SUPPLEMENTARY MATERIAL

Determination of mercury (thimerosal) in vaccines using different oxidants and cold vapor atomic fluorescence spectrometry in dilute acids

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Tables

Table S1. Instrumental parameters were used to determine total Hg by CV AFS.

Parameter	Value
Measurement mode (a. u.)	Peak height
Wavelength (nm)	253.7
Delay time (s)	15
Analysis time (s)	40
Memory wash time (s)	60
Gain	10
Reducer solution $SnCl_2$ (mM)	90
Carrier gas - Argon UHP 99.999% (mL min^{-1})	250
Dryer gas – Synthetic air 99.999% (mL min^{-1})	2500
Carrier solution (mL min^{-1})	9.0
Sample solution (mL min^{-1})	9.0
$SnCl_2$ solution (mL min^{-1})	4.5

Table S2. Heating program for closed system digestion of vaccine samples by microwave radiation.

Step	Time (min)	Power (W)	Temperature (ºC)
I	15*	700	RT - 130
II	20**	700	130
III	15*	1000	130 - 180
IV	20**	1000	180

RT = room temperature // *Ramp time // **Hold time

Table S3. Analyzed vaccine sample characteristics and the respective TH concentrations declared by the manufacturer.

Nº	Sample	TH declared (mg L^{-1})
1	Hepatite B: Hepatitis B Vaccine (Recombinant)	100
2	Td: Tetanus-diphtheria adsorbed vaccine.	100
3	DTP/Hib/HB: Diphtheria-tetanus-pertussis/Hemophilus influenzae type b conjugate and Hepatitis B vaccine	50
4	Influenza: Influenza vaccine (fragmented, inactivated)	4
5	Triple viral: measles, mumps, and rubella vaccine.	absent

Table S4. Residual acidity of digested TH solutions using the microwave in a closed system. The results refer to the analysis of 100 μL of the analytical blank in front of the different compositions (*n* = 3).

Condition	Volume HNO_3/H_2O_2 (mL)	Residual acidity (M)
1	8 / 0	6.23 ± 0.30
2	7 / 1	5.90 ± 0.05
3	6 / 2	3.38 ± 0.09
4	5 / 3	3.02 ± 0.10

Table S5. Acid concentration (HCl and HNO_3) influences the oxidizing systems (KBr / $KBrO_3$ and $KMnO_4$) regarding oxidation efficiency TH to Hg_{ino}. Experimental condition: $SnCl_2$ 90 mM (reducer solution) and HCl 2.40 M or HNO_3 1 M (carrier solution) (*n* = 3).

Condition	Acid	Concentration (M)	System	**Oxidation efficiency (%)
1	HCl	0.2 – 0.8	KBr / $KBrO_3$	75 - 105
2	HNO_3	0.06 – 0.7		66 - 95
3	HCl*	0.6	$KMnO_4$	98 ± 4
4	HNO_3	0.06 – 0.7		10 - 22

*For [HCl] < 0.2 M, there was no oxidation of TH to $Hg_{ino.}$
**Calculated based on the reference method procedure (microwave digestion).

Table S6. ANOVA for the linear model was obtained by the proposed method (systems 1 and 2).

System	Source	[a]SS	[b]DF	[c]MS	*F*
$KBr / KBrO_3$	Model	5783990.0	1	5783990	1509.6337
	Residual	84290.5	22	3831.4	
	Lack of Fit	15001.9	6	2500.3	0.5774
	Pure error	69288.6	16	4330.5	
$KMnO_4$	Model	2574890.0	1	2574890	1961.9962
	Residual	20998.1	16	1312.4	
	Lack of Fit	939.6	4	234.9	0.1405
	Pure error	20058.5	12	1671.5	

[a]SS: Sum of squares; [b]DF: Degrees of freedom; [c]MS: Mean squares.
System 1: F_{cal} (1509.6337) > F_{crit} (4.301) // System 2: F_{calc} (1961.9962) > F_{crit} (4.494).

Table S7. Analytical characteristics of different methodologies for determining TH in vaccines concerning the proposed method.

