On-site detection and laboratory verification of the presence of nerve agent biomarkers using dried blood spots

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1. Biotransformation pathways

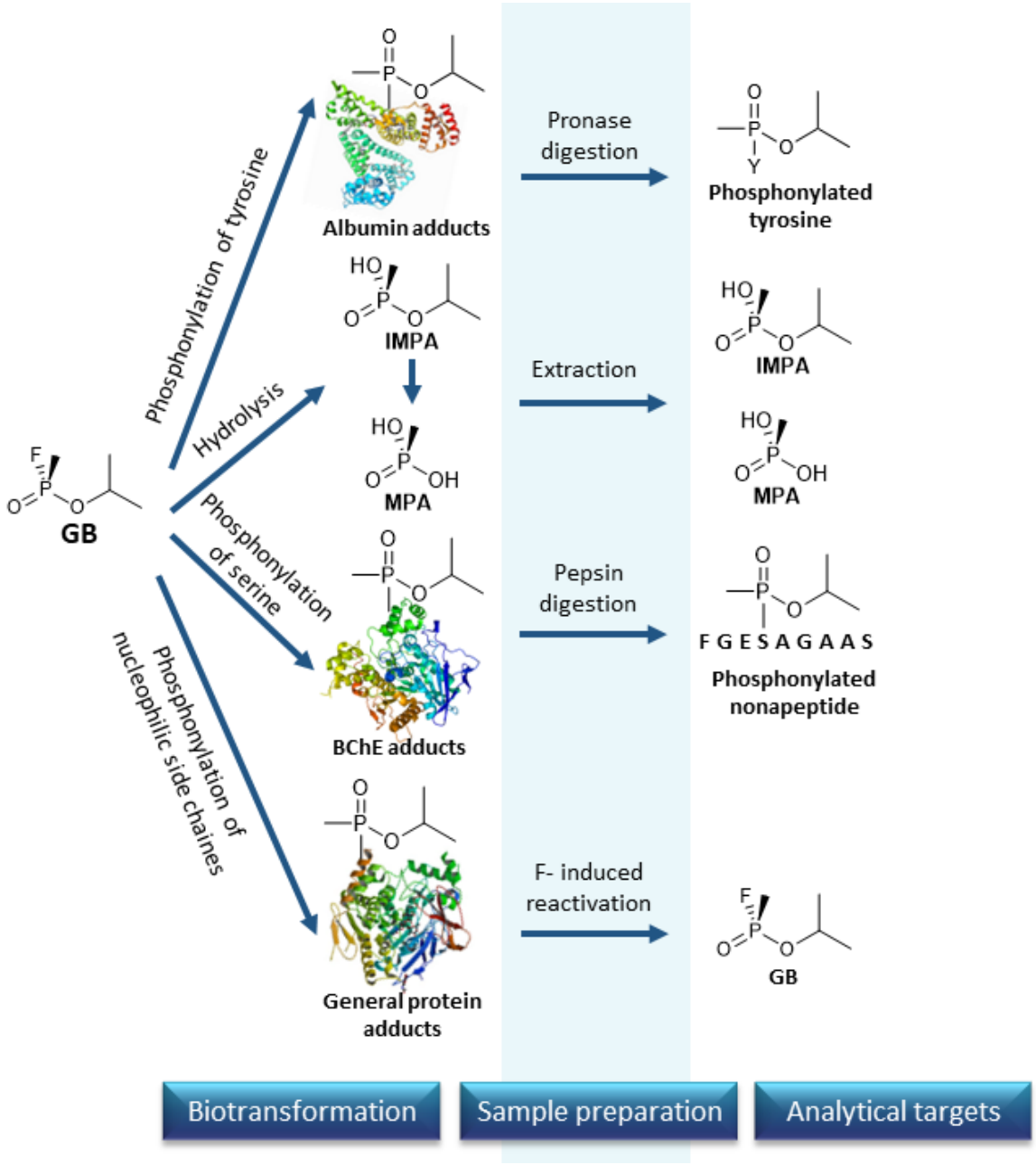

Figure S 1. Biotransformation pathways in humans after poisoning with sarin. Two main processes are hydrolysis, and the formation of protein adducts. Albumin, BChE and other protein adducts are then digested or reactivated by fluoride before the analysis.

