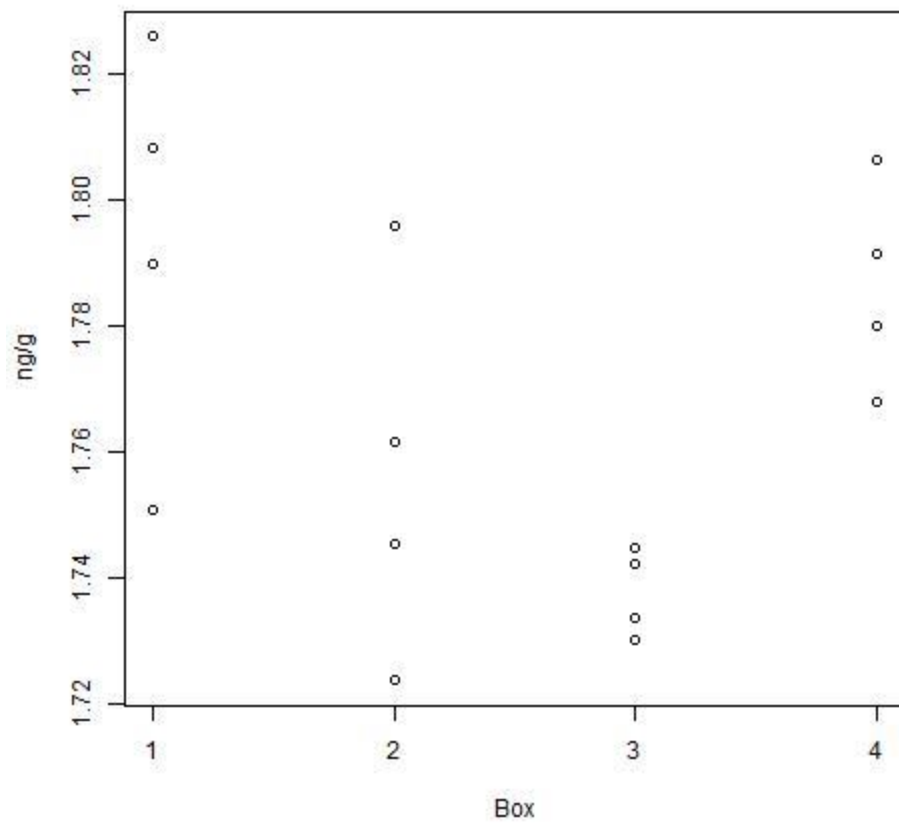
PGA-Level.2 : NIST LC-MS/MS (2)



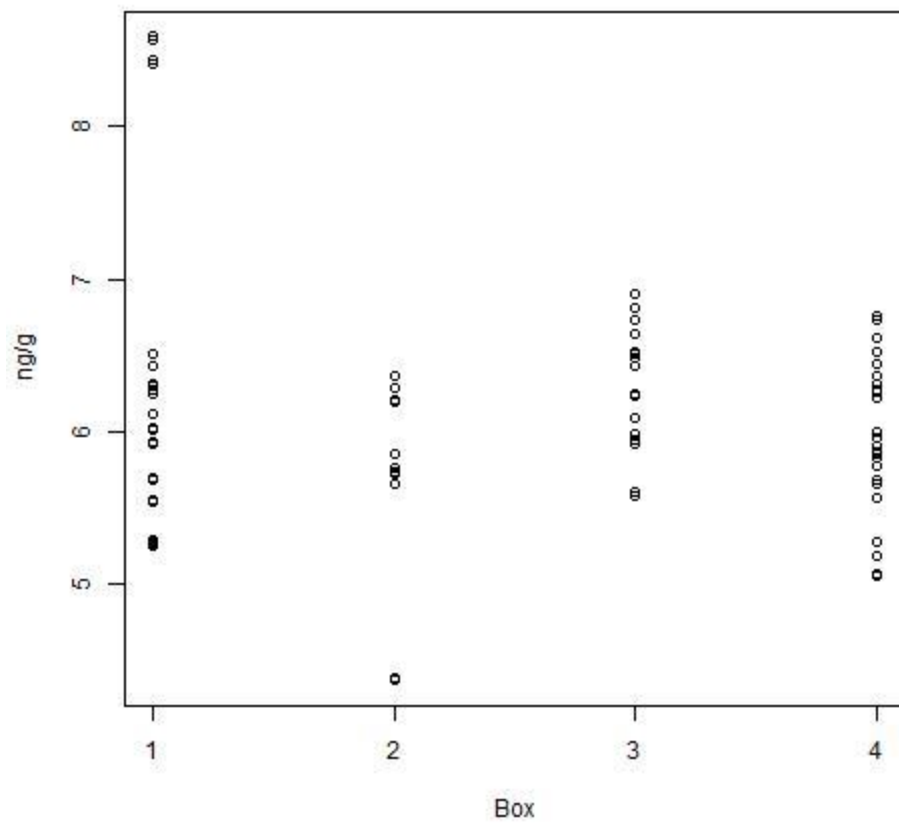
PGA-Level.3 : NIST LC-MS/MS (1)