[a]Analytical technique	Sample preparation	[b]Vaccine type analyzed (analyzed quantity)	Sample volume (µL)	RSD (%)	LOD (µg L^{-1})	Recovery (%)	Reference
ELCAD - AES	Dilution	Hepatitis B (1)	200	< 5.00	25	[c]–	[1]
ICP OES	Addition of formic acid and photochemical vapor generation	Anti-rabies (3), Td (1), Hepatitis B (1), and influenza (2)	500	2.90	0.30	93 - 102	[2]
DBD-PIV-AFS	Dielectric barrier discharge-plasma induced	Anti-rabies (1), Td (1), and Hepatitis B (3)	[c]–	2.50	0.03	94.3 - 100	[3]
PVG-DBD-OES	Addition of formic acid and photochemical vapor generation	Anti-rabies (1), Hepatitis B (3), and Influenza (2)	[c]–	0.99	0.17	[c]-	[4]
DP AdSV Hg(Ag)FE	Sonication	D (1), d (1), Td (1) and DTP (1),	40	2.20	0.36	99 - 105	[5]
CV AFS	Oxidation with $FeCl_3$	Anti-rabies (4) and Hepatitis B (1)	[c]–	2.80	0.02	96- 103.4	[6]
SWV	Photo-degradation (UV)	Influenza (2)	100	3.20	340	91.9 - 92.4	[7]
CV AFS	Oxidation with $KBr/KBrO_3$	Td (1), DTP/Hib/HB (1), Hepatitis B (1), Influenza (1) and Triple viral (1)	100	3.14	0.02	80.1 - 106.0	This work
	Oxidation with $KMnO_4$	dT (1), DTP/Hib/HB (1), Hepatitis B (1), Influenza (1) and Triple viral (1)	100	4.59	0.02	92.5 - 101.1	

[a]Techniques: ELCAD-AES: Electrolyte cathode glow discharge atomic emission spectrometry; ICP OES: Inductively coupled plasma optical emission spectrometry; CV AAS: Cold vapor atomic absorption spectrophotometry; DBD-PIV-AFS: Dielectric barrier discharge using plasma-induced vaporization coupled atomic fluorescence spectrometry; PVG-DBD-OES: Photochemical vapor generation using dielectric barrier discharge coupled to optical emission spectrometry; DP AdSV Hg(Ag)FE: Differential pulse adsorptive stripping voltammetry using a renewable mercury film electrode; CV AFS: Cold vapor atomic fluorescence spectrometry; SWV: Squarewave voltammetry. [b]Type of vaccine: Td = Tetanus-diphtheria adsorbed vaccine; D = Diphtheria adsorbed pediatric vaccine; d = Diphtheria adsorbed vaccine for teenagers and adults; DTP = Diphtheria-tetanus-pertussis adsorbed vaccine.; DTP/Hib/HB = Diphtheria-tetanus-pertussis/Haemophilus influenzae type b conjugate and Hepatitis B vaccine. [c]Uninformed.

Table S8. General comparison of the parameters obtained for systems 1 and 2 and microwave-assisted digestion.

Parameters	Unit	Oxidizing system		Microwave-assisted digestion (reference method)
		$KBr/KBrO_3$	$KMnO_4$	
Sample				
Vaccine volume	µL	100	100	500
Reagents				
Concentration system				
TH solution	µg L^{-1}	20	20	20
Complexing/oxidizing	mM	2/0.34	1.2	-
HCl	M	0.6	0.6	-
HNO_3	M	0.2	0.2	14
H_2O_2	m/m (%)	-	-	30
Ascorbic acid volume	µL	20	200	20
Reaction time	min	5	5	70
Cold vapor generation				
Solution Concentration				
Carrier HCl	M	2.4	2.4	
Carrier HNO_3	M	-	-	1
Reductant $SnCl_2$	mM	90	90	90

References (Table S7)

