Construction and use of a library of *bona fide* heparins employing ^{1}H NMR and multivariate analysis

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S1. Summary of FDA NMR protocol [Sodium Heparin Stage 2 monograph]

500 MHz Pulsed (FT) NMR spectrometer, 16 Scans, 90° pulse with a 20 s delay, recorded at a temperature of 25 °C. The N-acetyl peak positions are defined as 2.05±0.02 and 2.16±0.03 ppm, respectively for heparin and OSCS. The positions of H1 of GlcNAc/GlcNS, 6S [5.42 ppm], H1 of IdoA2S [5.21 ppm], H2 of GlcNS [3.28 ppm (doublet centered at 3.28 ppm)] and the methyl of GlcNAc [2.05 ppm] must not differ by more than ±0.03 ppm. No signals should be present in the ranges 0.10–2.00, 2.10–3.20, and 5.70–8.00 ppm, that have a height greater than 4 % of the mean signal height of H1 of GlcNAc/GlcNS, 6S [5.42 ppm] and H1 of IdoA2S [5.21 ppm]. There should also be no signal between 3.35–4.55 ppm with a height greater than 200 % signal height of the mean of H1 of GlcNAc/GlcNS, 6S [5.42 ppm] and H1 of IdoA2S [5.21 ppm], for porcine heparin.

S2: Single Heparin Library PCA of Single Heparin Library: Mean centered. Dataset was normalised to average spectral area and mean centered before PCA.

This analysis was performed on a small library contain the 'best' spectra [the spectrum with the smallest width at half height for the TSP peak] of the replicates that constituted the full library. This analysis shows that there are possible correlations between sulfation pattern and component derived though PCA, especially for 6-O-sulfation (S2 figure 7).

