

Supporting Information for:

High-Confidence Qualitative Identification of Organophosphorus Nerve Agent Adducts to Human Butyrylcholinesterase

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General	Setting
Time	0-60 minutes
Mode	Positive
In Source CID	0 eV
Default Charge State	1
Exclusion	-
Inclusion	-
Tags	-
Full MS	Settings
Microscans	1
Resolution	70,000
AGC Target	3e6
Maximum IT	30 ms
Number of Scan Ranges	1
Scan Range	700 to 1100 m/z
Spectrum Data Type	Profile
ddMS2/ddSIM	Settings
Microscans	1
Resolution	17,500
AGC Target	2e4
Maximum IT	150 ms
Loop Count	3
MSX Count	1
TopN	3
Isolation Window	2.0 m/z
Fixed First Mass	-
NCE	22.0
Stepped NCE	10%
Spectrum Type	Profile
dd Settings	Settings
Underfill Ratio	1.0%
Intensity Threshold	1.3e3
Apex Trigger	-
Charge Exclusion	-
Peptide Match	Preferred
Exclude Isotopes	On
Dynamic Exclusion	2.0 s

Table S1: Method Parameters for data-dependent Acquisition. The following settings were optimized for reproducible, data dependent fragmentation of OPNA-BChE adducted nonapeptides.

Acquisition	Parameter
Assay Type	Name
Injection Volume	10 µL
Mass Precision	4.00
Target Screening Processing	Parameter
Peaks S/N Ration Threshold	5
Peaks Mass Tolerance	5 ppm
Retention Time	Identify
Fragment Ions	Identify
Minimum Number of Fragments	1
Intensity Threshold	2000
Mass Tolerance	30 ppm
MS Order	MS2
Isotopic Pattern	Identify
Fit Threshold	90%
Allowed Mass Deviation	5 ppm
Allowed Intensity Deviation	10%
Peak Detection	Parameter
Detection Algorithm	ICIS
Detection Method	Highest Peak
Smoothing	1
Area Noise Factor	5
Peak Noise Factor	10
Baseline Window	40
Constrain Peak Width	No
Noise Method	Incos
Minimum Peak Width	3
Multiplet Resolution	10
Area Tail Extension	5
Area Scan Window	0
RMS	No

Table S2: Parameters for Post-Acquisition Processing. The following parameters were used for the processing of QC materials, convenience set samples, and unknown sera.

Compound Detail	Parameter
Experiment	XIC
Category	N/A
CAS	N/A
Formula: BChE	C ₃₃ H ₄₉ N ₉ O ₁₄
Formula: MeP-BChE	C ₃₄ H ₅₂ N ₉ O ₁₆ P
Formula: GB-BChE	C ₃₇ H ₅₈ N ₉ O ₁₆ P
Ionization	ESI
Response Threshold	0
Neutral Mass	[Based on Formula]
Compound Type	Target Compound
Internal Standard	N/A
Target Peaks	Parameter
Extracted Mass	[Based on Formula and Ionization]
MS Order	MS1
Polarity	Positive
Adduct	Hydrogen
Charge State	1
Window (sec)	120
RT (min): BChE	12.5
RT (min): MeP-BChE	14.5
RT (min): GB-BChE	23.5
Lens	0.0
Energy Ramp	0
Fragment Masses*	Compounds Using Fragment Masses
602.2569	BChE, MeP-BChE & GB-BChE
673.2940	MeP-BChE & GB-BChE
778.3366	MeP-BChE & GB-BChE
620.2602	BChE
691.3051	BChE

Table S3: Compound Database Settings. The following parameters were used for the QC Compound Database. This database was also used for the post-acquisition processing of convenience set samples. For unknown samples, a new database was made taking into account the high resolution unknown parent ion masses identified but keeping the masses and mass tolerances of fragments constant.

HESI parameter	Setting
Sheath Gas Flow Rate	50
Aux Gas Flow Rate	5
Sweep Gas Flow Rate	1
Spray Voltage	4.00 kV
Capillary Temperature	350C
S-Lens Rf Level	80
Heater Temperature	100

Table S4: Optimized Source Parameters for Data-Dependent Acquisition. These parameters were optimized using direct infusion of purified synthetic BChE nonapeptides of unadducted BChE, GB-BChE, and MeP-BChE. Analytes were introduced into the source of the instrument at the flow rate, solvent composition, and concentrations likely to be observed in processed sera or plasma.