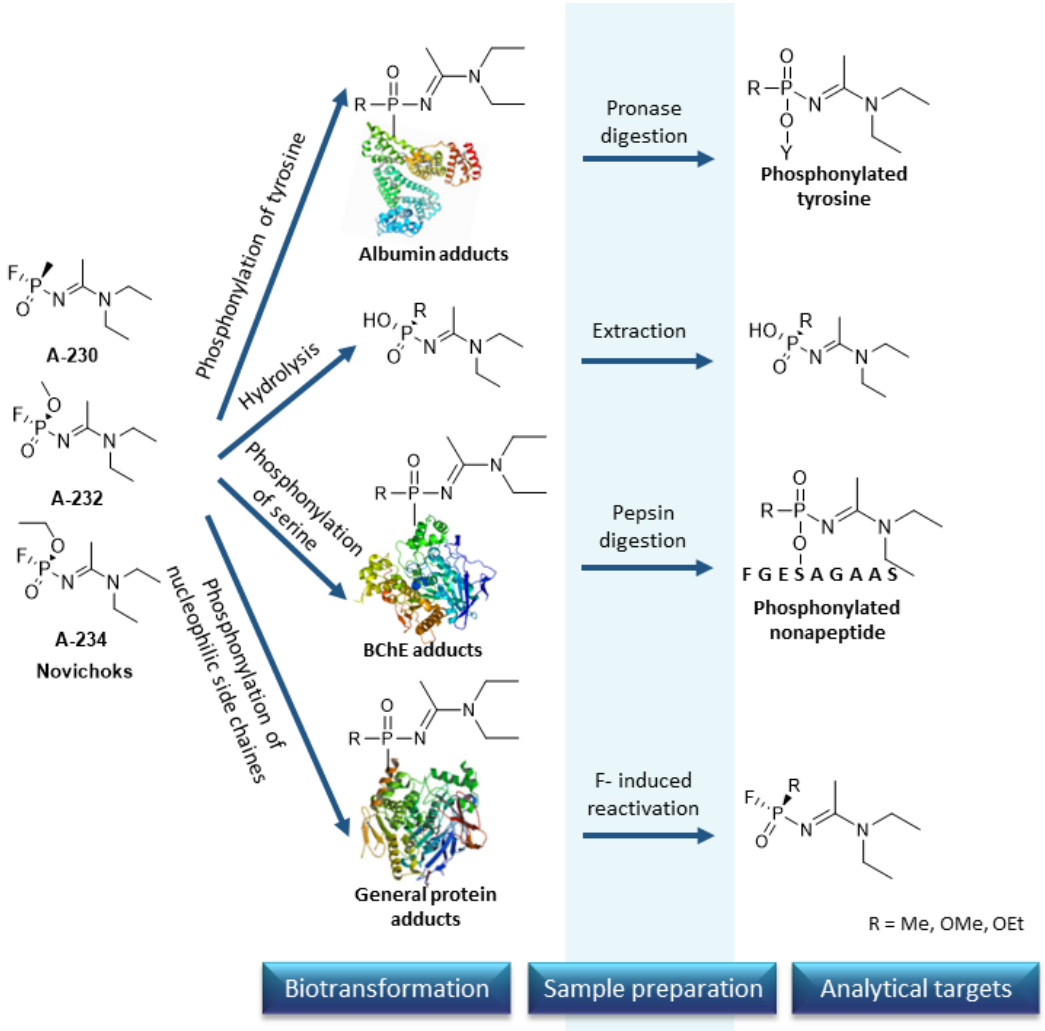

Figure S 2. To be investigated biotransformation pathways in humans after poisoning with Novichok nerve agents, based on sarin research. Two main processes are hydrolysis, and the formation of protein adducts. Albumin, BChE and other protein adducts are then digested or reactivated by fluoride before the analysis.

2. LC-MS/MS method optimization

Table S 1. Gradient elution settings for LC-MS/MS analyses. Eluent A is water with 0.2% v/v formic acid and eluent B is ACN with 0.2% v/v formic acid.