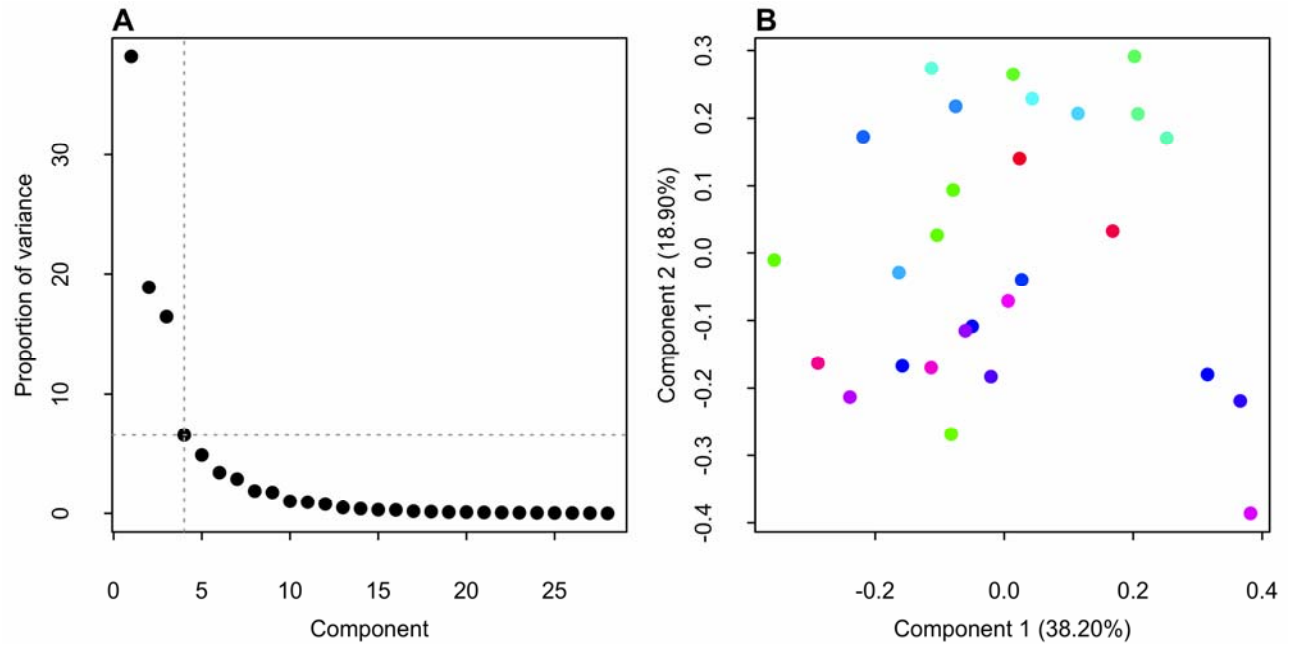

Figure 1. Scree and Loading Plot

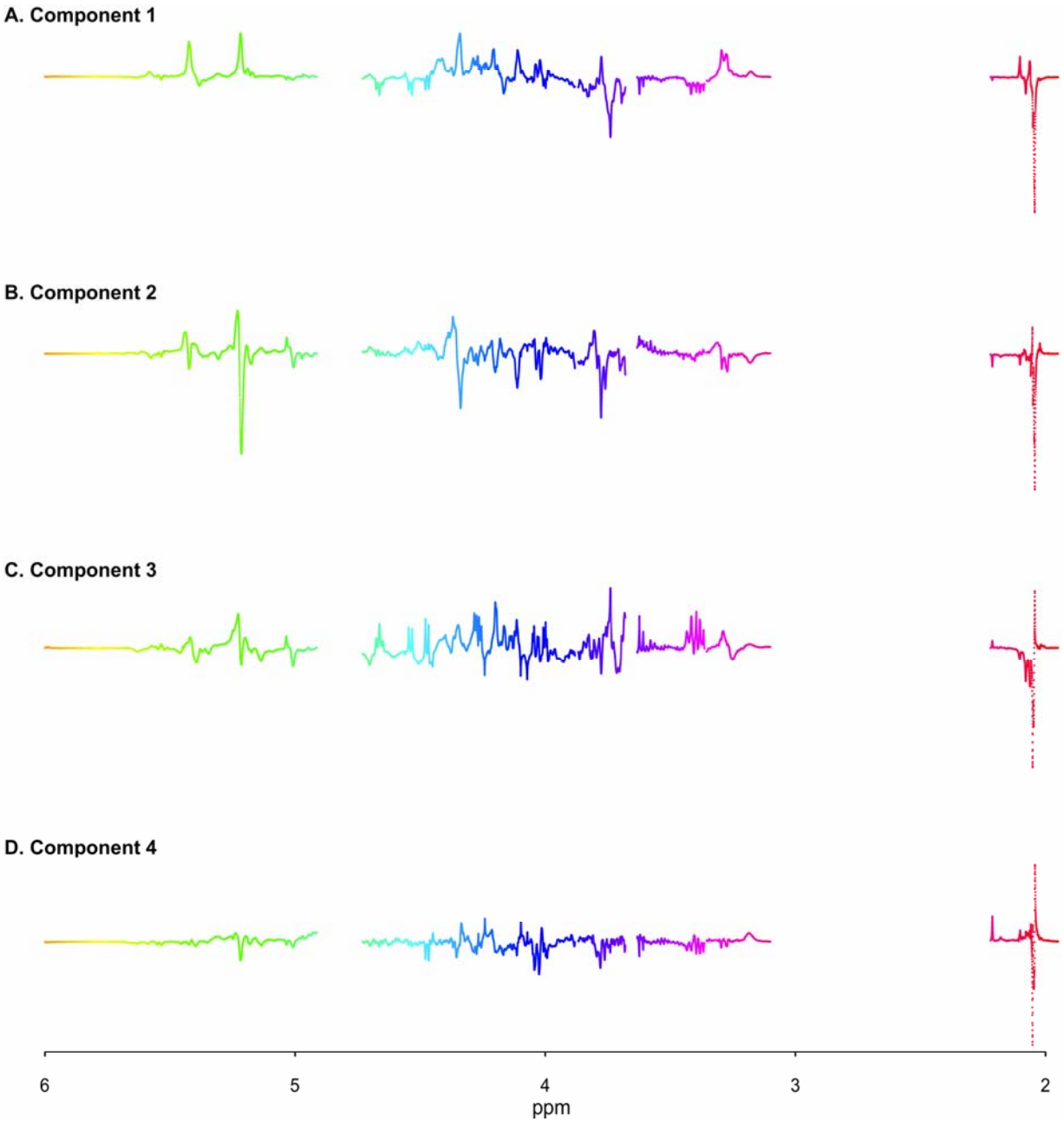

Figure 2. Component 1-4 Score plots

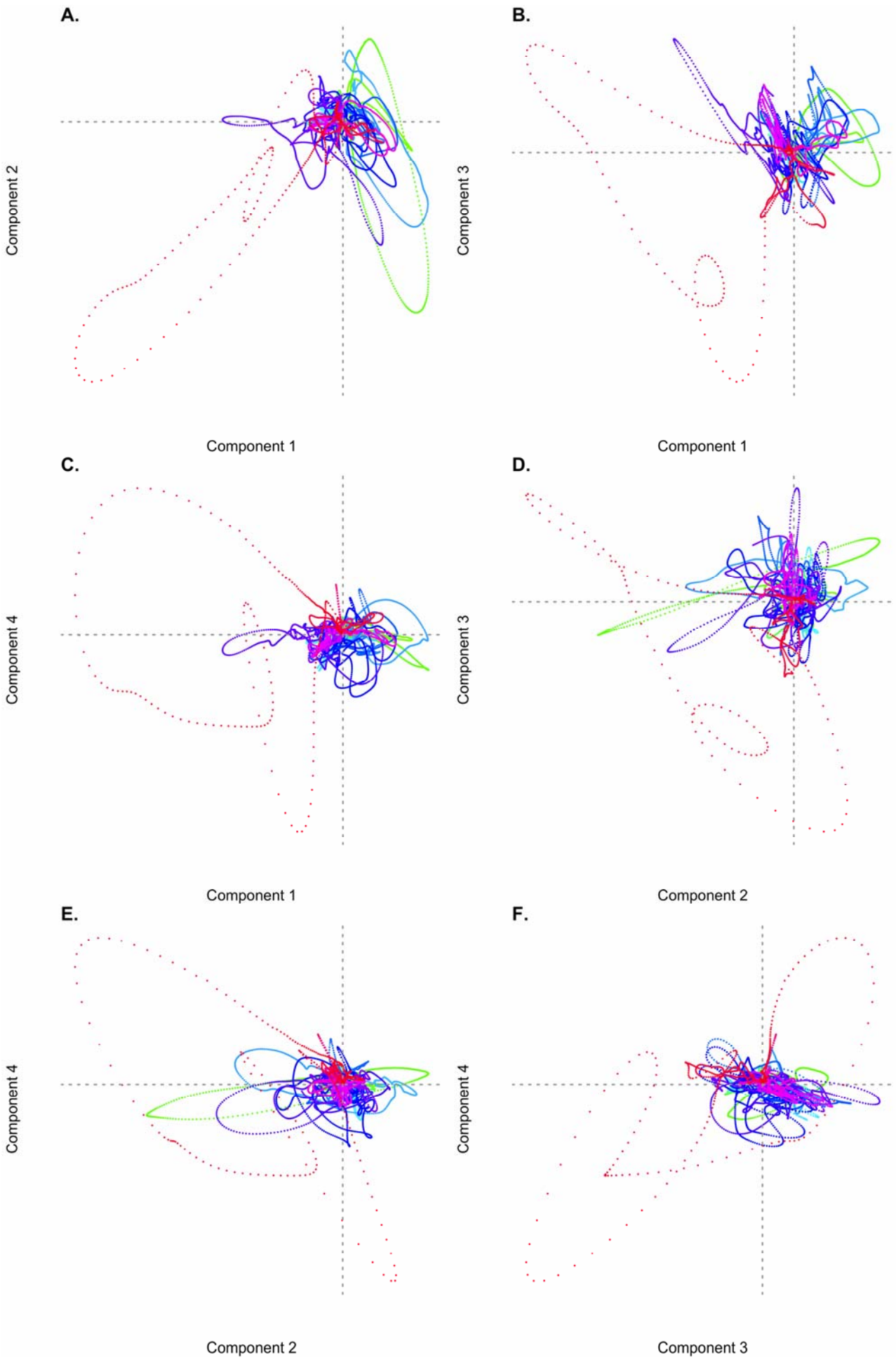

Figure 3. Component score v. component score plots

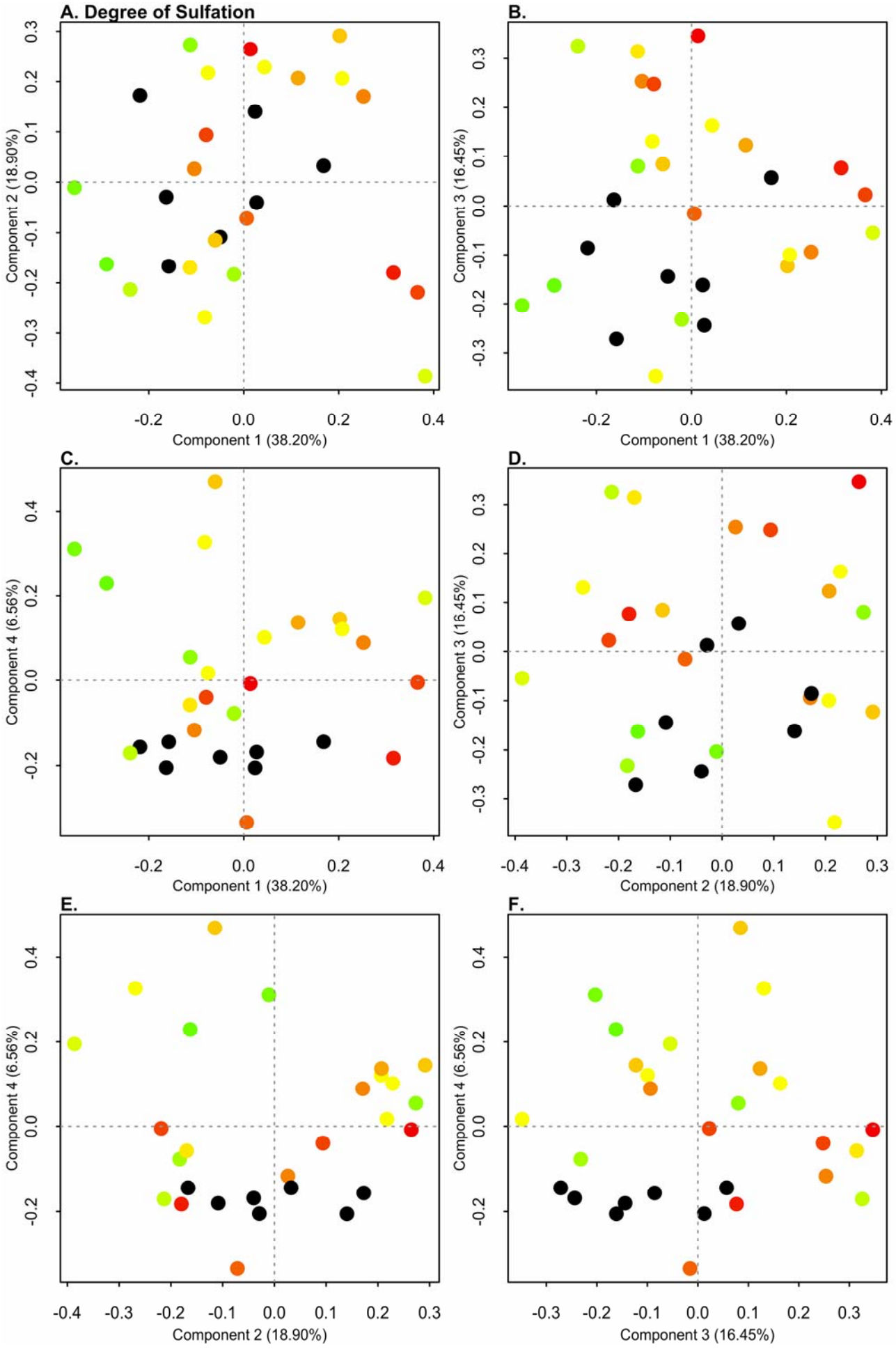

Figure 4 Degree of Sulfation colour gradient loading plots [green low sulfation to red high sulfation, samples with no data as black]

