THE DETECTION OF RICIN IN FOOD MATRICES USING ELISA TECHNOLOGY

Eric A.E. Garber and Michael A. McLaughlin

Division of Natural Products, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD 20740

ABSTRACT

Ricin is a potent ribosome inactivating protein (RIP-2) present in beans of the castor plant, Ricinus communis. Due to recent events, the detection of ricin has become a serious concern to the FDA. As part of an effort to develop protocols for the detection of ricin in food products, a commercially available ELISA assay was evaluated and compared with commercially available lateral flow devices (LFDs) in regards to specificity and sensitivity. Limits of detection (LOD) were determined for ricin added to solid and liquid food matrices. In all cases the ELISA was significantly more sensitive than the LFDs. Both the ELISA and the LFDs were capable of detecting ricin at levels below and equivalent to concentrations known to present a health risk. A two tier procedure is proposed for the screening of food samples in which the LFDs are employed as an initial screen followed by ELISA analysis of those samples that elicit either a positive or borderline response.

MATERIALS & METHODS

Ricin, A Chain, B Chain, and Agglutinin (RCA-120) were obtained from Vector Labs. Abrin C was obtained from Sigma.

Spiking All food samples, except chocolate, were prepared by spiking with the agent in PBS and allowing absorption into the food. Milk chocolate containing ricin was prepared by mixing 1.25, 3.75, 12.5, 50, or 100 ug ricin into 3g of melted chocolate. The chocolate was stored at 4ºC until used.

Extraction

Liquid food samples were diluted 5-fold with 200 mM sodium phosphate, pH 6.8 (NaPi).

Solid food samples (1g) were washed with 25 ml NaPi.

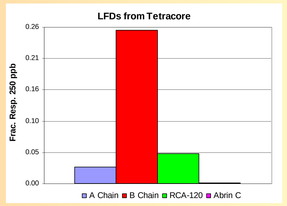
Chocolate samples (0.3g) were melted at 65 ºC (1min), mixed with 3 ml NaPi (30 sec.), and diluted 10-fold with NaPi,

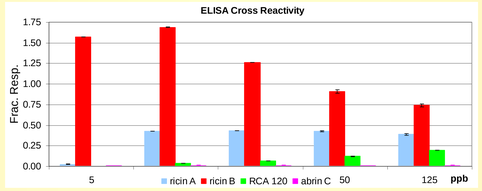
Cosmetic samples were mixed with 2 ml of NaPi (assume volume at 3 ml) and diluted 10-fold with NaPi.

Assays LFDs were obtained from a governmental supplier and Tetracore, Inc. (Gaithersburg, MD). The ELISA assay was obtained from Tetracore. All assays were used according to manufacturers recommendations. All samples were diluted 1:1 with PBS prior to analysis. The LFDs were read at 30 min, the ELISAs at 26 min.

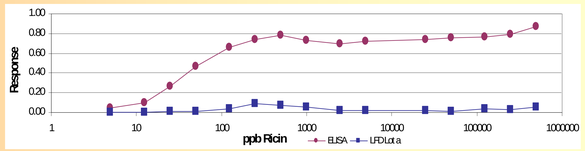
Data Analysis Fractional Response was calculated as the:

$\Delta\text{Response of food}_{\text{spiked vs unspiked}} / \Delta\text{Response of buffer}_{\text{spiked vs unspiked}}$





SENSITIVITY, SPECIFICITY, & CROSS-REACTIVITY



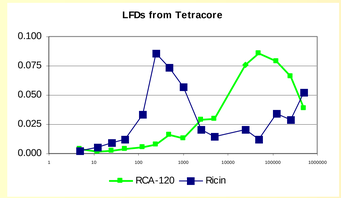
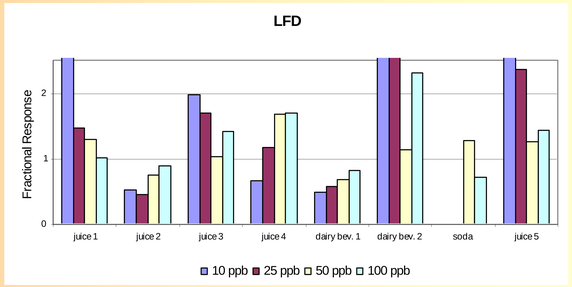


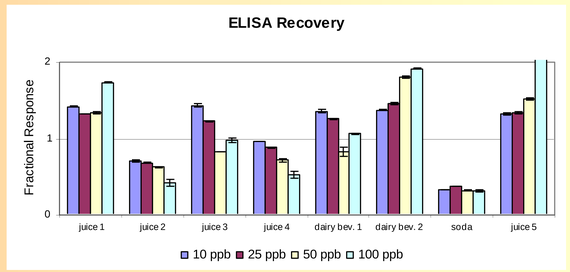
Table I: LFDs from Governmental Supplier

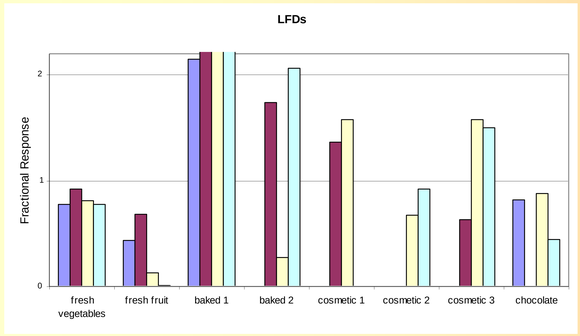
ppm	A Chain	B Chain	RCA-120
0	-	-	-
0.005	-	-	-
0.0125	-	-	-
0.025	-	-	-
0.05	+	-	-
0.125	+	-	-
0.25	+	-	+
0.5	+	+	+
1	+	+	+
2.5	+	+	+
5	+	+	+
25	+	+	+
50	+	+	+
125	+	+	+
250	+	+	+
500	+	+	+

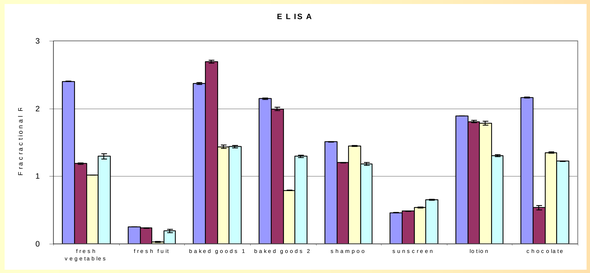
ANALYSIS OF FOODS AND COSMETICS

concentrations listed are after extraction









CONCLUSIONS

Both types of LFDs and the ELISA effectively detected ricin in food and cosmetic samples at levels orders of magnitude less than that known to be a health and safety concern.

The Hook Effect observed with both types of LFDs, was not observed with the ELISA. Inclusion of a serial dilution step in the LFD protocol obviated the problem.

The immunodiagnostic devices from the governmental supplier and Tetracore, Inc. displayed different (complementary) subunit specificity.

None of the immunodiagnostic devices cross reacted with another plant derived RIP-2.

Variability in the response observed with fresh fruit was dependent on whether spiking was on the skin or the flesh (data not shown).

Fractional Response values greater than 1 were observed with bakery and cosmetic products capable of undergoing hydration (loss of free solvent) which would increase the ricin concentration in the sample.

A protocol was established which entailed preliminary / field measurements using LFDs. Those samples that displayed ambiguous or positive responses were also analyzed using ELISA technology.

ACKNOWLEDGEMENTS

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FDA

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