Parameter	Final Method	Fragment Accurate Mass ($\pm 1\sigma$) (ID percentage)	Lower Level	Fragment Accurate Mass ($\pm 1\sigma$) (ID percentage)	Higher Level	Fragment Accurate Mass ($\pm 1\sigma$) (ID percentage)
Starting Solvent Composition	2% B	602.2551 (± 2.7 ppm) 620.2652 (± 4.3 ppm) 691.3018 (± 2.7 ppm) 100% Identified	0% B	N/A 0% Identified	4% B	602.2544 (± 4 ppm) 620.2677 (± 3.8 ppm) 691.3031 (± 1.1 ppm) 100 % Identified
Collision Energy (NCE); NCE Stem	22; 10% Step	602.2551 (± 2.7 ppm) 620.2652 (± 4.3 ppm) 691.3018 (± 2.7 ppm) 100% Identified	17.6; 8% Step	602.2552 (± 0.23 ppm) 620.2661 (± 1.1 ppm) 691.3022 (± 2.5 ppm) 89% Identified	26.4; 12% Step	602.2534 (± 7.2 ppm) 620.2678 (± 2.1 ppm) 56% Identified
LC Gradient Slope	0.53% B/min; 1.47% B/min	602.2551 (± 2.7 ppm) 620.2652 (± 4.3 ppm) 691.3018 (± 2.7 ppm) 100% Identified	0.43% B/min; 1.17% B/min	602.2565 (± 2.4 ppm) 620.2653 (± 3.1 ppm) 691.3042 (± 2.9 ppm) 100% Identified	0.63% B/min; 1.77% B/min	602.2567 (± 1.1 ppm) 620.2662 (± 2.8 ppm) 691.3030 (± 4.8 ppm) 100% Identified
LC Flow Rate	100 μ L/min	602.2551 (± 2.7 ppm) 620.2652 (± 4.3 ppm) 691.3018 (± 2.7 ppm) 100% Identified	80 μ L/min	602.2560 (± 1.7 ppm) 620.2664 (± 6.3 ppm) 691.3021 (± 2.3 ppm) 100% Identified	120 μ L/min	602.2562 (± 3.5 ppm) 691.3033 (± 4.1 ppm) 620.2669 (± 1.5 ppm) 100% Identified
MS Calibration**	<48 Hours	602.2551 (± 2.7 ppm) 620.2652 (± 4.3 ppm) 691.3018 (± 2.7 ppm) 100% Identified	>168 Hours	602.2518 (-5.5 ppm) 620.2645 (-1.1 ppm) 691.3002 (-2.3 ppm) 100% Identified	>288 Hours	602.2507 (-7.3 ppm) 620.2630 (-3.4 ppm) 691.3019 (0.14 ppm) 100% Identified

Table S5: Ruggedness Testing for BChE nonapeptide QC Material (QCL). Method parameters were altered for QCL analysis. Mass deviations (ppm) are provided for each analyte and parameter. In addition, an ID percentage is also included as a measure of efficiency.

Parameter	Final Method	Fragment Accurate Mass ($\pm 1\sigma$) (ID percentage)	Lower Level	Fragment Accurate Mass (ID percentage)	Higher Level	Fragment Accurate Mass ($\pm 1\sigma$) (ID percentage)
Starting Solvent Composition	2% B	602.2568 (± 2.6 ppm) 673.2929 (± 2 ppm) 778.3362 (± 2.2 ppm) 100% Identified	0% B	602.2560 (± 2.7 ppm) 673.2915 (± 0.3 ppm) 778.3345 (± 2.3 ppm) 100% Identified	4% B	602.2581 (± 3.3 ppm) 673.2934 (± 2.7 ppm) 778.3344 (± 3.4 ppm) 100% Identified
Collision Energy (NCE); NCE Stem	22; 10% Step	602.2568 (± 2.6 ppm) 673.2929 (± 2 ppm) 778.3362 (± 2.2 ppm) 100% Identified	17.6; 8% Step	602.2570 (± 2 ppm) 673.2928 (± 2.8 ppm) 778.3350 (± 0.8 ppm) 100% Identified	26.4; 12% Step	602.2563 (± 2.7 ppm) 673.2938 (± 1 ppm) 778.3344 (± 2.1 ppm) 100% Identified
LC Gradient Slope	0.53% B/min; 1.47% B/min	602.2568 (± 2.6 ppm) 673.2929 (± 2 ppm) 778.3362 (± 2.2 ppm) 100% Identified	0.43% B/min; 1.17% B/min	602.2561 (± 1 ppm) 673.2929 (± 1.6 ppm) 778.3369 (± 2.8 ppm) 100% Identified	0.63% B/min; 1.77% B/min	602.2558 (± 4.8 ppm) 673.2941 (± 0.6 ppm) 778.3361 (± 2.4 ppm) 100% Identified
LC Flow Rate	100 μ L/min	602.2568 (± 2.6 ppm) 673.2929 (± 2 ppm) 778.3362 (± 2.2 ppm) 100% Identified	80 μ L/min	602.2551 (± 4.4 ppm) 673.2950 (± 2.5 ppm) 778.3363 (± 3.5 ppm) 100% Identified	120 μ L/min	602.2561 (± 3.4 ppm) 673.2952 (± 3.3 ppm) 778.3340 (± 8.2 ppm) 100% Identified
MS Calibration**	<48 Hours	602.2568 (± 2.6 ppm) 673.2929 (± 2 ppm) 778.3362 (± 2.2 ppm) 100% Identified	>168 Hours	602.2539 (-4.8 ppm) 673.2928 (-0.1 ppm) 778.3409 (6.0 ppm) 100% Identified	>288 Hours	602.2548 (-3.3 ppm) 673.2919 (-1.5 ppm) 778.3308 (-6.9 ppm) 66% Identified