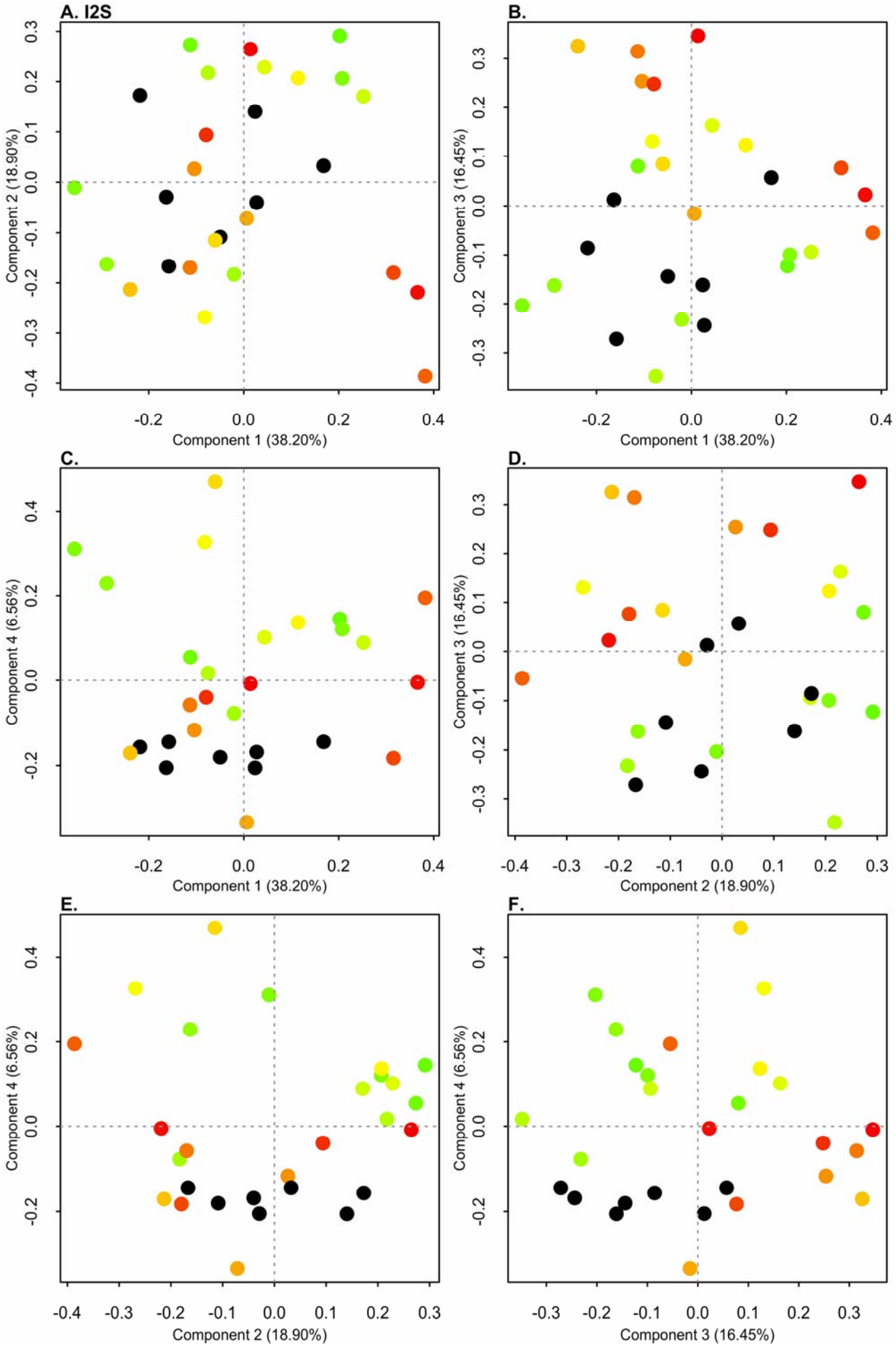

Figure 5. Level of sulfation at I2 colour gradient loading plots [green low sulfation to red high sulfation, samples with no data as black].

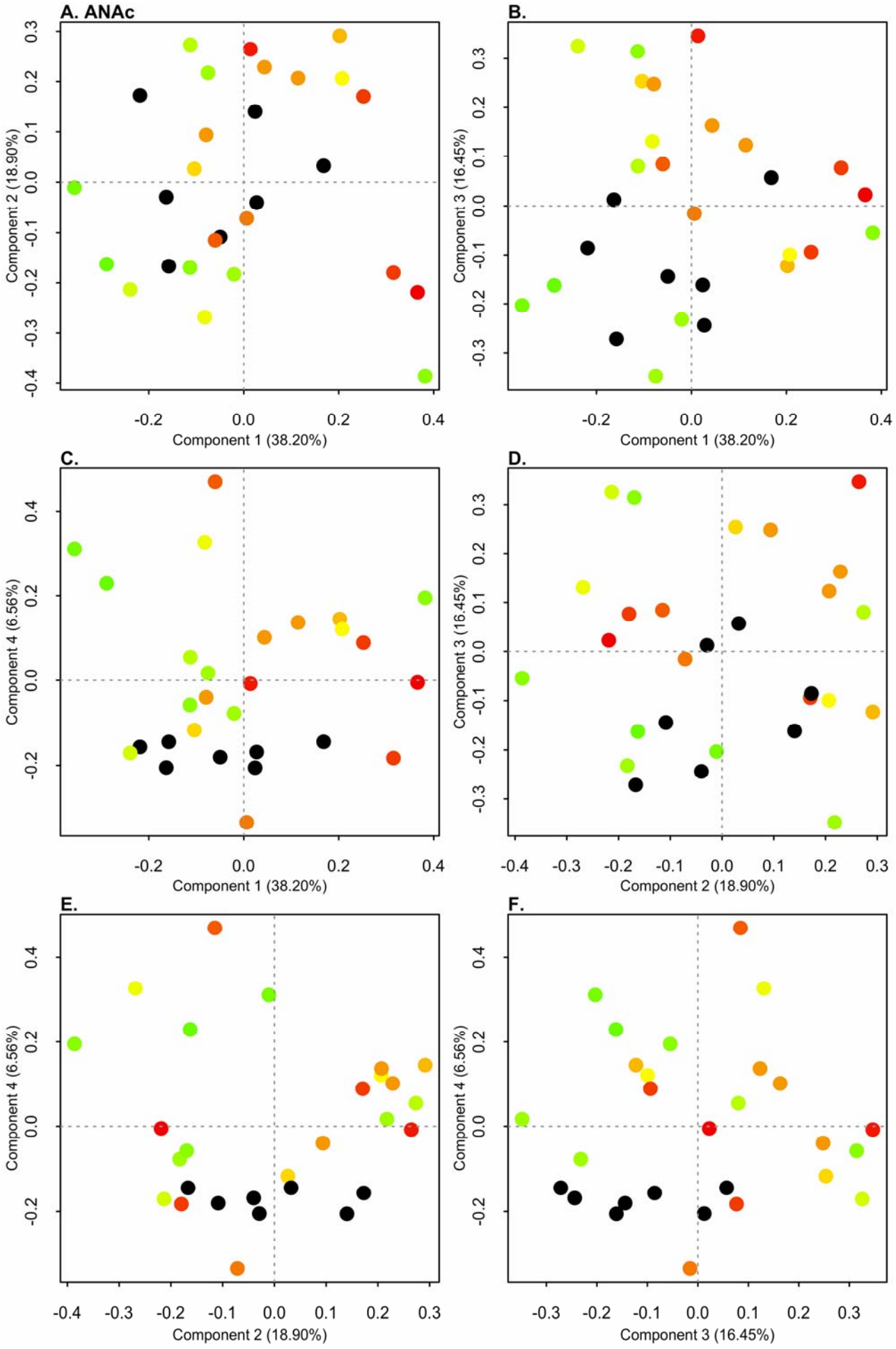

Figure 6. Level of Sulfation at A2 colour gradient loading plot [green low sulfation to red high sulfation, samples with no data as black]

