Supplementary Information

A robust method to quantify low molecular weight contaminants in heparin: detection of tris(2-*n*-butoxyethyl) phosphate

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S1:Table 1 Values of integrated contaminant/H-1 heparin[a] ratio in ppm and R^2 of linearized data from concentration and H-1 heparin/contaminant ratio

Contaminants	20000	10000	5000	2500	1250	625	155	R^2
Acetate- C3	0.113	0.053	0.032	0.017	0.010	0.006	0.002	0.997
Benzyl alcohol- C3	0.213	0.095	0.056	0.028	0.017	0.007	0.002	0.996
*Phenol- C1	0.245	0.107	0.064	0.032	0.018	0.008	0.002	0.995
Ethanol- C3	0.303	0.134	0.075	0.041	0.024	0.012	0.021	0.992
Methanol- C2	0.435	0.208	0.144	0.091	0.052	0.047	0.030	0.974

C1, Class 1, solvents to be avoided; C2, Class 2, solvents to be limited; C3, Class 3, solvents with low toxic potential. * Non-volatile contaminant. [a] heparin from bovine intestine mucosa H-1 region from 5.70 to 4.90 ppm. All statistical analyses were calculated for $p < 0.001$.

S2:Table 2 Values of sum from signal height of contaminant/H-1 heparin[a,b] ratio in ppm and R^2 of linearized data from concentration and H-1 heparin/contaminant ratio

Contaminants	20000	10000	5000	2500	1250	625	155	R^2
Acetate- C3[c]	0.679	0.355	0.149	0.098	0.048	0.031	0.008	0.998
Benzyl alcohol- C3[d]	0.410	0.194	0.095	0.046	0.027	0.013	0.003	0.999
*Phenol- C1[e]	0.253	0.112	0.060	0.028	0.015	0.009	0.002	0.997
Ethanol- C3[f]	0.904	0.474	0.245	0.135	0.064	0.042	0.045	0.999
Methanol- C2[g]	2.405	1.294	0.674	0.385	0.213	0.153	0.082	0.999

C1, Class 1, solvents to be avoided; C2, Class 2, solvents to be limited; C3, Class 3, solvents with low toxic potential. * Non-volatile contaminant. [a] heparin from bovine intestine mucosa. [b] A-1, C-1 and I-1(a,b/c). [c] signal height of the singlet at 1.91 ppm. [d] signal height of the main peak at 7.43 ppm. [e] signal height of the triplet at 7.32 ppm. [f] signal height of the triplet at 1.18 ppm. [g] signal height of the singlet at 3.35 ppm. All statistical analyses were calculated for $p < 0.001$.

S3: PCA analysis from 1H-NMR spectra of heparin samples at different contaminant concentrations
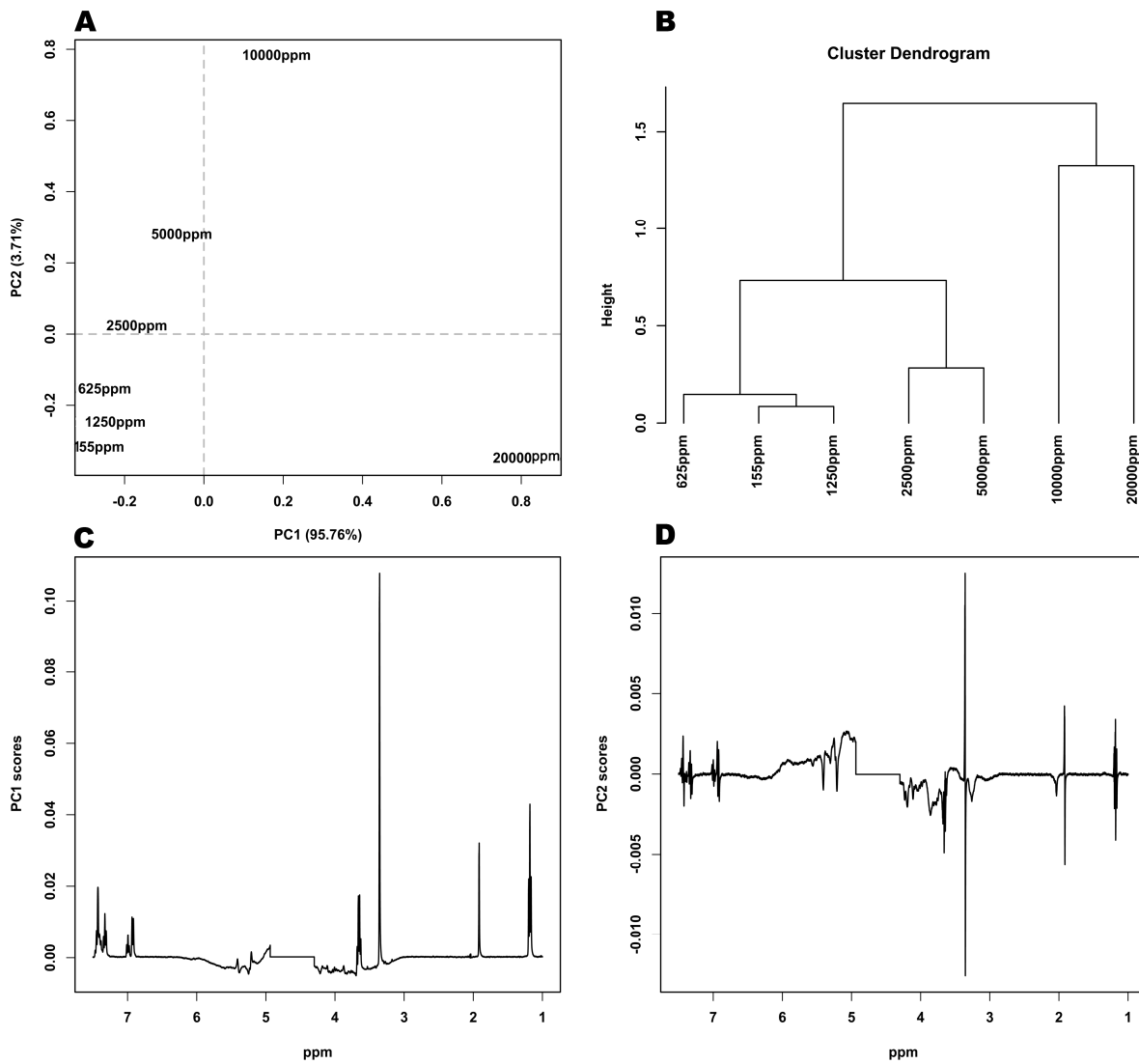

Fig. 1. (A) PCA analysis from ^{1}H-NMR spectra of heparin samples at different contaminant concentrations ranging from 155 to 20000 ppm (B) Cluster analysis performed by PCA from contaminated heparins; (C) PC1 and PC2 (D) scores which gave main differences from the ^{1}H NMR signals.