Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Gas Chromatography-Tandem Mass Spectrometry based Detection of Half Nitrogen Mustards in Plasma as a New Biomarker of Nitrogen Mustards exposure

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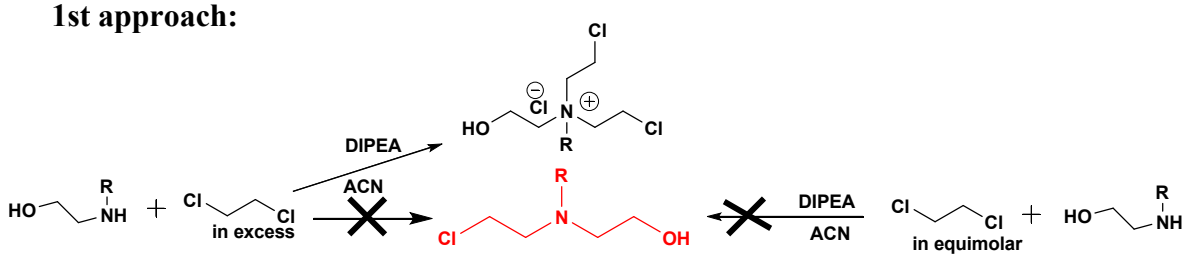
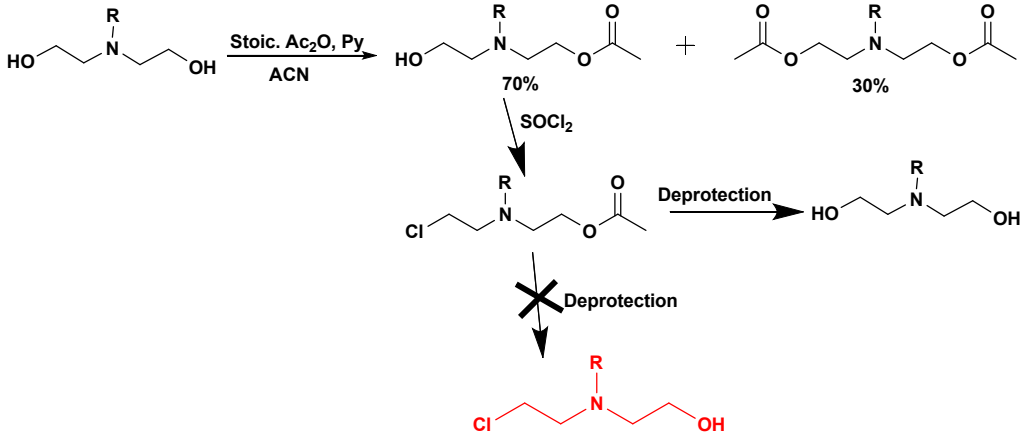
1st approach:



2nd approach:



Scheme S1: Different approaches for the synthesis of halfNMs.

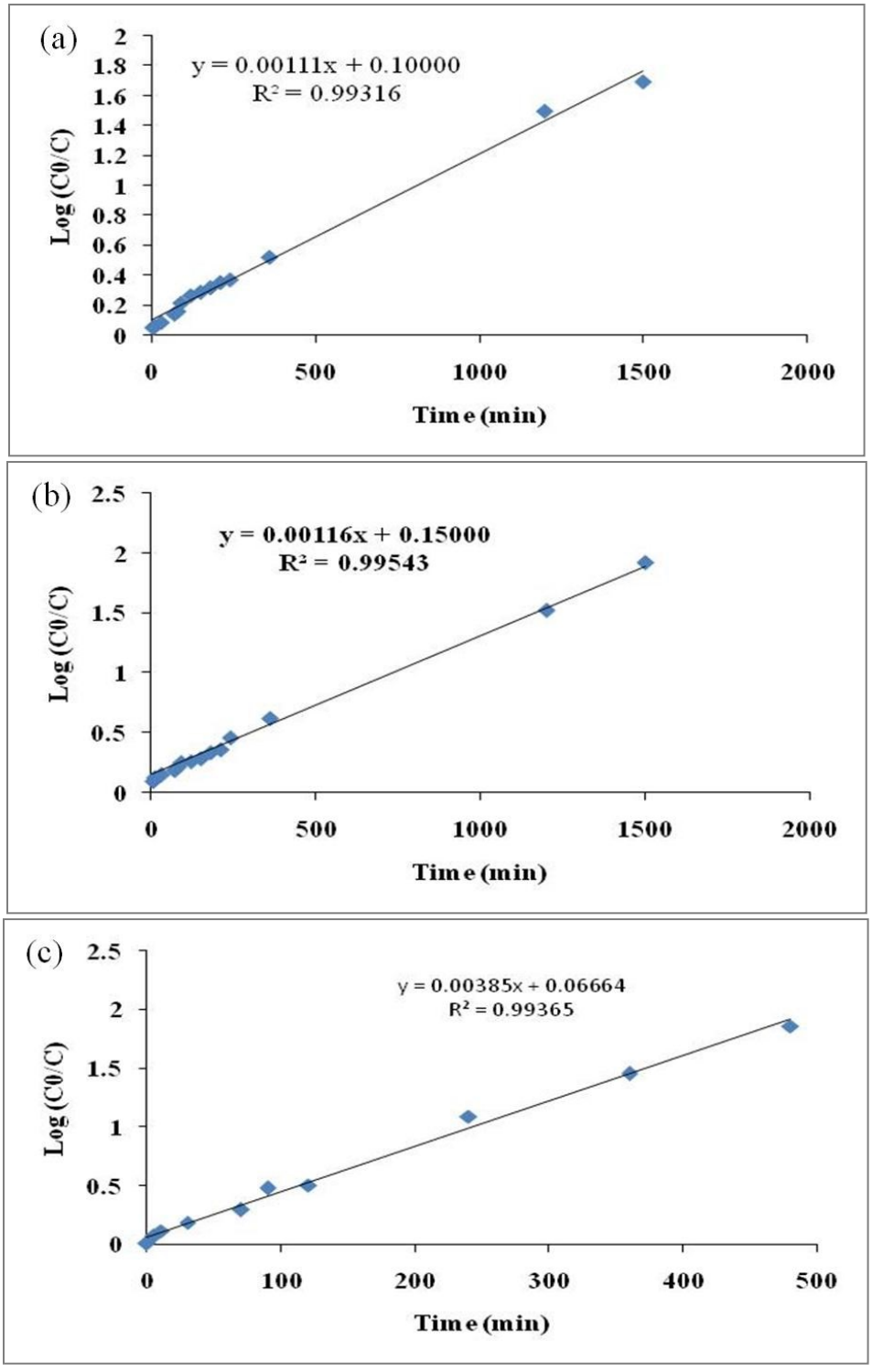


Figure S1: Log (C_0/C) vs time (min) co-ordinates for the hydrolysis of (a) HN1 salt (b) HN2 salts and (c) HN3 salts during hydrolysis of each salt in 2:1 (v/v), acetone-water solution. Here C_0 is the concentration of NM at time = 0 min. and C is the concentration of NM at time = t min.