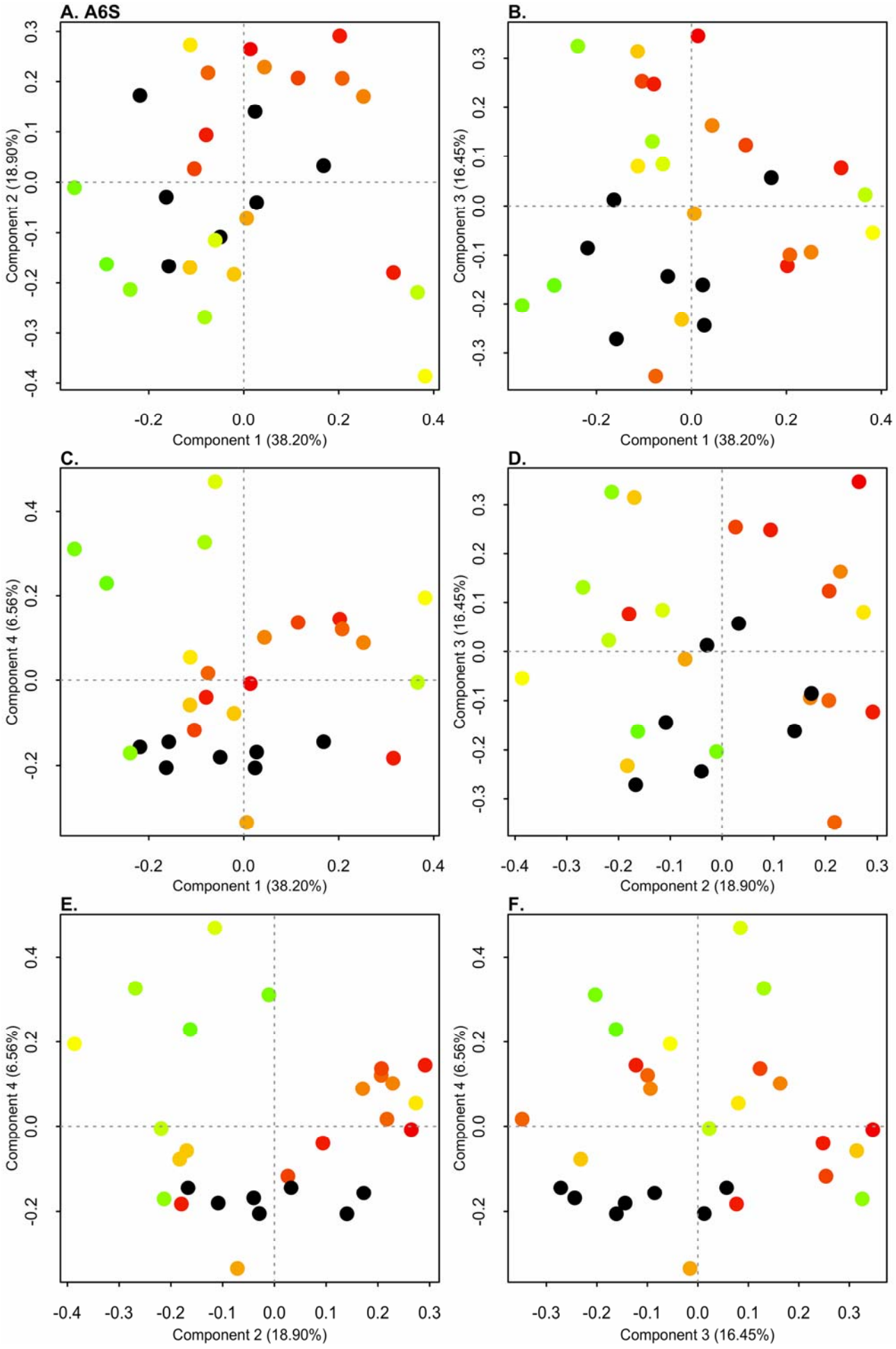

Figure 7. Level of Sulfation at A6 colour gradient loading plot [green low sulfation to red high sulfation, samples with no data as black]

S3: Multiple Heparin Library PCA of Multiple Heparin Library: Dataset was normalised to average spectral area and mean centered before PCA. The library contains replicates for each sample.

