Supplementary materials

Sensitive immunochromatographic assay for detection of dimethachlone flungicide in tomatoes and lettuces

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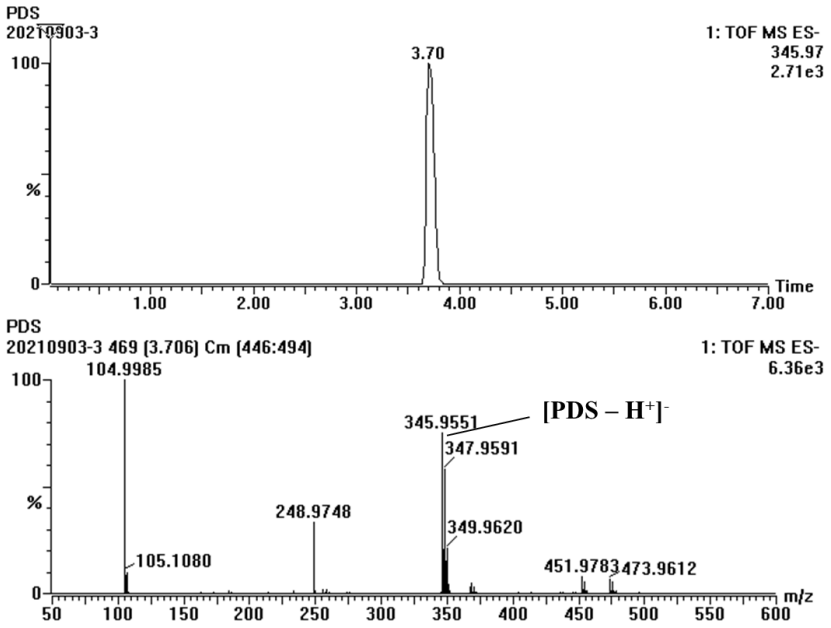

Figure S1. The HPLC-MS spectra of hapten PDS.

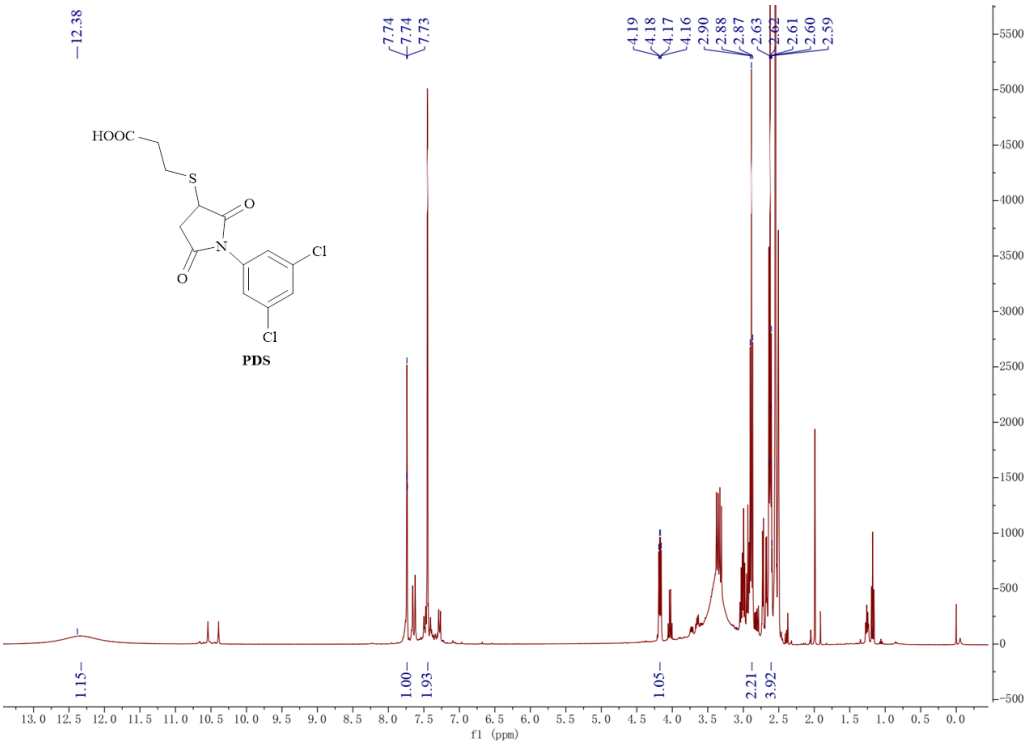

Figure S2. The ^{1}H NMR spectra of hapten PDS.