Table S1: Observed half-life time of salts of NM in 2:1 (v/v), acetone-water solution.

SL No.	Salts of NM	Slope (m)	K = m x 2.303	Half-life time ($t_{1/2}$) = 0.693/K
1	HN1	0.00111	0.00255633	271.09 min
2	HN2	0.00116	0.00267148	259.41 min
3	HN3	0.00385	0.00886655	78.16 min

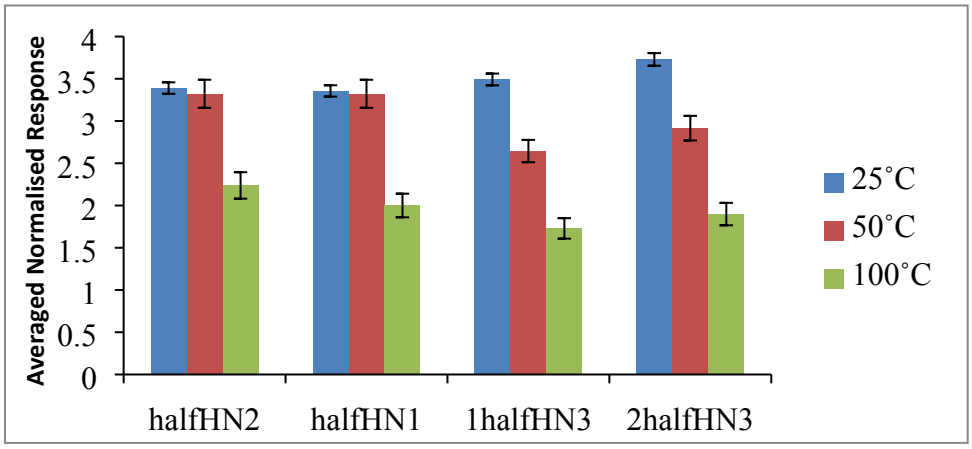


Figure S2: Screening of the temperature for the heptafluorobutyrylation of half NMs.

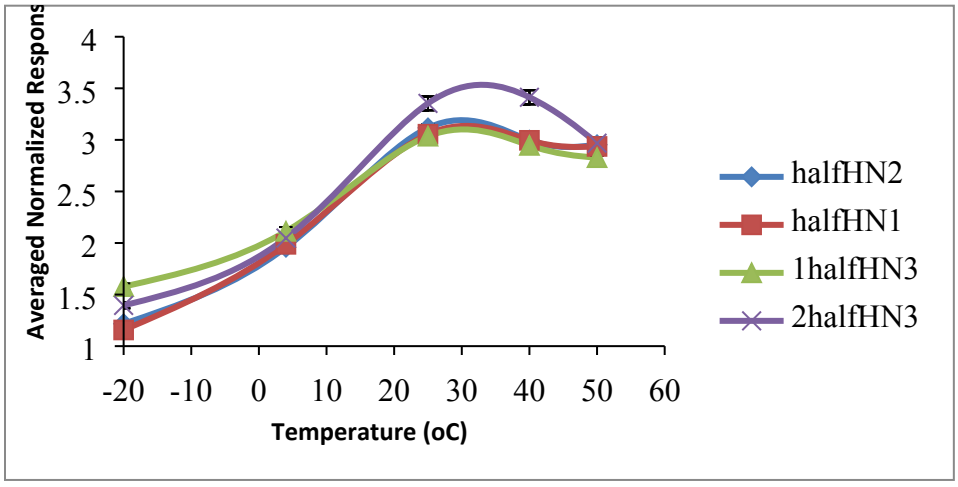


Figure S3: Fine optimization of the temperature for the heptafluorobutyrylation of halfNMs.

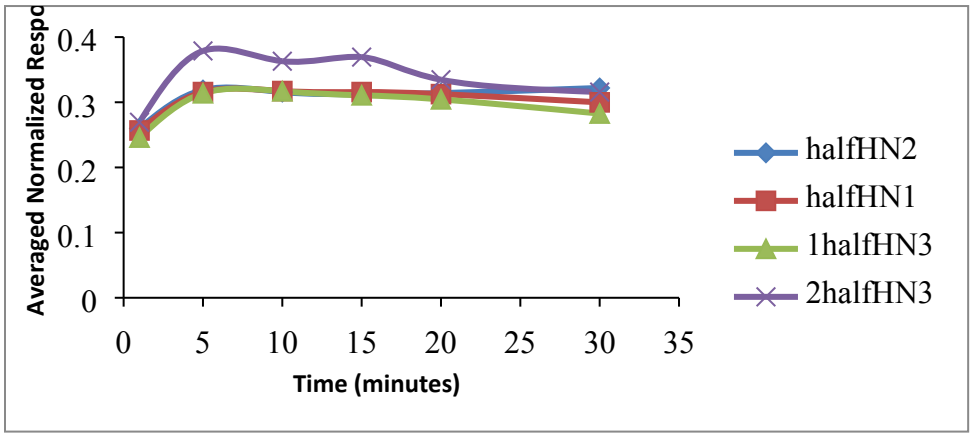


Figure S4: Study of the effect of time on the heptafluorobutyrylation of halfNMs.