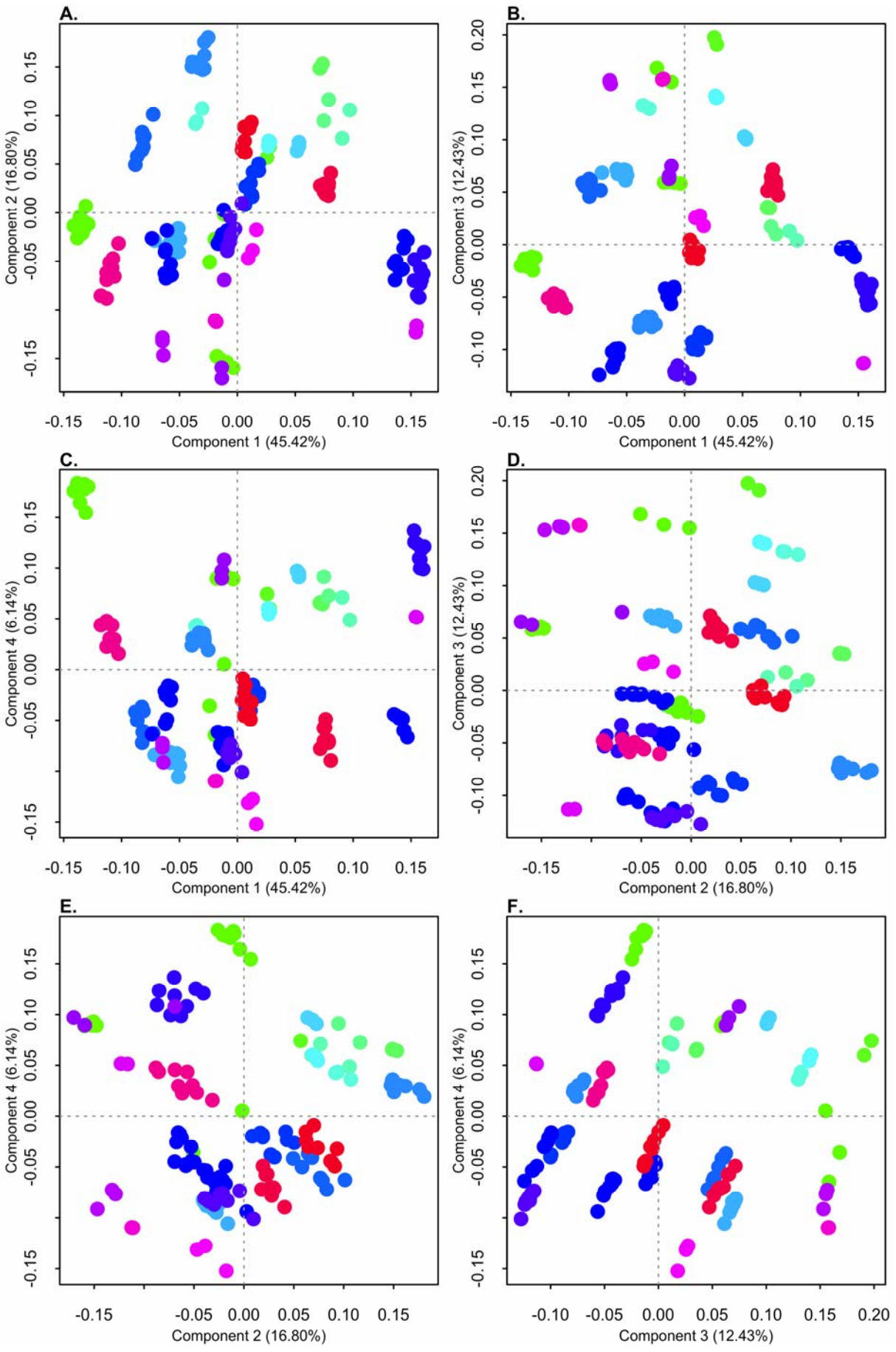

Figure 1. Loading plots of the PCA of the Heparin Library

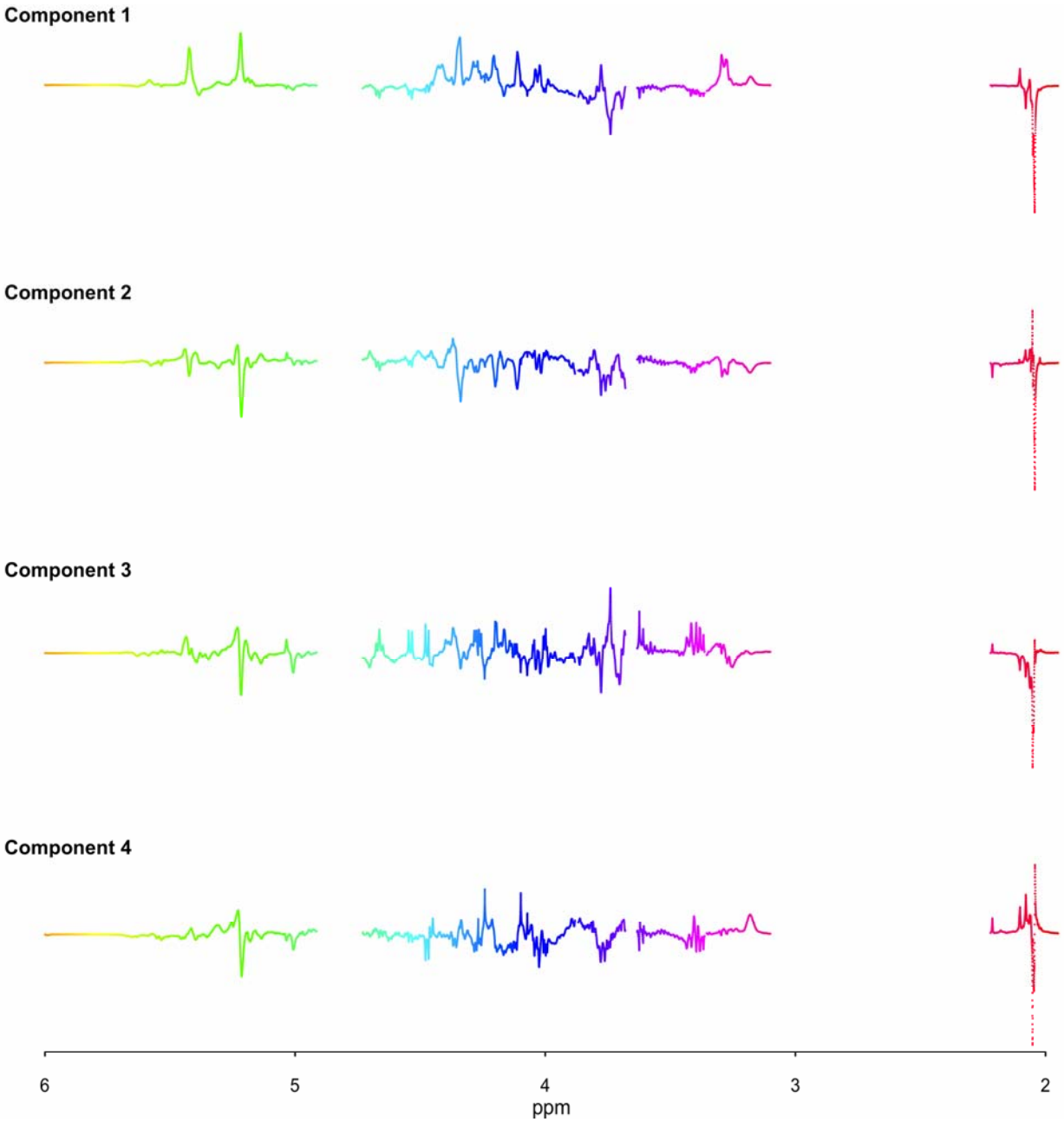

Figure 2. Component Scores of the PCA of the Multiple Heparin Library.

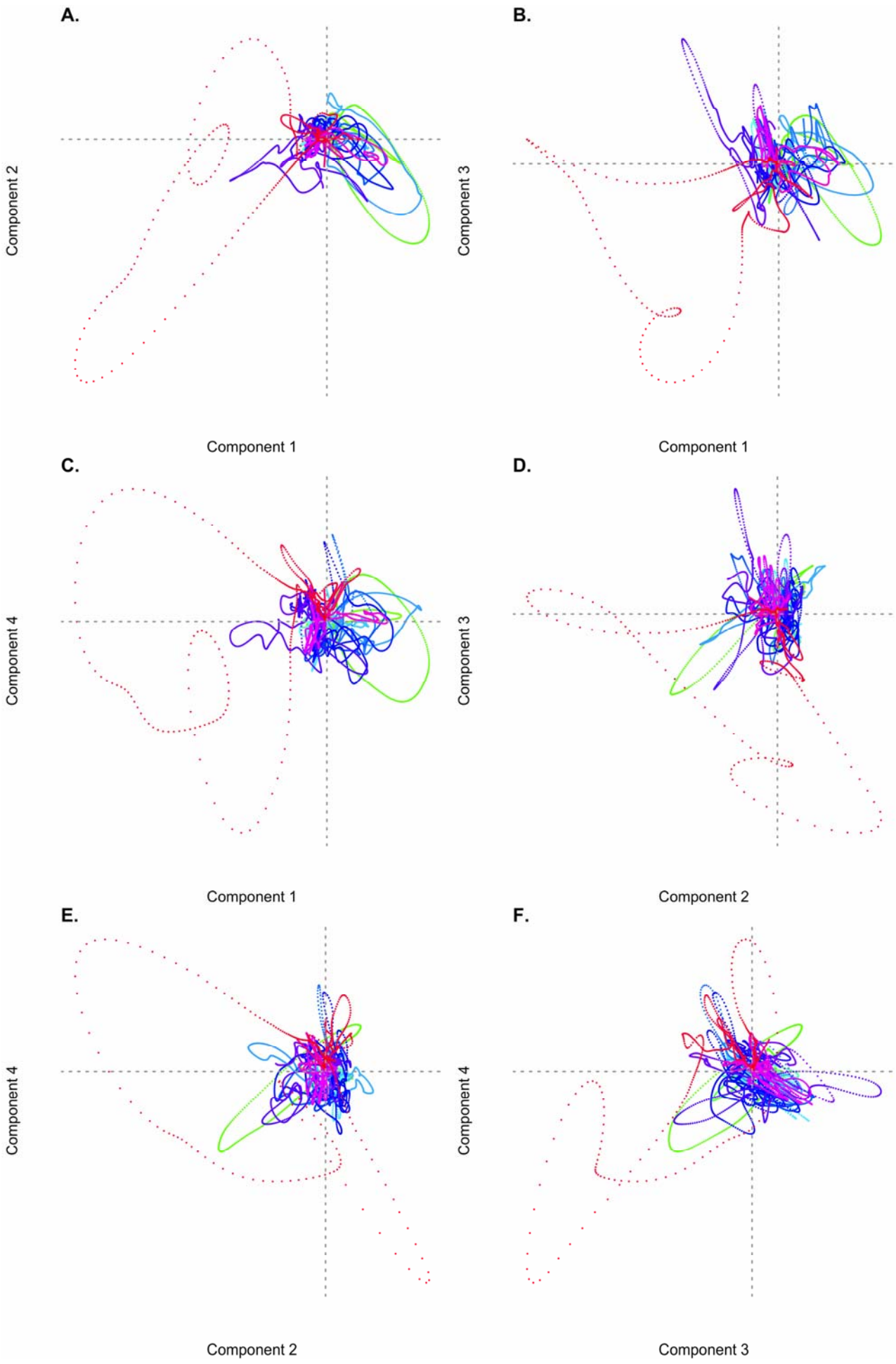

Figure 3. Component Score v. Component Score plots for PCA of the multiple heparin Library.

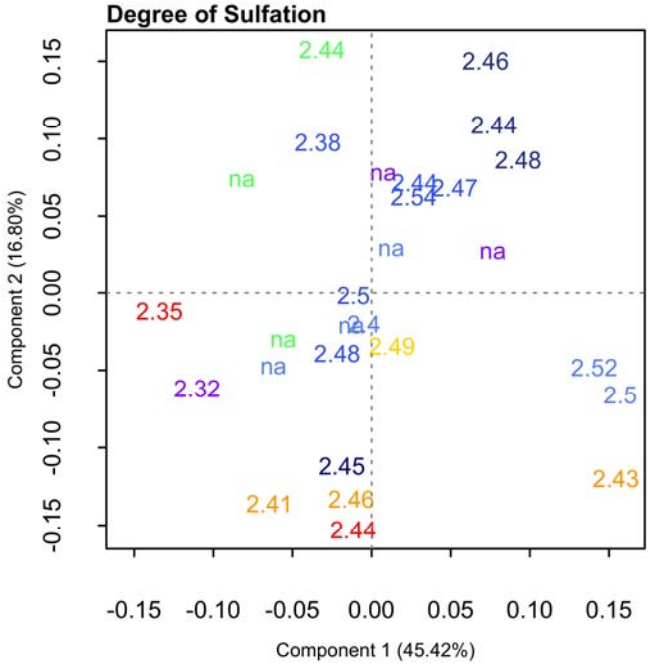

Figure 4. Loading plot (Component 1 v Component 2) of the PCA of the Heparin Library annotated with the Degree of Sulfation of the compounds. Colour coordinated for manufacturer as in Figure 1 of the manuscript. The figure illustrates that there are possible correlations between the components derived from the PCA and the degree of sulfation of each sample.

S4-1: OSCS sample one by one versus Heparin Library [Full spectrum] Dataset was normalised to average spectral area, mean centered and Pareto scaled before PCA.