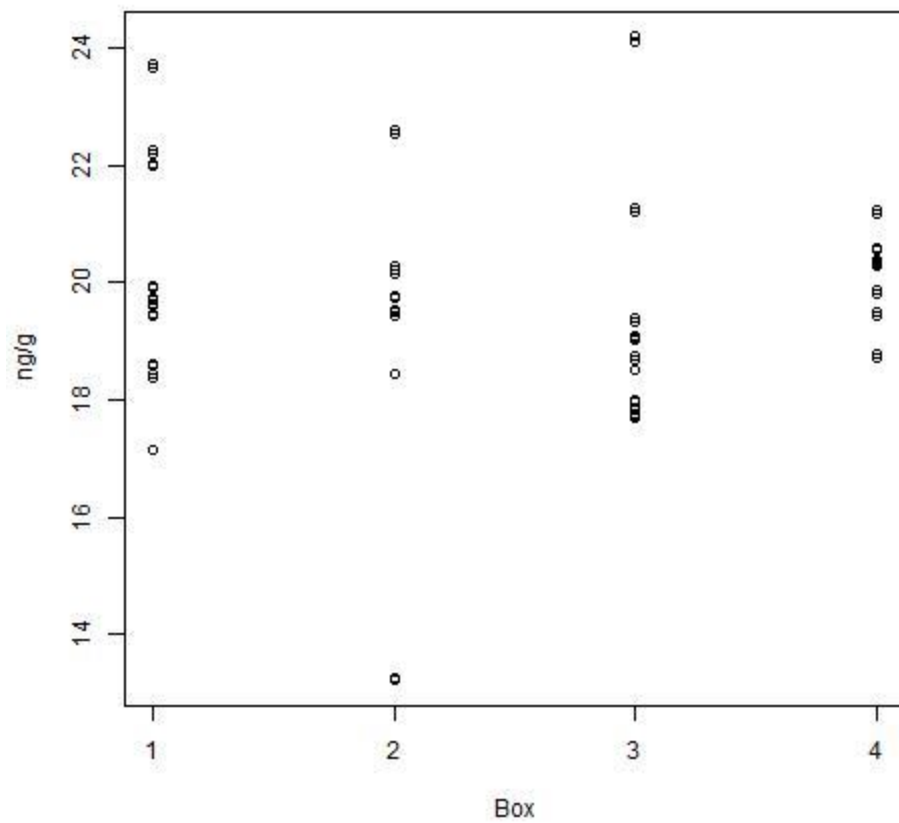
PGA-Level.3 : NIST LC-MS/MS (2)



