UGA145 Part I: Development and Validation of a Non-antibody Method for Rapid Detection of Serum Aflatoxin B_1-lysine Adduct

The first part of the UGA 145 project is to develop and validate a non-antibody based method for measurement of serum AFB_1-Lysine adduct. Solid-phase extraction based purification and high pressure liquid chromatography with fluorescence detection were developed. Procedures of enzyme digestion, recovery, accuracy and precision rates were optimized. Standards and samples were confirmed through liquid-chromatography/mass-spectrometry (LC/MS) method. This method was further validated in animal study and is ready to use for human studies.

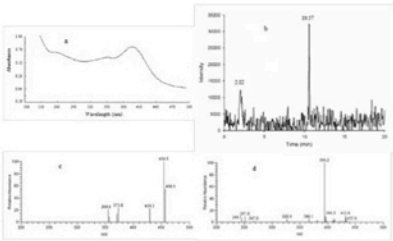

Figure 1. Quantification and confirmation of AFB1-Lys adduct

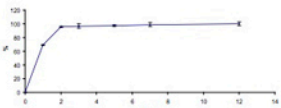

Figure 2. Kinetics of enzymatic digestion of serum (n=4), AFB-Lys content at 12h represent 100%.

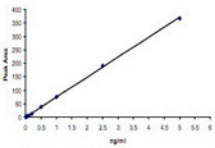

Figure 3. Calibration curve of AFB-Lys in nine concentrations from 1 to 5000pg/ml

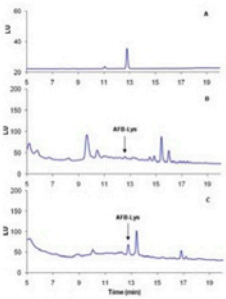

Figure 4. Typical chromatograms of serum AFB-Lys adduct : A, 1ng/ml standard; B, low level of AFB-Lys in human serum; C, high level of AFB-Lys in human serum

Table 1. Binding efficacy among different cartridges (ng/ml, n=3)

Concentration	MAX	HLB	Sep-Pak
Low	0.21±0.01	0.09±0.01*	0.20±0.00
High	1.28±0.03	0.70±0.01*	1.27±0.11

*: $P < 0.05$, compared with other cartridges at same concentration.

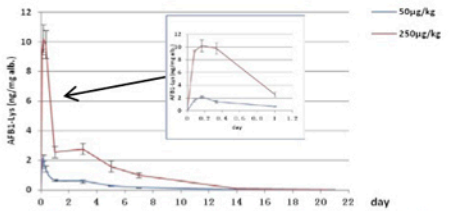

Figure 5. Kinetics of AFB-Lys adduct after single dose of AFB1

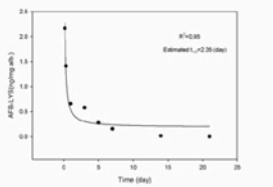

Figure 6. Curve fitting of AFB-Lys concentration after a single dose of 50μg/kg AFB1 treatment

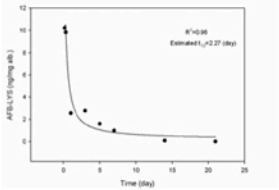

Figure 7. Curve fitting of AFB-Lys concentration after a single 250μg/kg AFB1 treatment

Table 2. Inaccuracy and imprecision rates of diluted rat serum (n= 5)

	AFB1-lys (ng/ml)	Inaccuracy rate (%)	Imprecision rate (%)
Day 1	low (0.16)	3.75	5.55
	high (0.8)	7.77	2.76
Day 2	AFB1-lys (ng/ml)	Inaccuracy rate (%)	Imprecision rate (%)
	low (0.16)	4.48	8.60
	high (0.8)	2.80	2.39
Day 3	AFB1-lys (ng/ml)	Inaccuracy rate (%)	Imprecision rate (%)
	low (0.16)	6.68	3.65
	high (0.8)	2.75	3.47

Table 3. Recovery rates of AFB-Lys adduct (n=3)

AFB_1-Lys (ppb)	Peak area (LU)		Recovery rate (%)
	Processed	In buffer	
0.05	4.53±0.38	4.8±0.2	94.44
0.2	14.77±0.55	20.27±0.12	72.87
1	73.2±1.35	101.67±0.51	72.00

Table 4. R.T stability of AFB-Lys adduct in serum (n=3)

Concentration (ng/ml)	Time (hour)		
	0	12	24
Low	0.21±0.00	0.20±0.01	0.19±0.01
High	1.24±0.01	1.21±0.02	1.11±0.02*

*: $P<0.05$ compared with 0 hour at same concentration

Contributors

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