Analyte	Eluent A (%)	Linear ramping (min)	Eluent A (%)	Linear ramping (min)	Eluent A (%)
FGESAGAAS **MPA-FGES(mpa)AGAAS** **GB-FGES(GB)AGAAS** **A-230-FGES(A-230)AGAAS** **A-232-FGES(A-232)AGAAS** **A-234-FGES(A-234)AGAAS**	100	20	20	0.1	100 (hold 5 min)
MPA-Tyr **GB-Tyr** **A-230-Tyr** **A-232-Tyr** **A-234-Tyr**	100 (hold 1 min)	10	50 (hold 2 min)	0.1	100 (hold 3 min)
MPA **IMPA** **GB** **Methyl phosphate** **Ethyl phosphate**	100	5	20 (hold 1 min)	0.1	100 (hold 3 min)

Table S 2. Mass spectrometric parameters for nerve agent adducts and metabolites analyzed by LC-MS/MS

Analyte	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)
FGESAGAAS	796.4	492.2	60	32
		620.3	60	32
		691.4	60	32
FGES(mpa)AGAAS	874.3	602.3	60	31
		673.3	60	27
		778.3	60	26
FGES(GB)AGAAS	916.3	602.3	60	31
		673.3	60	27
		778.3	60	26
FGES(A-230)AGAAS	970.4	602.3	60	31
		673.3	60	27
		778.3	60	26
FGES(A-232)AGAAS	986.4	602.3	60	31
		673.3	60	27
		778.3	60	26
FGES(A-234)AGAAS	1000.4	602.3	60	31
		673.3	60	27
		778.3	60	26
MPA-Tyr	260.1	197.1	40	13
		214.1	40	13
GB-Tyr	302.1	214.1	40	13
		260.1	40	13
A-234-Tyr	386.2	74.1	40	15
		244.1	40	15
		285.0	40	15
		313.0	40	15
MPA	97.0	65.0	20	26
		79.0	20	26
IMPA	139.0	79.0	20	7
		97.0	20	7
GB	141.0	99.0	20	7
A-230	195.0	73.8	20	9
		121.6	20	10
		177.0	20	15
A-232	211.0	56.2	20	15
		73.8	20	10
		138.1	20	11
A-234	255.1	74.2	20	12
		123.9	20	13
		197.0	20	11

3. GC-MS/MS method optimization and validation

Table S 3. Mass spectrometric parameters for nerve agents analyzed by GC-MS/MS

Analyte	Retention time (min.)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)
GB	7.70	125	99	10
		99	81	15
		99	47	30
A-230	19.19	194	72	5
		165	68	10
		122	81	10
A-232	20.00	210	181	10
		181	68	15
		138	97	15
A-234	20.65	224	195	5
		224	72	5
		195	68	10

Three different blood samples with a relatively low sarin inhibition of 20% were prepared for fluorite reactivation (n=3-5). Also, sarin standards, solvent blanks and blank samples without the addition of KF were added. OPCW criteria for signal-to-noise (S/N) and ion ratio were evaluated. The S/N should be larger than 5 and the ion ratio for a ratio of >50% should be maximum ±20%. The standard has an ion-ratio, for 125 → 99 with respect to 99 → 81, of 83%. The variation must not be larger than ±20% * 83.2 = ±16.6%. The obtained ion-ratios and the S/N as shown in Table S4 were according to the guidelines.

Table S 4. Evaluation of ion-ratios and signal-to-noise (S/N) ratios of blood incubated with 20% sarin after fluorite reactivation.

	99 → 81		125 → 99		Ion-ratio (%)	Deviation (%)
	Area	S/N	Area	S/N		
Solventblank1	0		0			
Standard_GB_0.5ng/mL	20964	612	17447	743	83	
Solventblank2	0		0			
Blood1_noKF	0		0			
Blood1_KF_1	26396	392	21500	1028	81	-2
Blood1_KF_2	22880	928	16395	251	72	-14
Blood1_KF_3	27267	1150	23724	202	87	5
Blood1_KF_4	31794	1617	26594	1735	84	1
Blood1_KF_5	31528	1201	25106	1682	80	-4
Solventblank3	0		0			
Blood2_noKF	0		0			
Blood2_KF_1	29042	7187	22301	341	77	-8
Blood2_KF_2	33697	522	26626	760	79	-5
Blood2_KF_3	31312	411	24139	304	77	-7
Solventblank4	0		0			
Blood3_noKF	0		0			
Blood3_KF_1	26677	534	18892	350	71	-15
Blood3_KF_2	30705	241	21918	298	71	-14
Blood3_KF_3	23437	190	18197	3067	78	-7
Solventblank5	0		0			