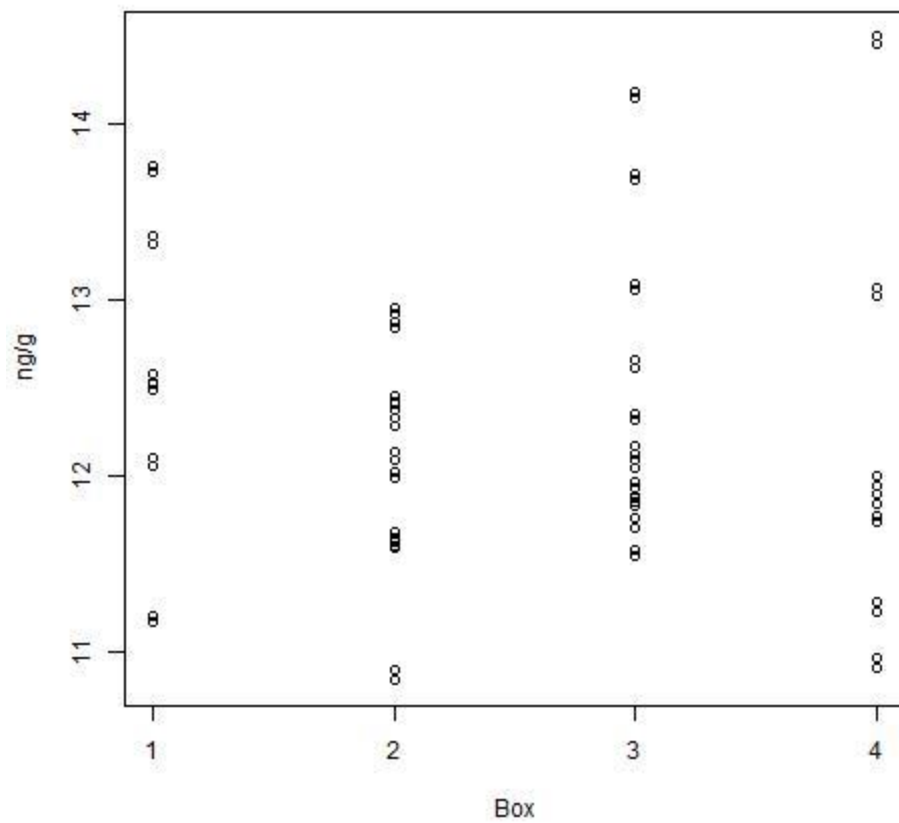
5mTHF Level 1 : NIST LC-MS/MS



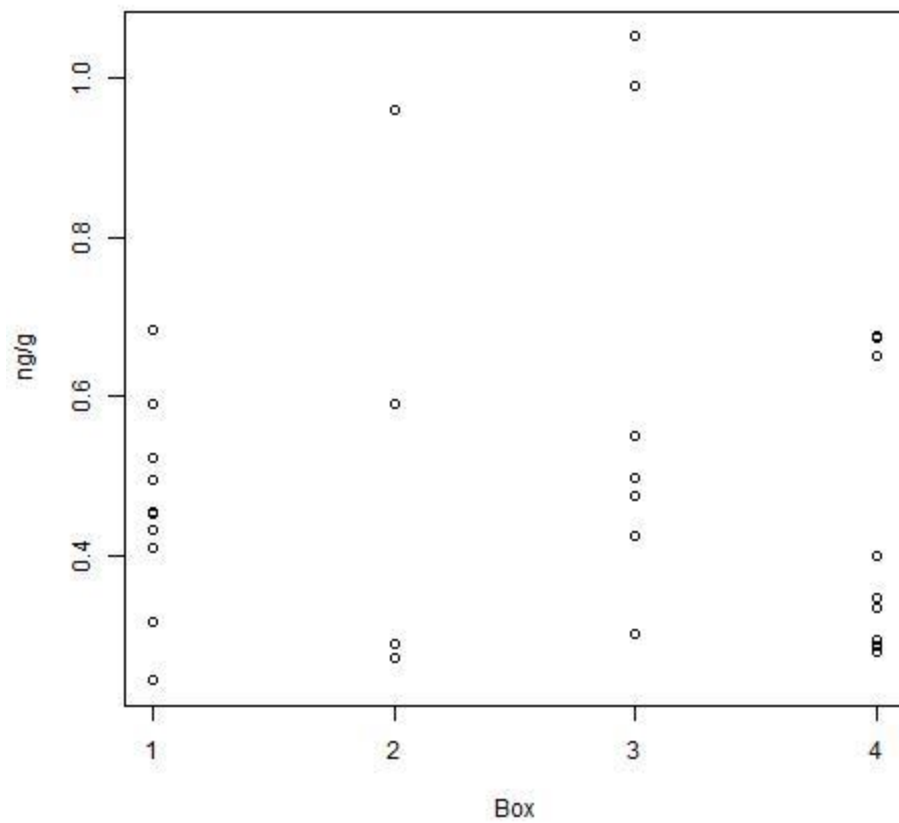
5mTHF Level 2 : NIST LC-MS/MS