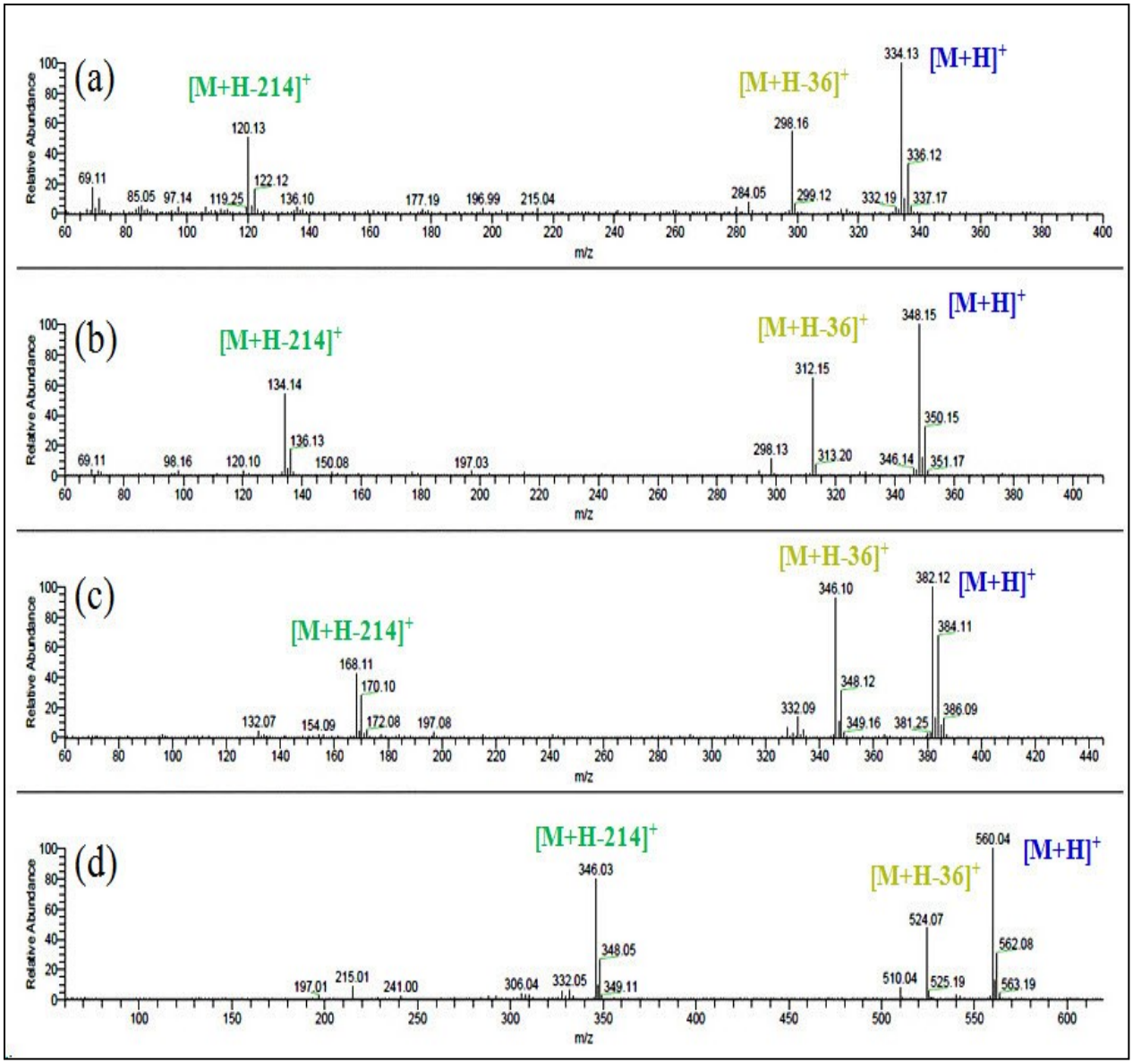


Figure S5: PCI-MS spectra of a) halfHN2HFB, b) halfHN1HFB, c) 1halfHN3HFB and d) 2halfHN3HFB respectively