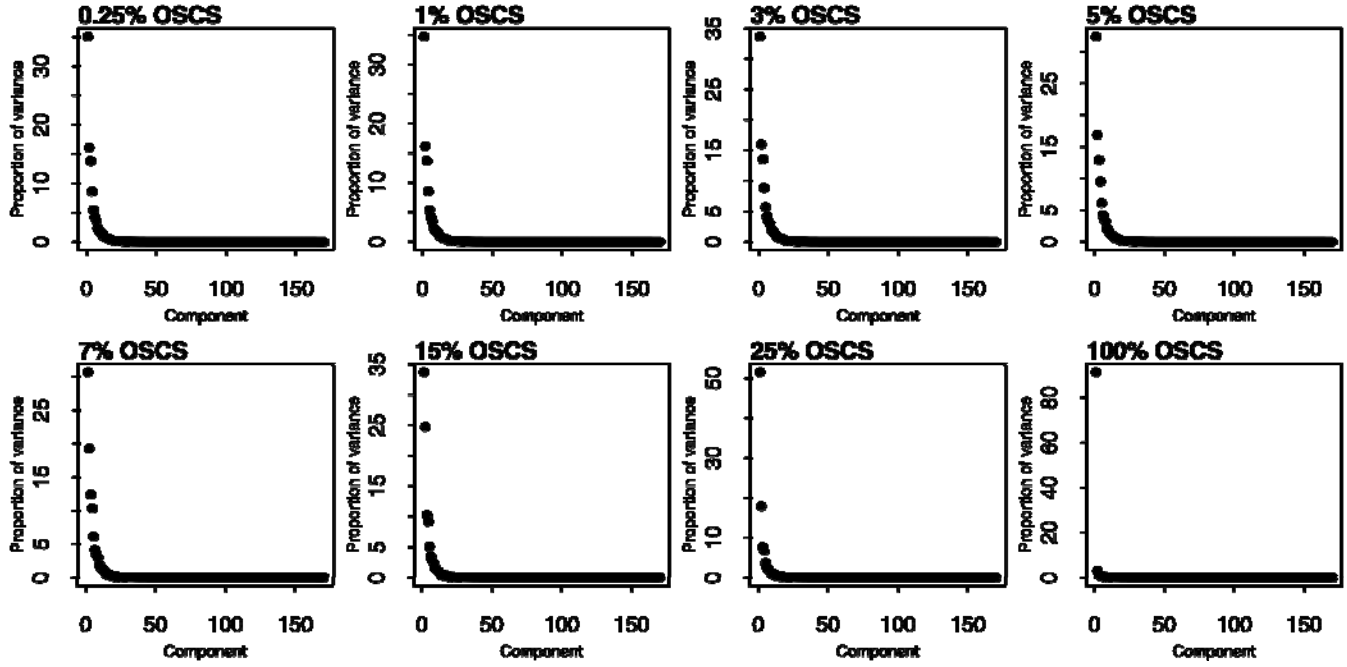

Figure 1. Scree plots derived from the PCA analysis of the OSCS samples with the heparin library.

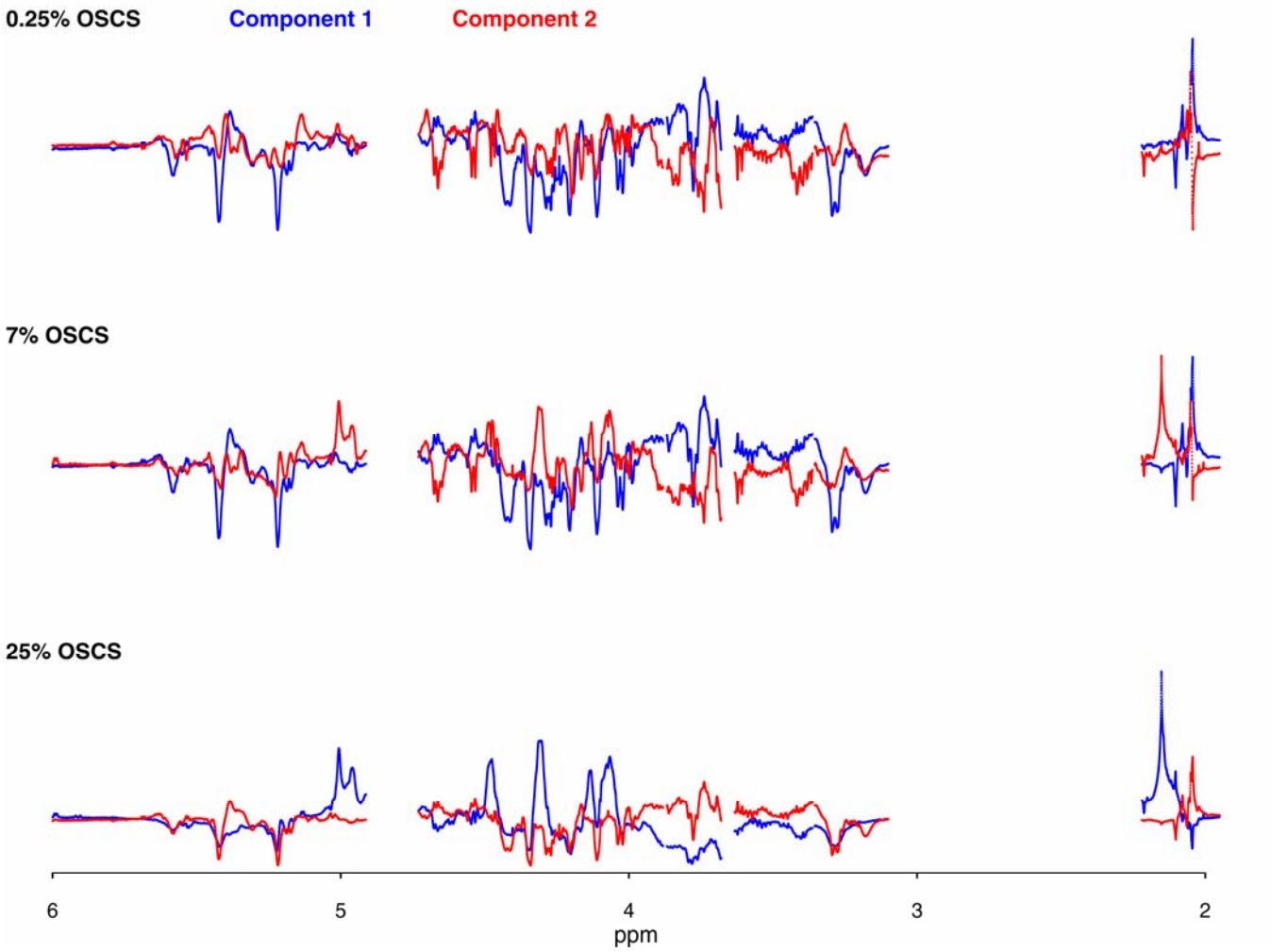

Figure 2. Score plots derived from the PCA analysis of the OSCS samples with the heparin library.