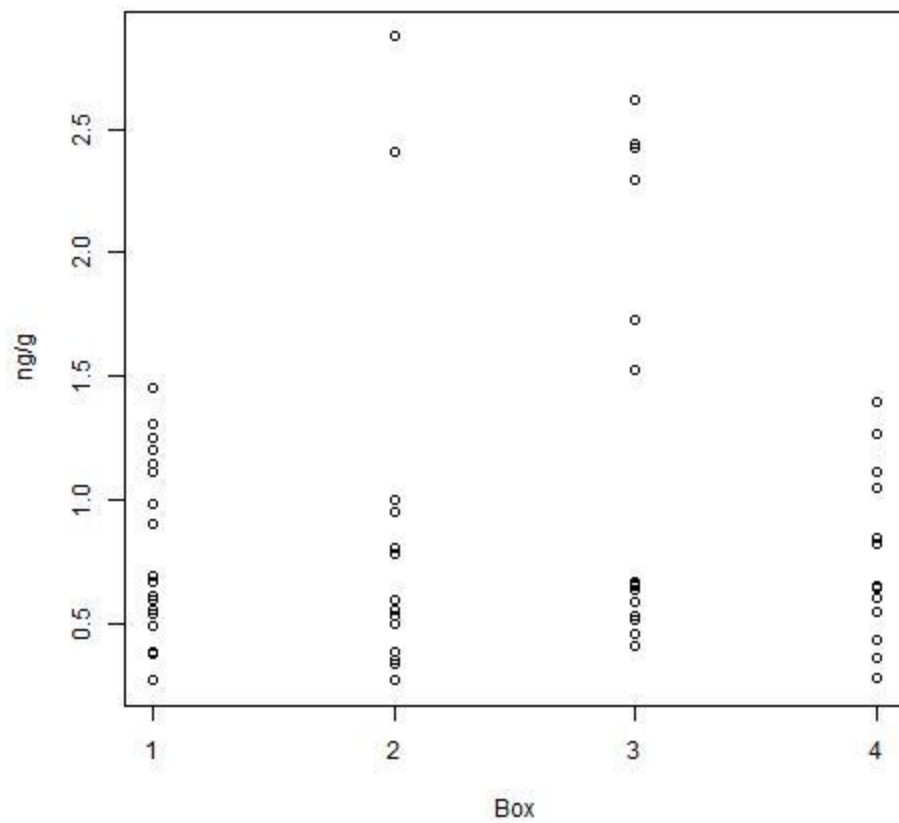
5mTHF Level 3 : NIST LC-MS/MS



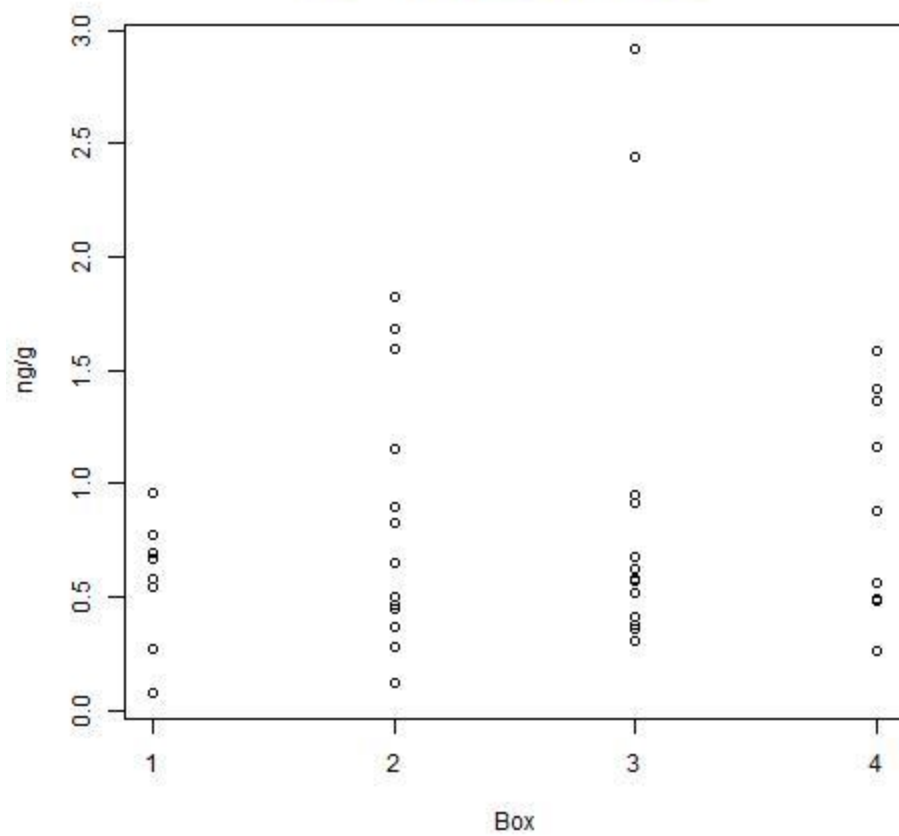
THF Level 1 : NIST LC-MS/MS