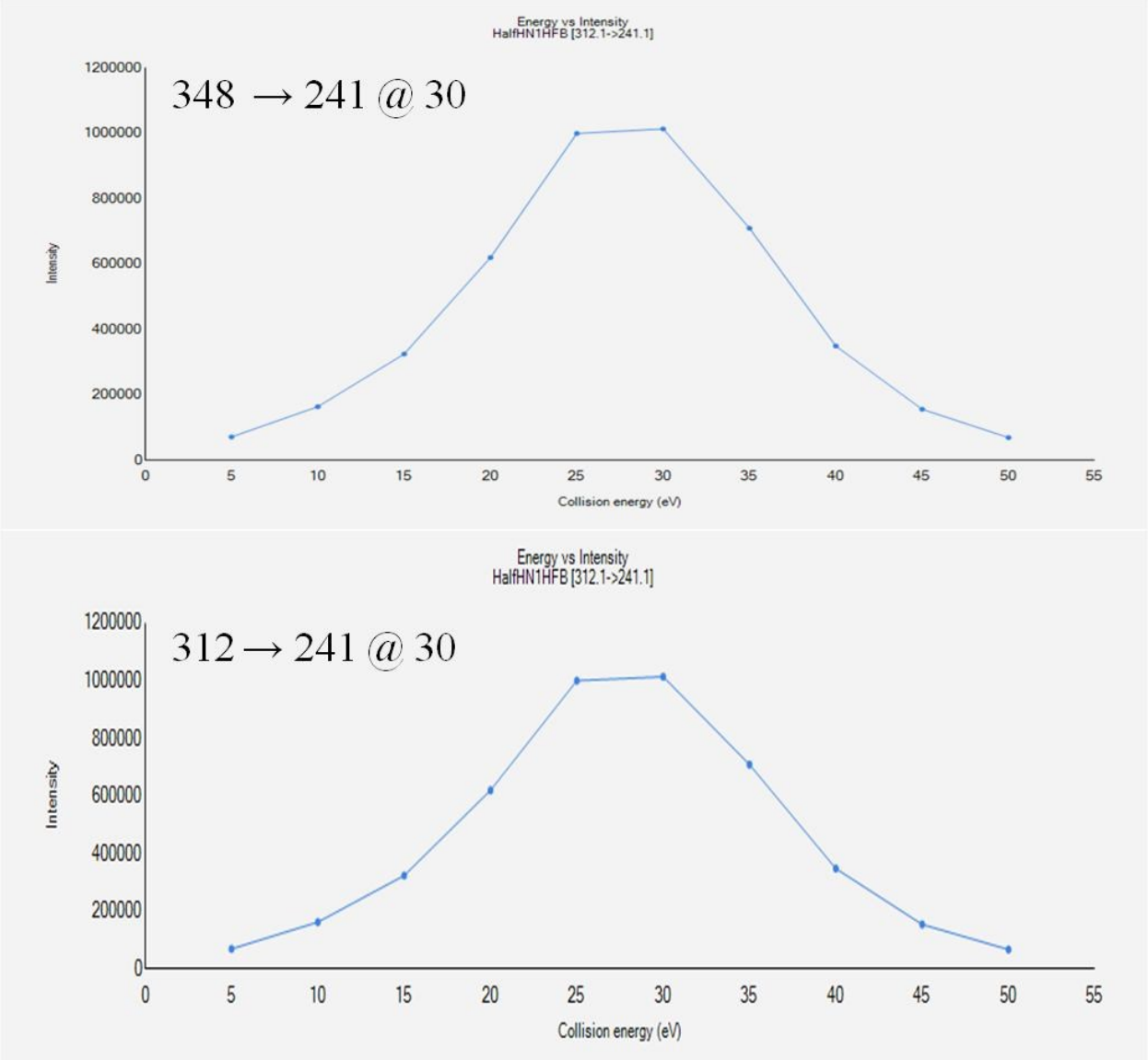


Figure S6a: Optimization of collision energy for the SRM transitions halfHN1HFB.

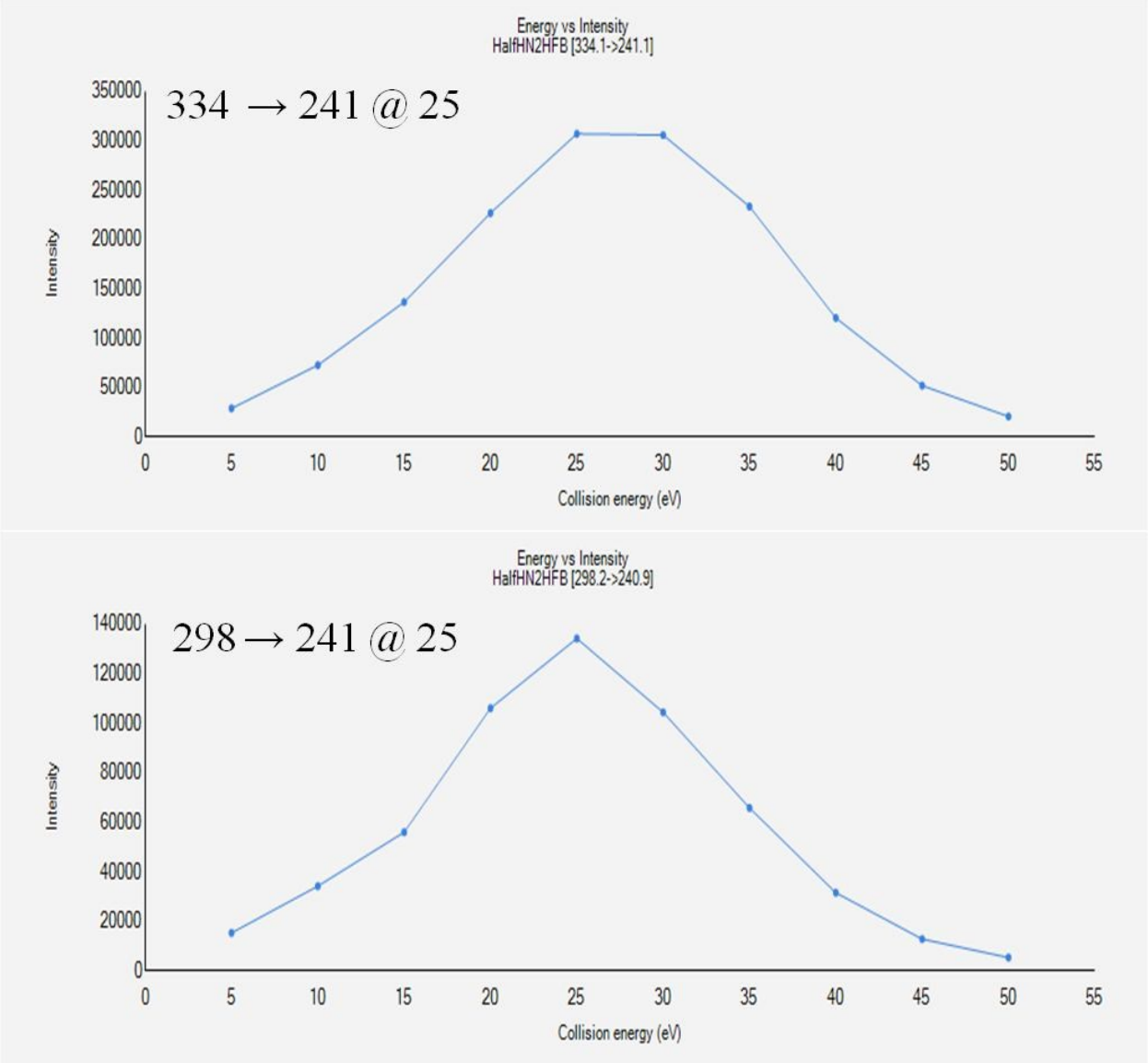


Figure S6b: Optimization of collision energy for the SRM transitions halfHN2HFB.