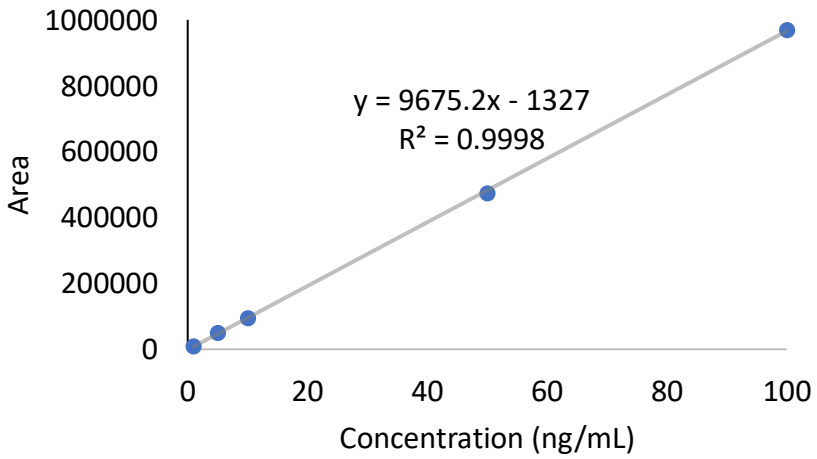

Figure S 3. Calibration curve of 1-100 ng/mL sarin analyzed by GC-MS/MS (n=1, m/z 99 → 81).

4. Cholinesterase activity

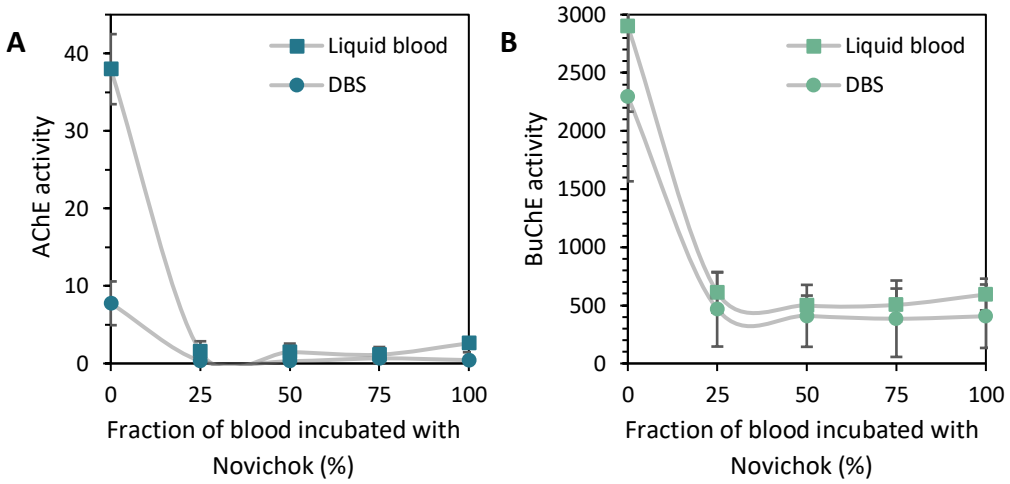

Figure S 4. Cholinesterase activity of 10 µL liquid blood (□) and 12.5 µL-based DBS (○) extracted after one month following nerve agent incubation. The ratio between blood incubated with 1.3 µM Novichock nerve agent and non-exposed blood is shown. A) AChE activity after Novichok inhibition (n=3), B) BChE activity after Novichok inhibition (n=3). Line is of indicative nature only, error bars represent ±1 standard deviation.

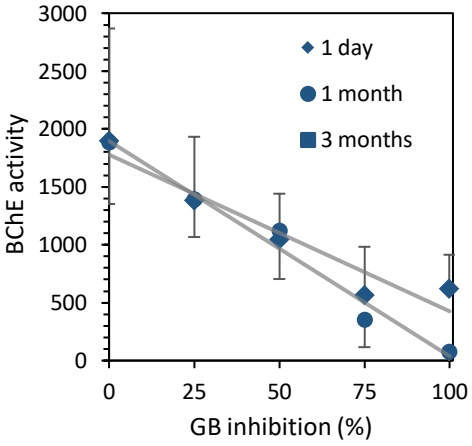

Figure S 5. BChE activity of DBS inhibited with sarin and extracted after one day (n=3, positive error bars), one month (n=8, negative error bars) and three months (n=1) of storage at room temperature.