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Figures

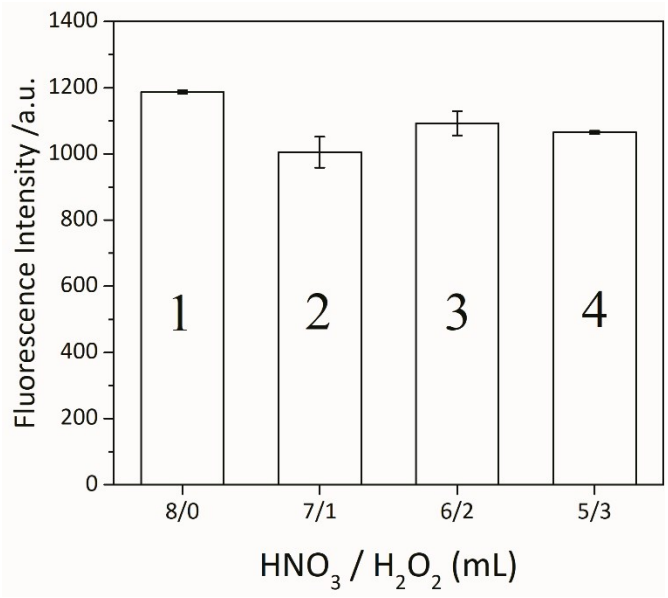

Fig. S1. Analytical signal comparison after microwave digestion of TH (20 µg L^{-1}) using different oxidizing mixture compositions (HNO_3 / H_2O_2) (*n* = 3).

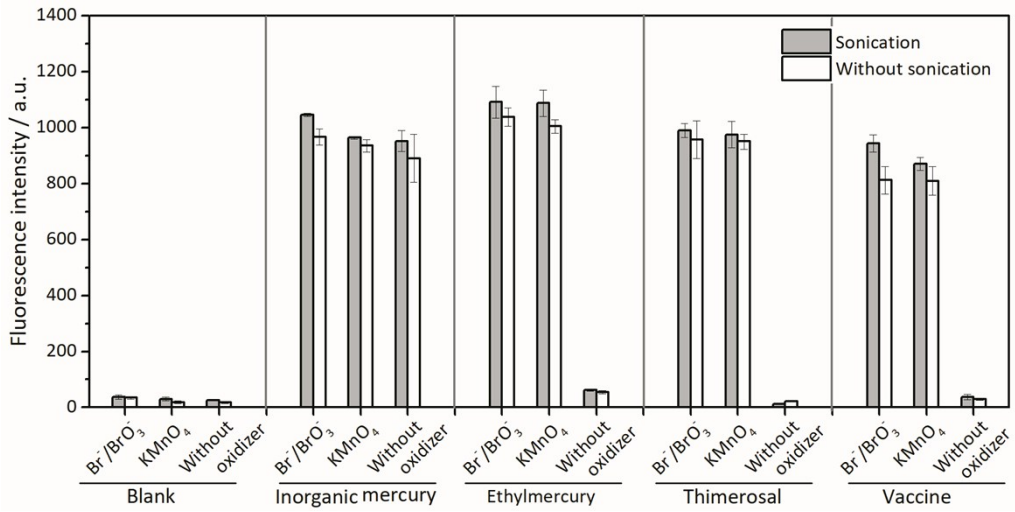

Fig. S2. Analytical signals evaluation using oxidizing systems (1 and 2) and sonication to determine total mercury in different standard solutions content mercury species and determination of TH in vaccines. In all systems assessed, the total mercury content concentration was adjusted to 15 µg L^{-1} (*n* = 3), except for the black solutions.

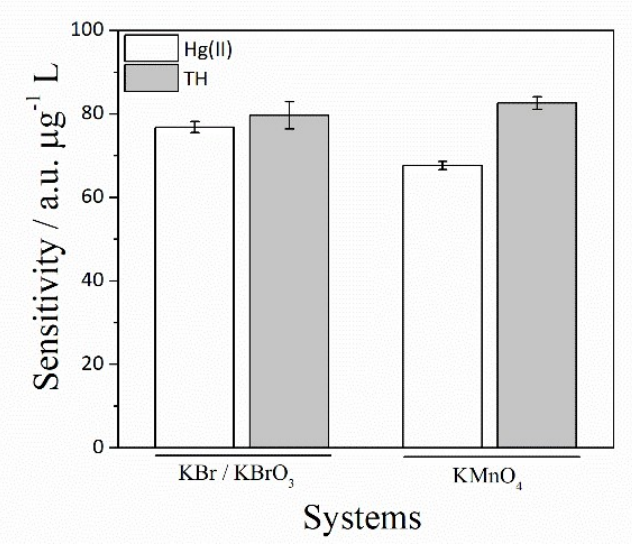

Fig S3. The sensitivity of the proposed method using different mercury species (Hg(II) and TH) (*n* = 3).