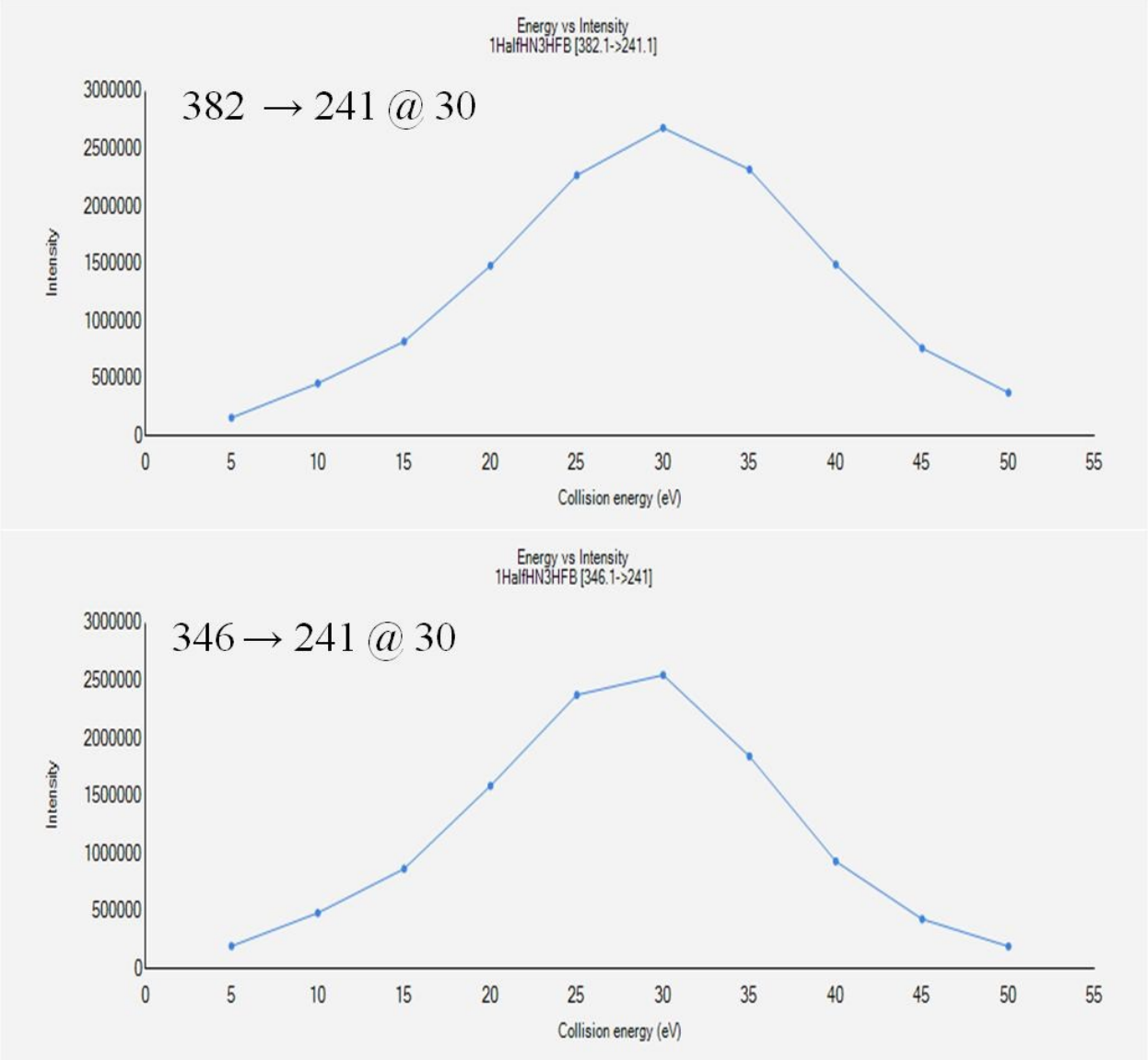


Figure S6c: Optimization of collision energy for the SRM transitions 1halfHN3HFB.

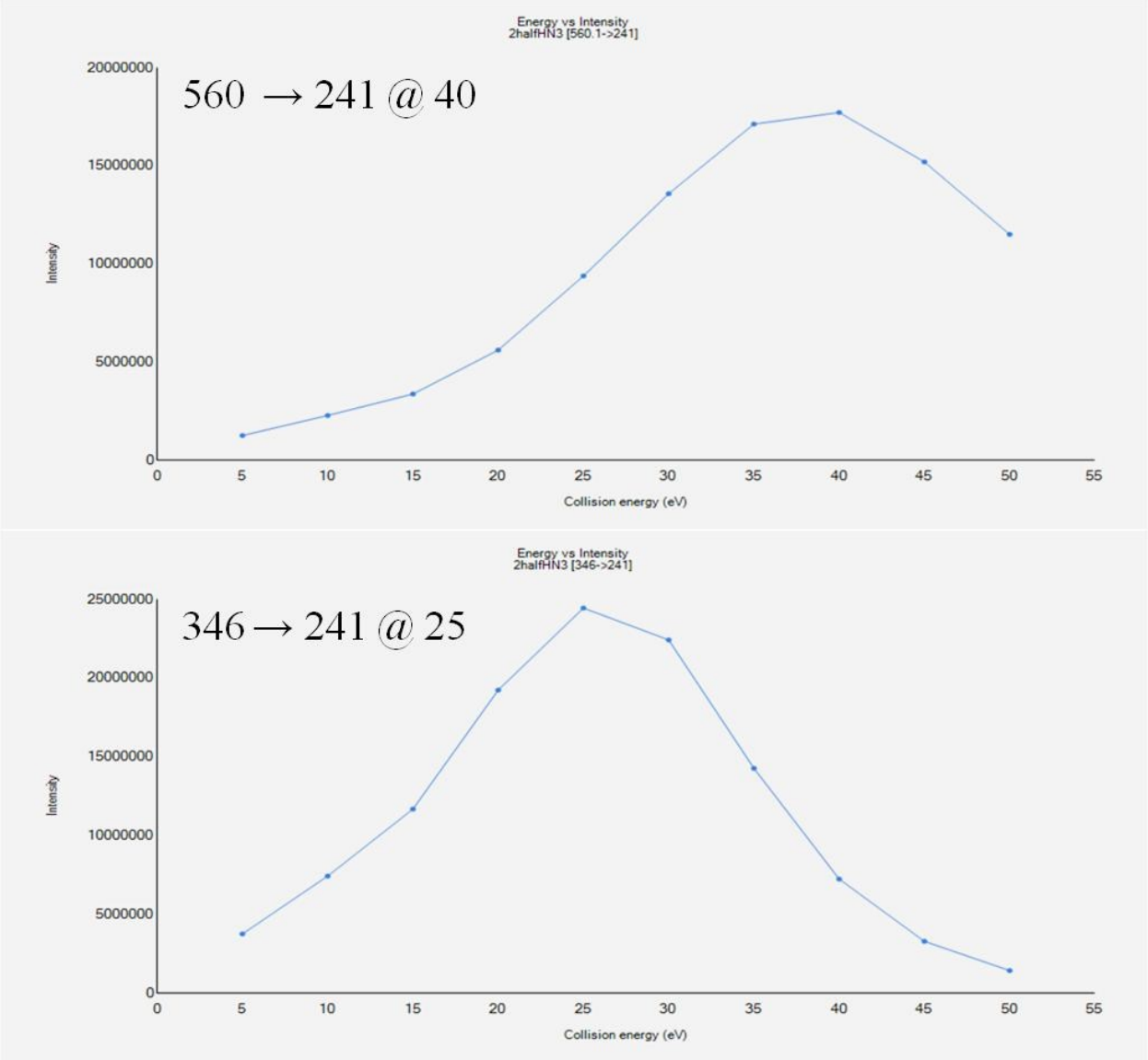


Figure S6d: Optimization of collision energy for the SRM transitions 2halfHN3HFB.