5. Analysis of free and regenerated nerve agent in liquid blood

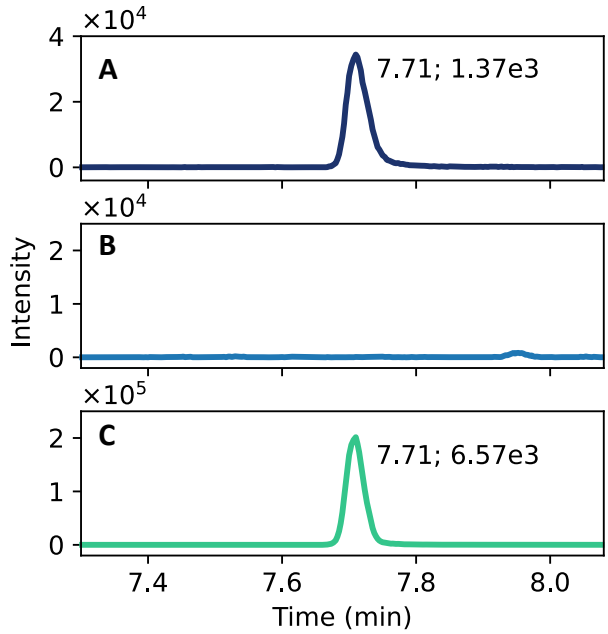

Figure S 6. Extracted ion chromatograms of regenerated sarin in 400 µL liquid whole blood exposed to sarin, after fluoride reactivation analyzed by GC-MS/MS (m/z 99 → 81, with corresponding area of the peak). A) Reference standard of sarin, B) Blood exposed to sarin without KF addition (control), C) Blood exposed to sarin with KF addition.

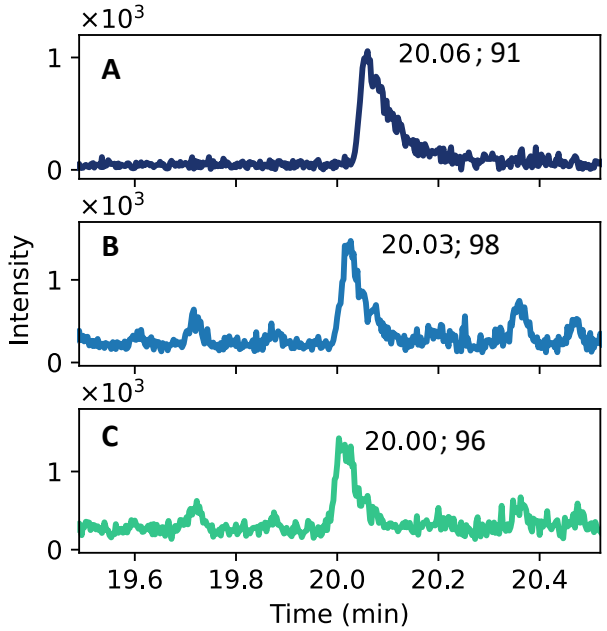

Figure S 7. Extracted ion chromatograms of intact Novichok A-232 in 400 µL liquid whole blood exposed to A-232, after fluoride reactivation analyzed by GC-MS/MS (m/z 138 → 97). A) Reference standard A-232, B) Dried blood spots exposed to A-232 without KF addition (control), C) DBS exposed to A-232 with KF addition.

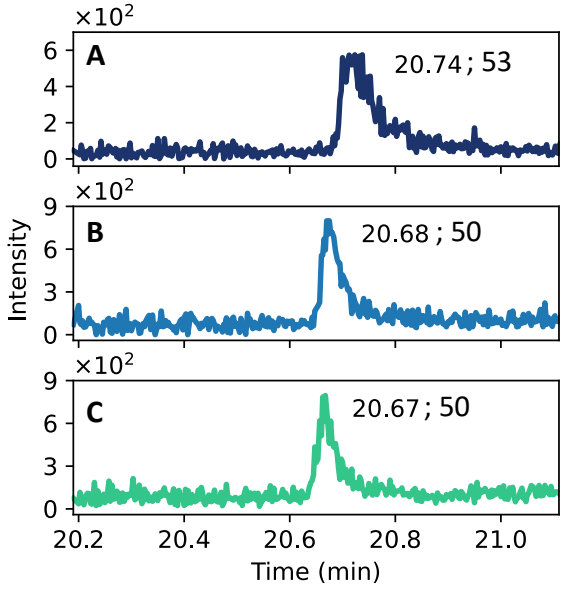

Figure S 8. Extracted ion chromatograms of intact Novichok A-234 in 400 µL liquid whole blood exposed to A-234, after fluoride reactivation analyzed by GC-MS/MS (m/z 224 → 195). A) Reference standard A-234, B) Dried blood spots exposed to A-234 without KF addition (control), C) DBS exposed to A-234 with KF addition.