Table S6: Ruggedness Testing for MeP-BChE QC Material (QCM). Method parameters were altered for QCM analysis. Mass deviations (ppm) are provided for each analyte and parameter. In addition, an ID percentage is also included as a measure of efficiency.

Parameter	Final Method	Fragment Accurate Mass ($\pm 1\sigma$) (ID percentage)	Lower Level	Fragment Accurate Mass ($\pm 1\sigma$) (ID percentage)	Higher Level	Fragment Accurate Mass ($\pm 1\sigma$) (ID percentage)
Starting Solvent Composition	2% B	602.2564 (± 1.6 ppm) 673.2935 (± 2.1 ppm) 778.3360 (± 2 ppm) 100% Identified	0% B	602.2580 (± 1.8 ppm) 673.2944 (± 0.6 ppm) 778.3370 (± 0.5 ppm) 33% Identified	4% B	602.2574 (± 1.5 ppm) 673.2915 (± 1.6 ppm) 778.3363 (± 3.8 ppm) 66% Identified
Collision Energy (NCE); NCE Stem	22; 10% Step	602.2564 (± 1.6 ppm) 673.2935 (± 2.1 ppm) 778.3360 (± 2 ppm) 100% Identified	17.6; 8% Step	602.2568 (± 4.6 ppm) 673.2937 (± 1.3 ppm) 778.3355 (± 0.8 ppm) 100% Identified	26.4; 12% Step	602.2562 (± 1.3 ppm) 673.2952 (± 1.3 ppm) 778.3353 (± 5.9 ppm) 100% Identified
LC Gradient Slope	0.53% B/min; 1.47% B/min	602.2564 (± 1.6 ppm) 673.2935 (± 2.1 ppm) 778.3360 (± 2 ppm) 100% Identified	0.43% B/min; 1.17% B/min	N/A 0% Identified	0.63% B/min; 1.77% B/min	602.2551 (± 0.1 ppm) 673.2916 (± 3.0 ppm) 778.3369 (± 2.7 ppm) 66% Identified
LC Flow Rate	100 μ L/min	602.2564 (± 1.6 ppm) 673.2935 (± 2.1 ppm) 778.3360 (± 2 ppm) 100% Identified	80 μ L/min	602.2573 (± 3.8 ppm) 673.2936 (± 1.6 ppm) 778.3374 (± 2.3 ppm) 66% Identified	120 μ L/min	602.2562 (± 2.6 ppm) 673.2939 (± 2.3 ppm) 778.3360 (± 2.3 ppm) 100% Identified
MS Calibration**	<48 Hours	602.2564 (± 1.6 ppm) 673.2935 (± 2.1 ppm) 778.3360 (± 2 ppm) 100% Identified	>168 Hours	602.2538 (-4.3 ppm) 673.2927 (-1.2 ppm) 778.3344 (-2.1 ppm) 100% Identified	>288 Hours	602.2555 (-1.5 ppm) 673.2933 (-0.3 ppm) 778.3376 (2.1 ppm) 100% Identified

Table S7: Ruggedness Testing for GB-BChE QC Material (QCH). Method parameters were altered for QCH analysis. Mass deviations (ppm) are provided for each analyte and parameter. In addition, an ID percentage is also included as a measure of efficiency.