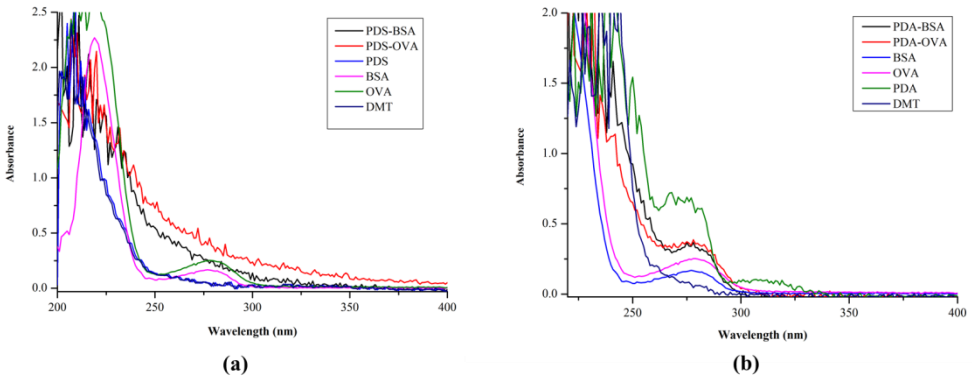

Figure S3. The UV-Vis spectrogram of DMT antigens. (a) The UV-Vis spectrogram of PDS-BSA and PDS-OVA; (b) The UV-Vis spectrogram of PDA-BSA and PDA-OVA.

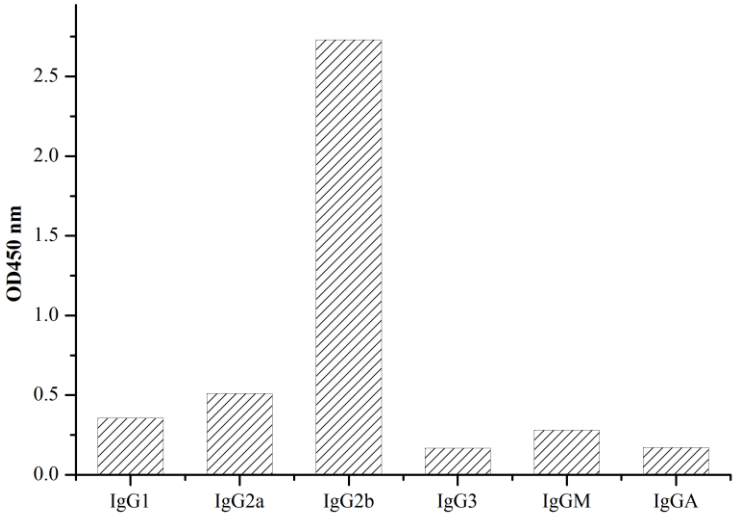

Figure S4. The subtype of anti-DMT mAb detected by ELISA.