6. Analysis of free nerve agent in dried blood spots

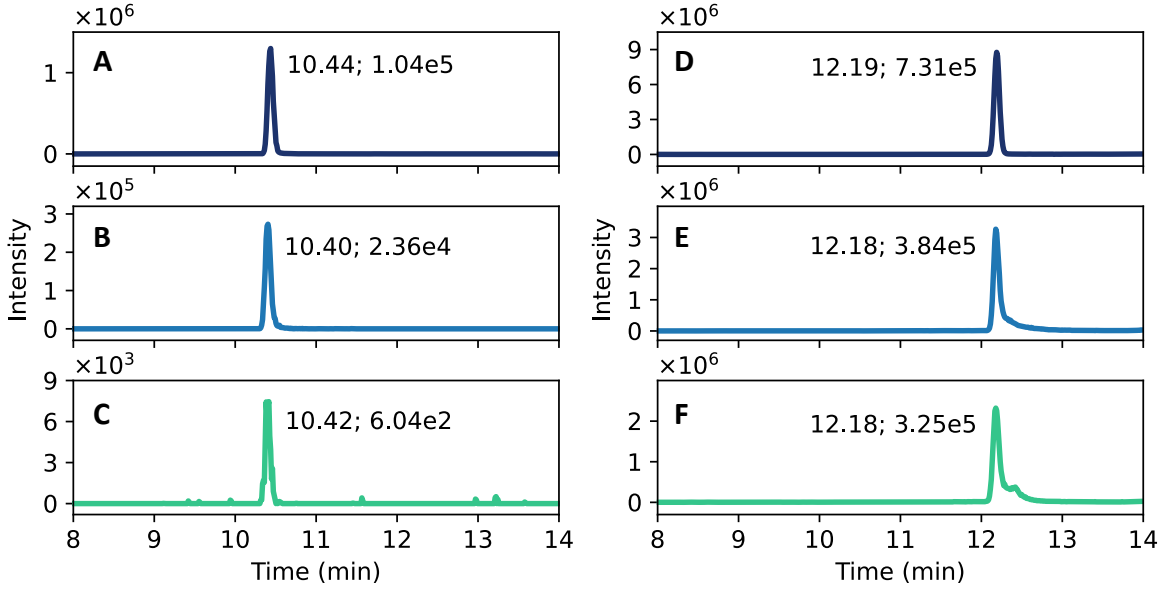

Figure S 9. Extracted ion chromatograms of intact Novichok A-C) A-230 (195.0 → 73.8) and D-F) A-234 (255.1 → 74.2) in dried blood spots (50 µL) analyzed by LC-MS/MS, three days after storage of the dried spots at ambient conditions. A, D) Reference standard, B, E) Dried blood spots exposed to nerve agent without KF addition (control), C, F) DBS exposed to nerve agent with KF addition.

7. Analysis of hydrolysis metabolites in DBS

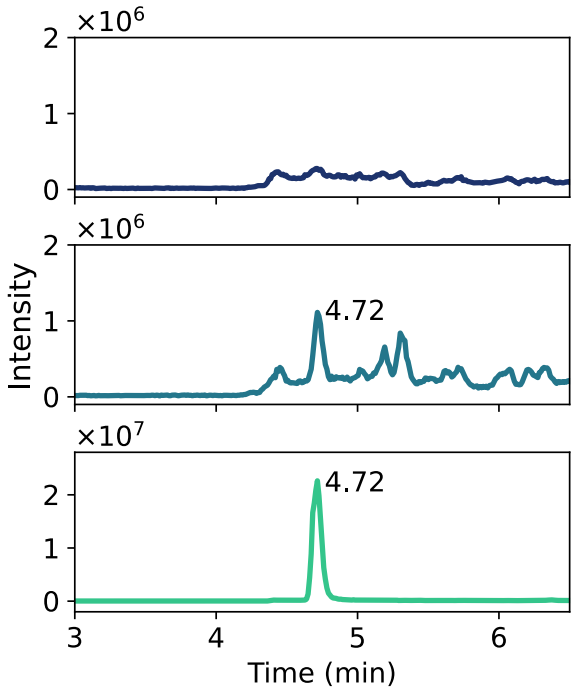

Figure S 10. Extracted ion chromatograms (139 → 97) of 100% inhibited dried blood spots after sarin exposure. A) sample preparation blank, B) potential presence of IMPA in dried blood spots, C) standard of IMPA.