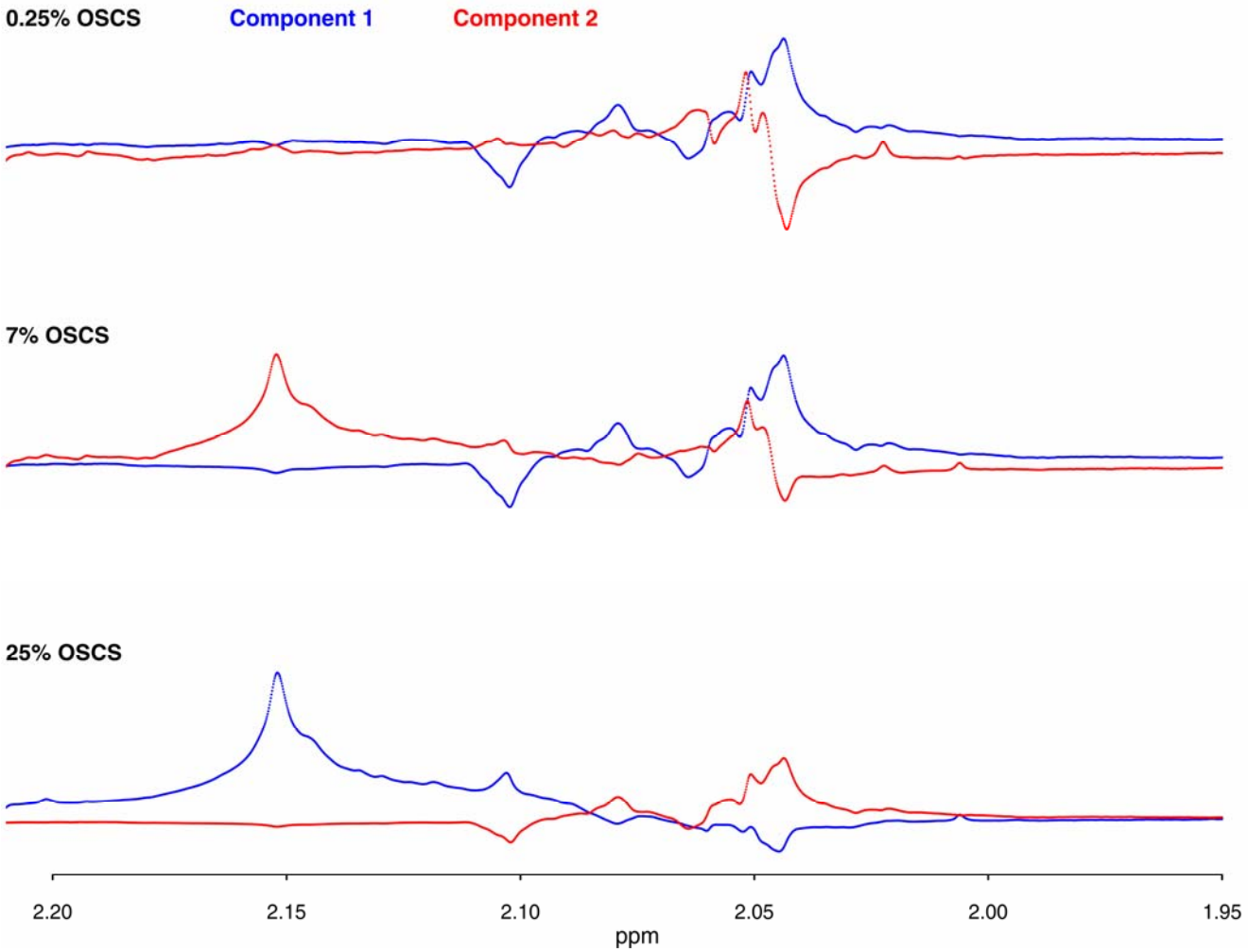

Figure 3. Score plots derived from the PCA analysis of the OSCS samples with the heparin library, expanded for *N*-acetyl region. The components are composed of signals related to heparin (2.04 ppm), dermatan sulfate (2.08 ppm) and oversulfated chondroitin sulfate N-acetyl signal (2.16 ppm) and a signal at 2.1 ppm which is the consequence of oxidation.

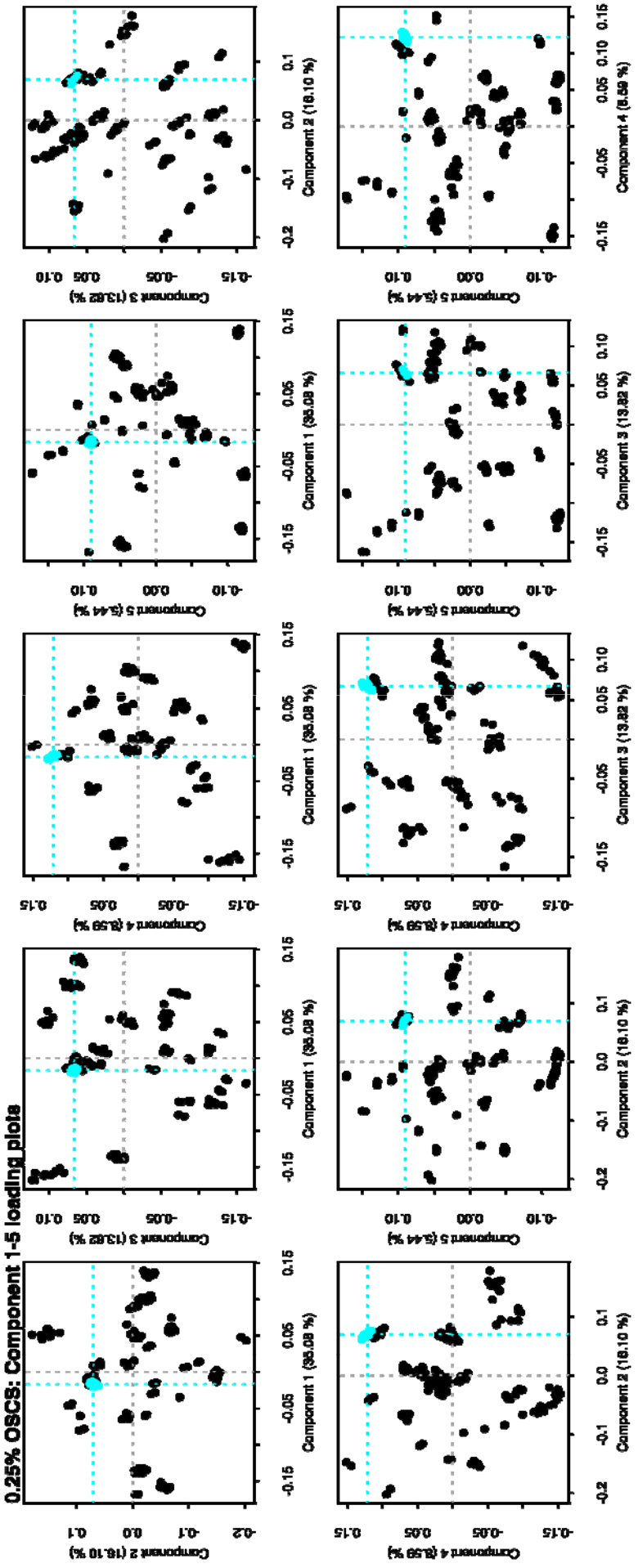

Figure 4 Combinatorial loading plots (Component 1-5) for the PCA of 0.25 % OSCS contaminated heparin and a heparin library.

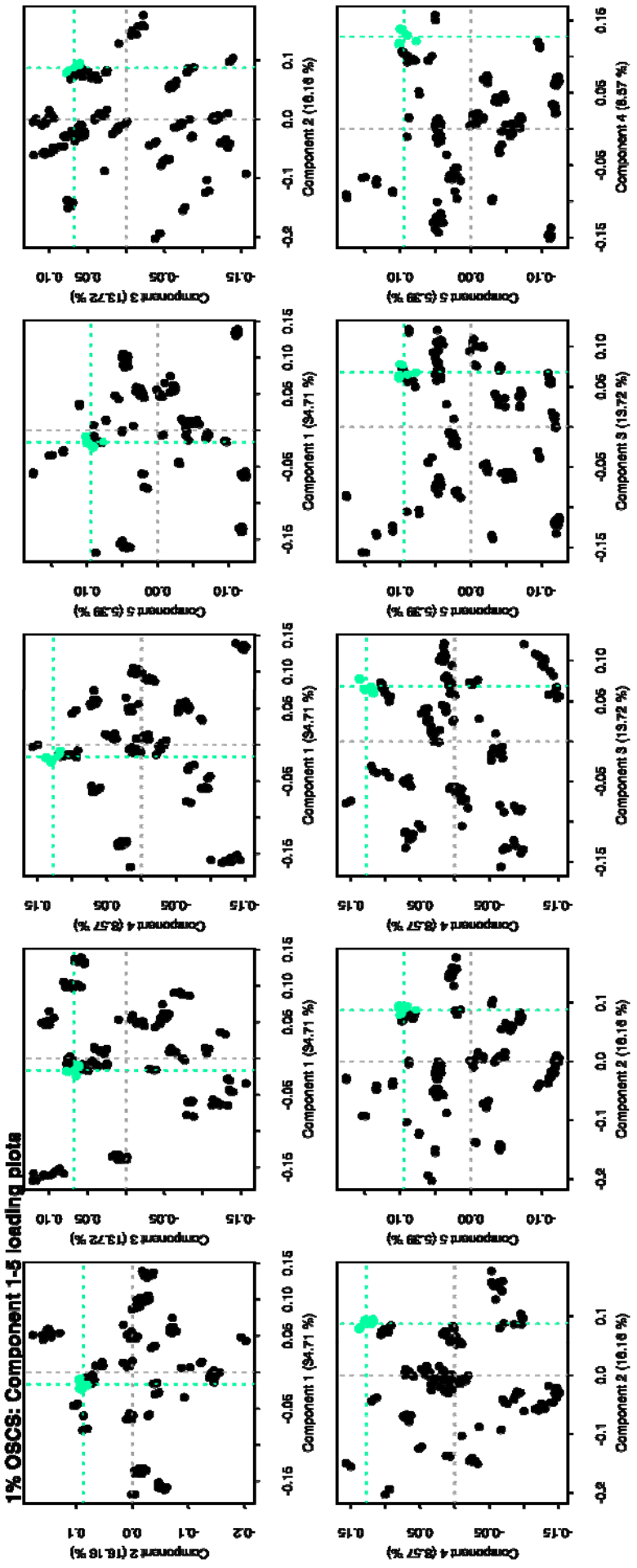

Figure 5 Combinatorial loading plots (Component 1-5) for the PCA of 1 % OSCS contaminated heparin and a heparin library.