Table S2: Parameters for Multiple Reaction Monitoring (MRM)

Analytes	Nature of ions	Ionic transition	Fragmentation energy (eV)
halfHN1HFB	Q	348 >241	30
	q	312 > 241	30
halfHN2HFB	Q	334 > 241	25
	q	298 > 241	25
1halfHN3HFB	Q	382 > 241	30
	q	346 > 241	30
2halfHN3HFB	Q	560 > 241	40
	q	346 > 241	25
HCB (IS)	Q	284.9 > 249.8	20
	q	284.9 > 248	20

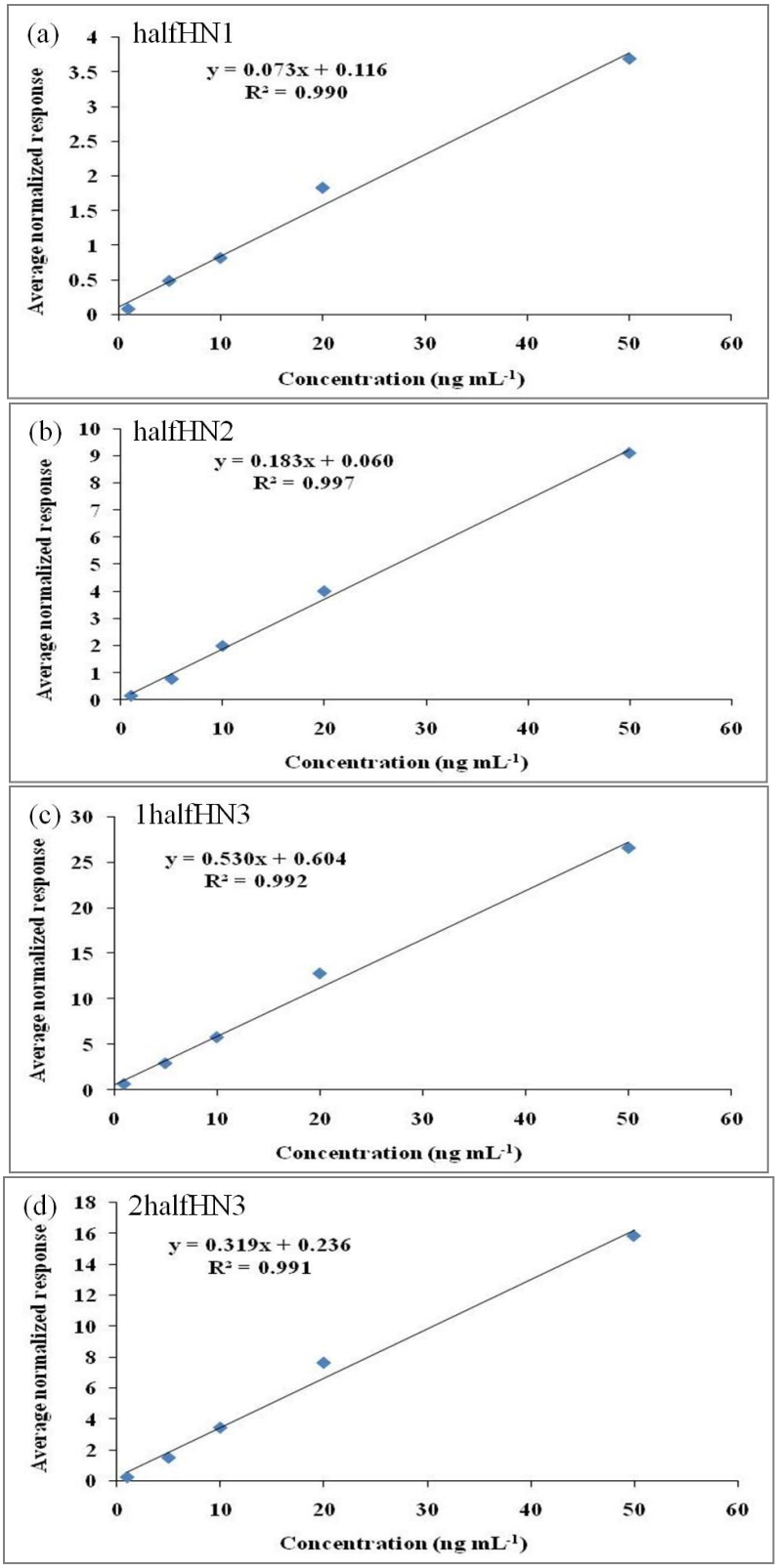


Figure S7: Calibration curve for (a) halfHN1, (b) halfHN2, (c) 1halfHN1 and (d) 2halfHN3 taking plasma as a matrix.