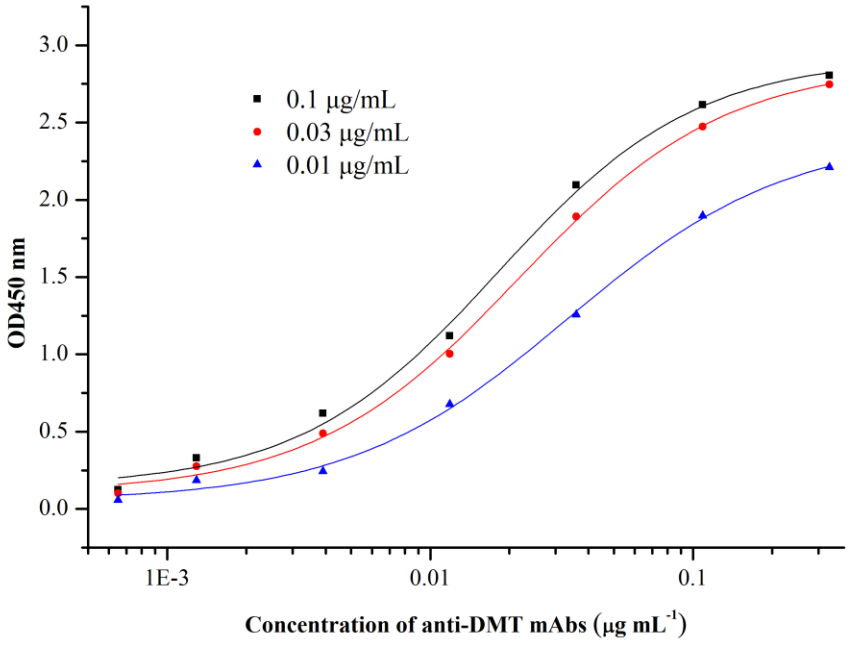

Figure S5. Affinity curve for anti-DMT mAb.

The average affinity constant was calculated to be 9.56×10^9 L mol^{-1}.

Sample preparation for the analysis of DMT in tomatoes by LC-MS/MS

A total of six samples comprising grape, apple, okra, tomato, cucumber, and potato were purchased from different supermarkets, located in Pune (India). In order to ensure homogeneity, samples were comminuted in two steps: firstly, the sample was milled thoroughly in a blender and secondly, a portion of this milled sample was subjected to high-speed homogenization at 15,200 g for 2 min using a Robot-coupe® processor (Robot Coupe USA Inc., Ridgeland, MS, USA). In all cases, the effect of sample comminution on the compound stability was assessed at ambient temperature (~ 25 °C), and also by cryogenic milling with liquid nitrogen (~ −196 °C) for an optimum duration, to obtain a fine homogenized texture. The samples were then analyzed immediately and the extent of target compound degradation during comminution was estimated at various stages of analysis. During analysis, the consequence of acidification was also estimated for up to 10 h.

Briefly, the samples with higher moisture content (for instance, grape, apple, tomato, and cucumber) were cryogenically milled [10 (±0.1) g] and extracted directly with 10 mL ethyl acetate (+1% formic acid). For the matrices having relatively lower moisture contents (specifically okra and potato), 5 (±0.1) g of cryogenically milled sample was mixed with 5 mL water (+1% formic acid) before extraction. After the addition of 10 g of anhydrous sodium sulfate, the samples were vortexed for 1 min and then centrifuged at 1200 g for 5 min at room temperature. For the tomatoes, after a dispersive SPE cleanup with PSA (50 mg mL^{-1}), and Na_2SO_4 (150 mg mL^{-1}), their supernatant (1 mL) was evaporated to dryness under a gentle stream of nitrogen gas. This cleanup step was not necessary for the other matrices. The dried extracts were reconstituted in 0.5 mL acetonitrile, vortexed for 30 s, and sonicated to redissolve the analytes. The final extract was diluted with an equal volume of water (+0.1% formic acid), followed by centrifugation at 5040 g for 5 min. The supernatant was then passed through a 0.2 μm PTFE filter (Chromatopak Analytical Instruments Pvt. Ltd., Mumbai, India) and thereafter, 10 μL was injected into the LC-MS/MS instrument. The grape, apple, tomato, and cucumber generated 1 g matrix mL^{-1} extract. For okra, and potato, extracts, 0.5 g of matrix mL^{-1} was obtained.

Then, cryogenically milled samples (the same amount as mentioned above for the ethyl acetate method) were extracted with acetonitrile (10 mL), and vortexed for 1 min. After the addition of salts (4 g $MgSO_4$, and 1 g NaCl), the samples were again vortexed for 1 min, and centrifuged at 3450 g for 5 min. In the case of tomatoes, a combination of PSA (50 mg mL^{-1}), and $MgSO_4$ (150 mg mL^{-1}) was used for cleanup. The cleaned extract (0.5 mL) was diluted with acidified water (0.5 mL) and the extracts (10 μL) were measured using LC-MS/MS.