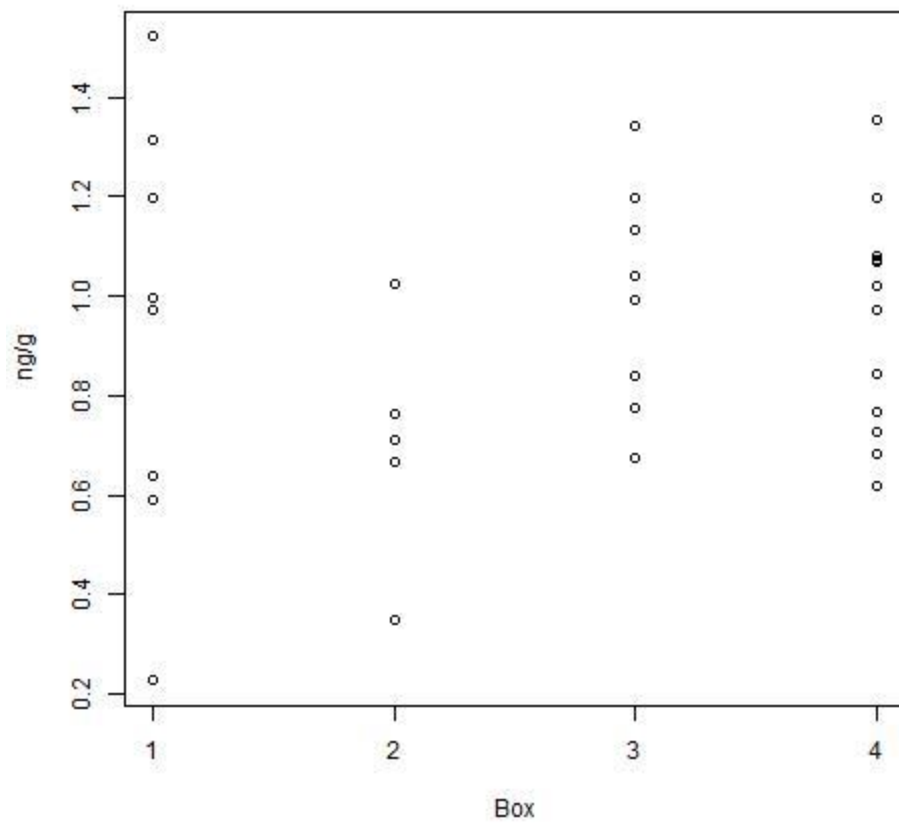
THF Level 2 : NIST LC-MS/MS



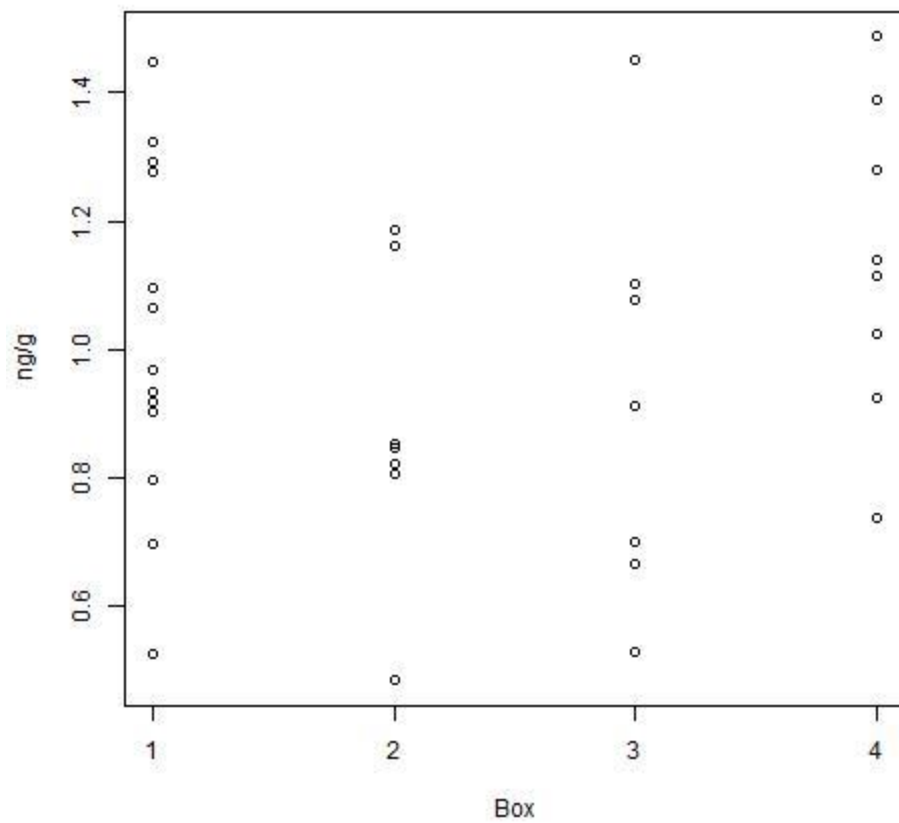
THF Level 3 : NIST LC-MS/MS