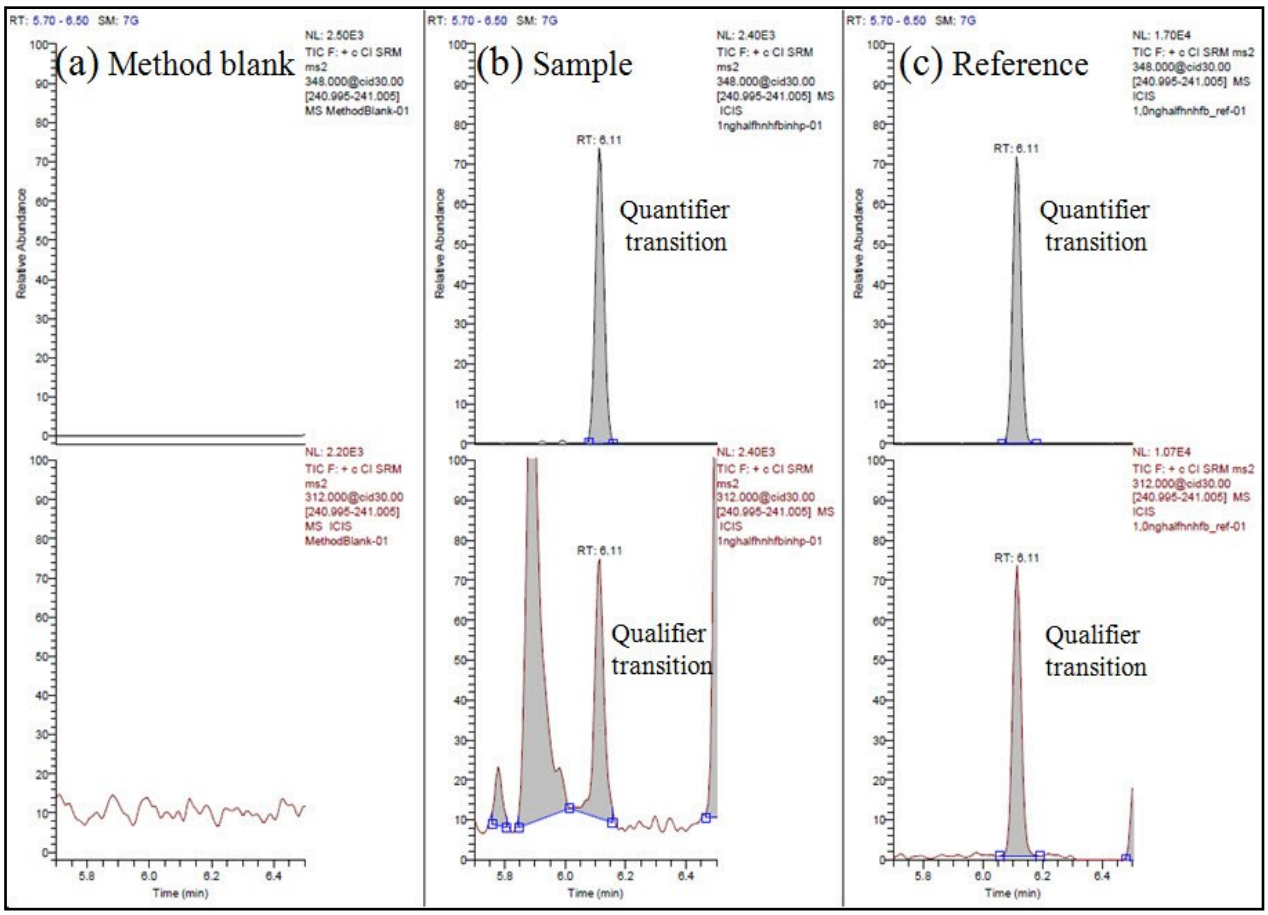


Figure S8: SRM chromatograms, with transition 348 → 241 (quantifier ion) and 312 → 241 (qualifier ion), obtained during *in-vitro* analysis of halfHN1 in human plasma; (a) blank sample, (b) HN1 spiked sample, and (c) Standard reference sample.

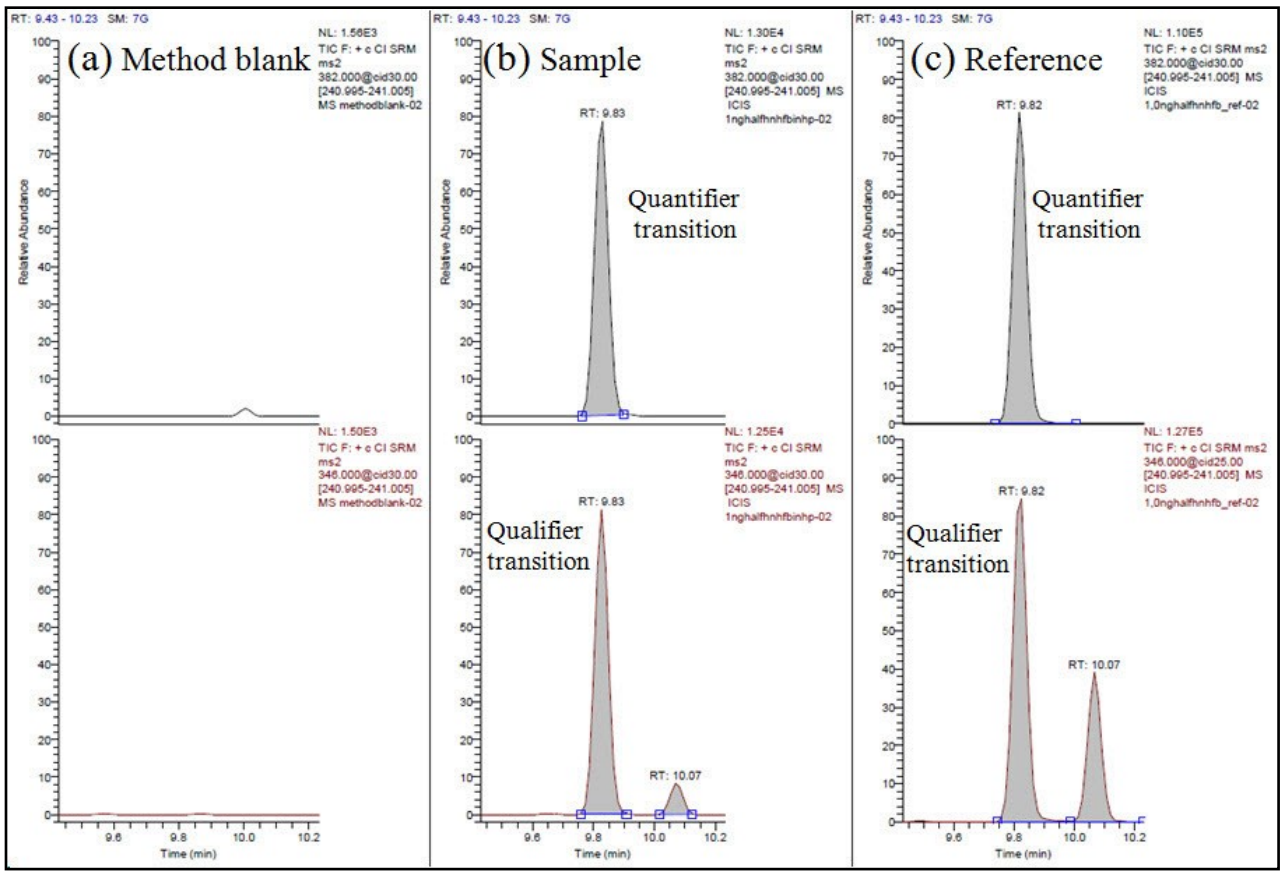


Figure S9: SRM chromatograms, with transition 382 → 241 (quantifier ion) and 346 → 241 (qualifier ion), obtained during the *in-vitro* analysis of 1halfHN3 in human plasma; (a) blank sample, (b) HN1 spiked sample, and (c) Standard reference sample.