Then, cryogenically milled samples (the same amount as mentioned above for the ethyl acetate method) were extracted with acetonitrile (10 mL), vortexed for 1 min. After addition of salts (4 g MgSO4, and 1 g NaCl), the samples were again vortexed for 1 min, and centrifuged at 3450×g for 5 min. In the case of tomato, a combination of PSA (50 mg/mL), and MgSO4 (150 mg/mL) was used for cleanup. The cleaned extract (0.5 mL) was diluted with acidified water (0.5 mL). Extracts (10 μL) were measured using LC-MS/MS.

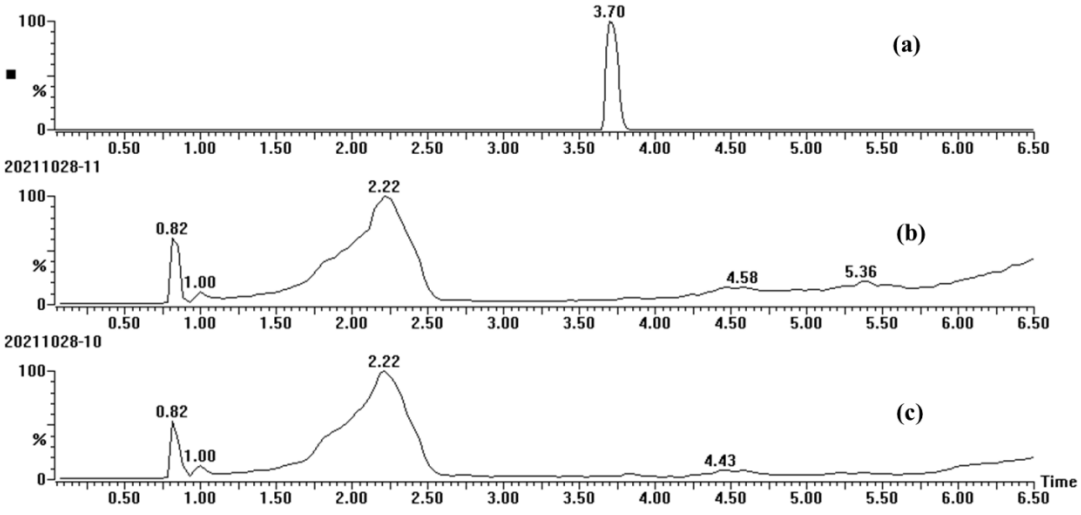

Figure S6. LC-MS/MS chromatograms of DMT in acetonitrile, tomato and lettuce samples. (a) the total ion current chromatograms of 1 μg mL^{-1} of DMT in acetonitrile; (b) the total ion current chromatograms of blank tomato sample; (c) the total ion current chromatograms of blank lettuce sample.

Table S1. The antisera of immunized mice detected by *ic*ELISA.

Coating antigen	IC_{50} (ng mL^{-1})	
	Immunized by PDS-BSA	Immunized by PDA-BSA
PDS-OVA	72.3	121.7
PDA-OVA	57.6	166.9

Table S2. Titer of purified anti- DMT mAb detected by ELISA.

	Dilution of purified anti-DMT mAb									
OD_{450}	5.0×10^3	1.0×10^4	2.0×10^4	4.0×10^4	8.0×10^4	1.6×10^5	3.2×10^5	6.4×10^5	1.28×10^6	Blank
	2.92	2.73	2.42	2.12	1.87	1.63	0.96	0.63	0.46	0.032

As shown in Table S1, the titer of purified anti-DMT mAb was up to 1.28×10^6.

Table S3. The cross-reactivity of anti-DMT mAb with analogues determined by *ic*ELISA.

Competitive analogues	IC_{50} (ng mL^{-1})	CR (%)
DMT	2.84	100
3, 5-DCA	--	< 1.0
IPD	195.9	1.45
PM	--	< 1.0
Vinclozolin	94.7	3.0

-- stands for inhibition up to 300 ng mL^{-1}.