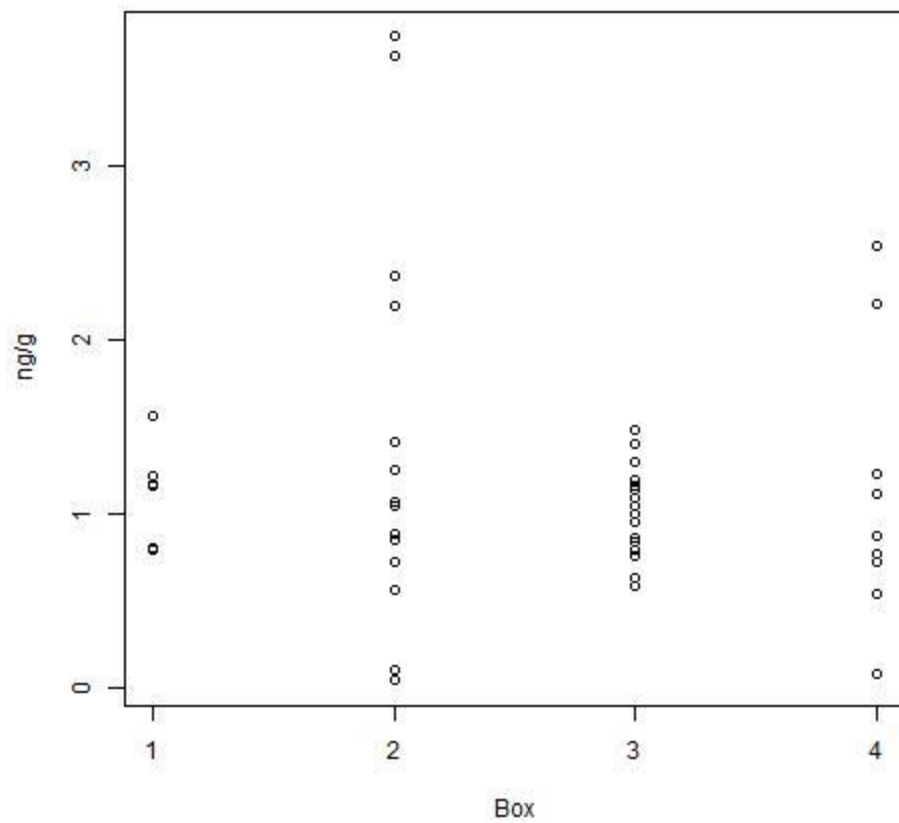
MeFox Level 1 : NIST LC-MS/MS



MeFox Level 2 : NIST LC-MS/MS



MeFox Level 3 : NIST LC-MS/MS



5fTHF Level 3 : NIST LC-MS/MS