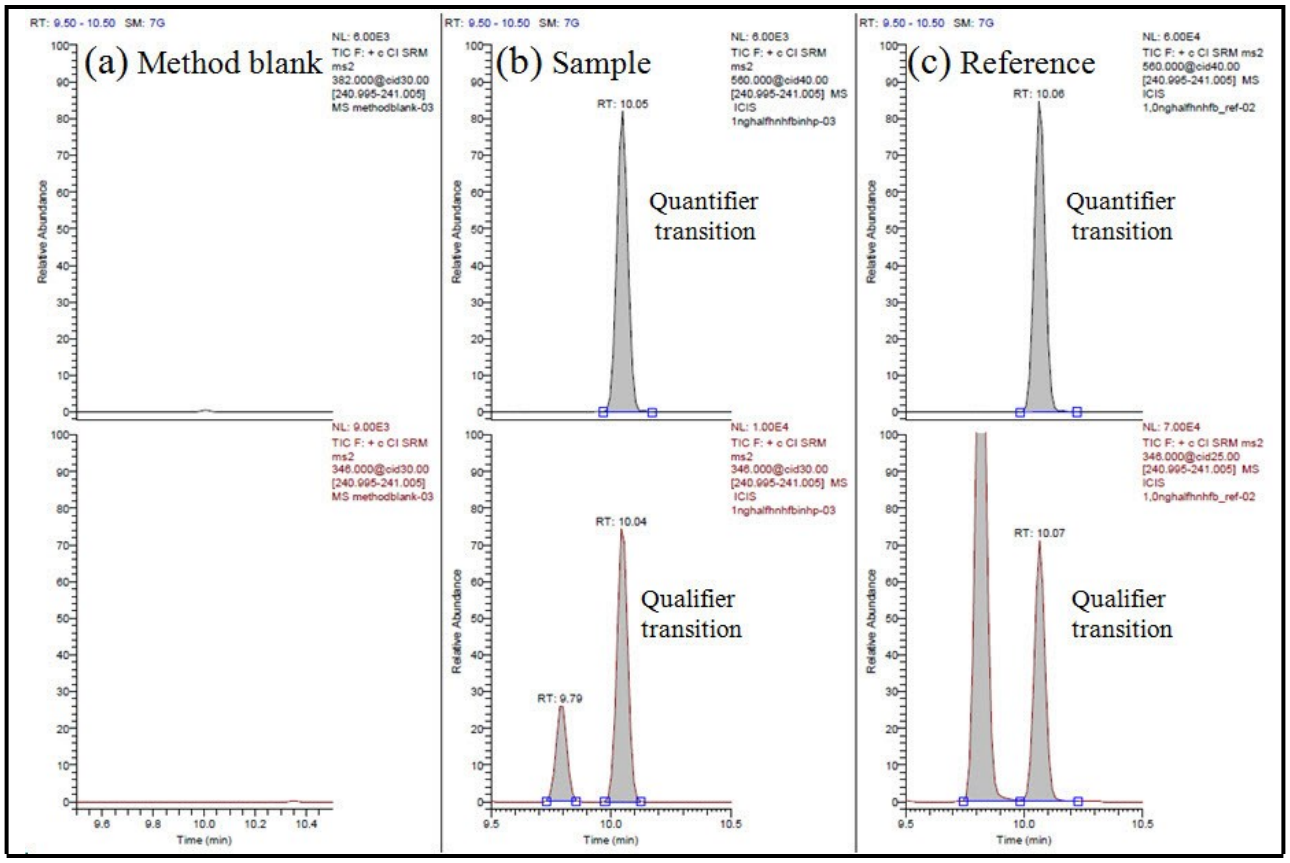


Figure S10: SRM chromatograms, with transition 560 → 241 (quantifier ion) and 346 → 241 (qualifier ion), obtained during the *in-vitro* analysis of 2halfHN3 in human plasma; (a) blank sample, (b) HN1 spiked sample, and (c) Standard reference sample.

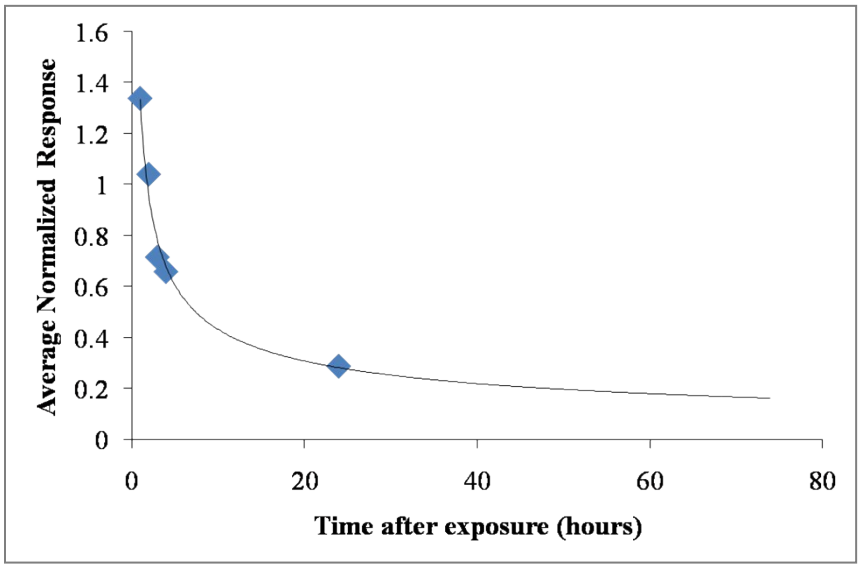


Figure S11: Response of halfHN2 with respect to time after exposure of Wister rat with 1X LD_{50} of HN2 (in *vivo* analysis).