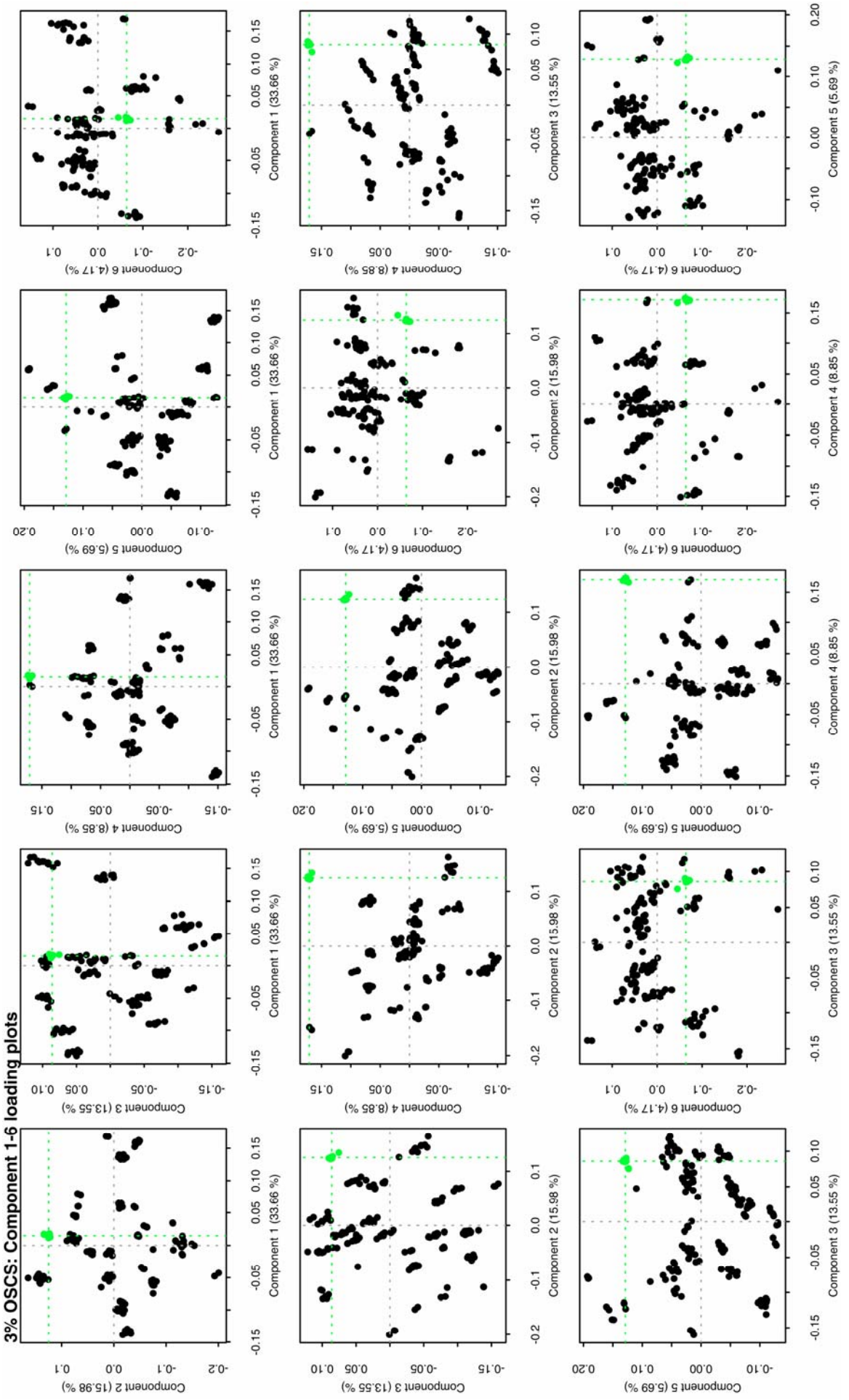

Figure 6 Combinatorial loading plots (Component 1-6) for the PCA of 3 % OSCS contaminated heparin and a heparin library.

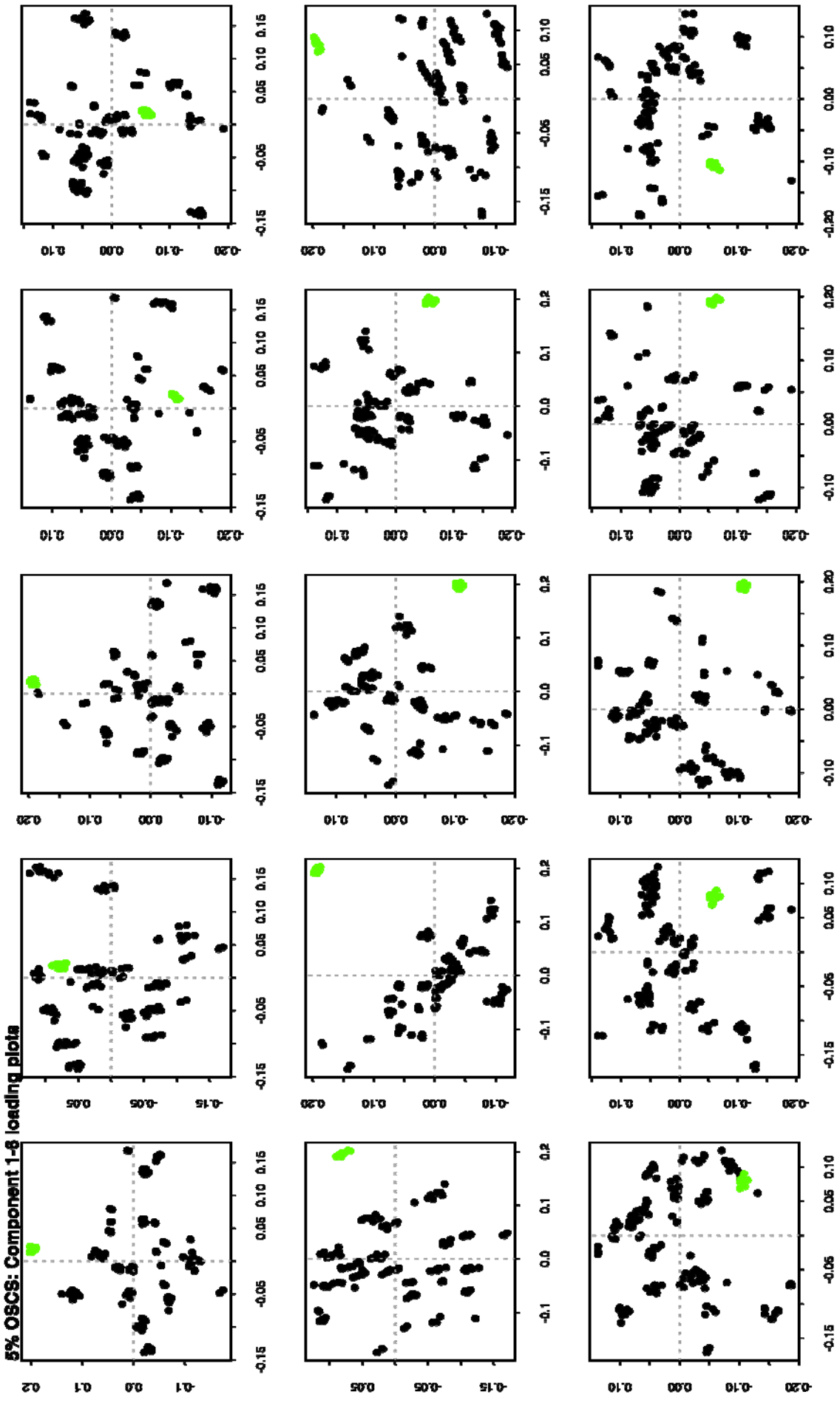

Figure 7 Combinatorial loading plots (Component 1-6) for the PCA of 5 % OSCS contaminated heparin and a heparin library.

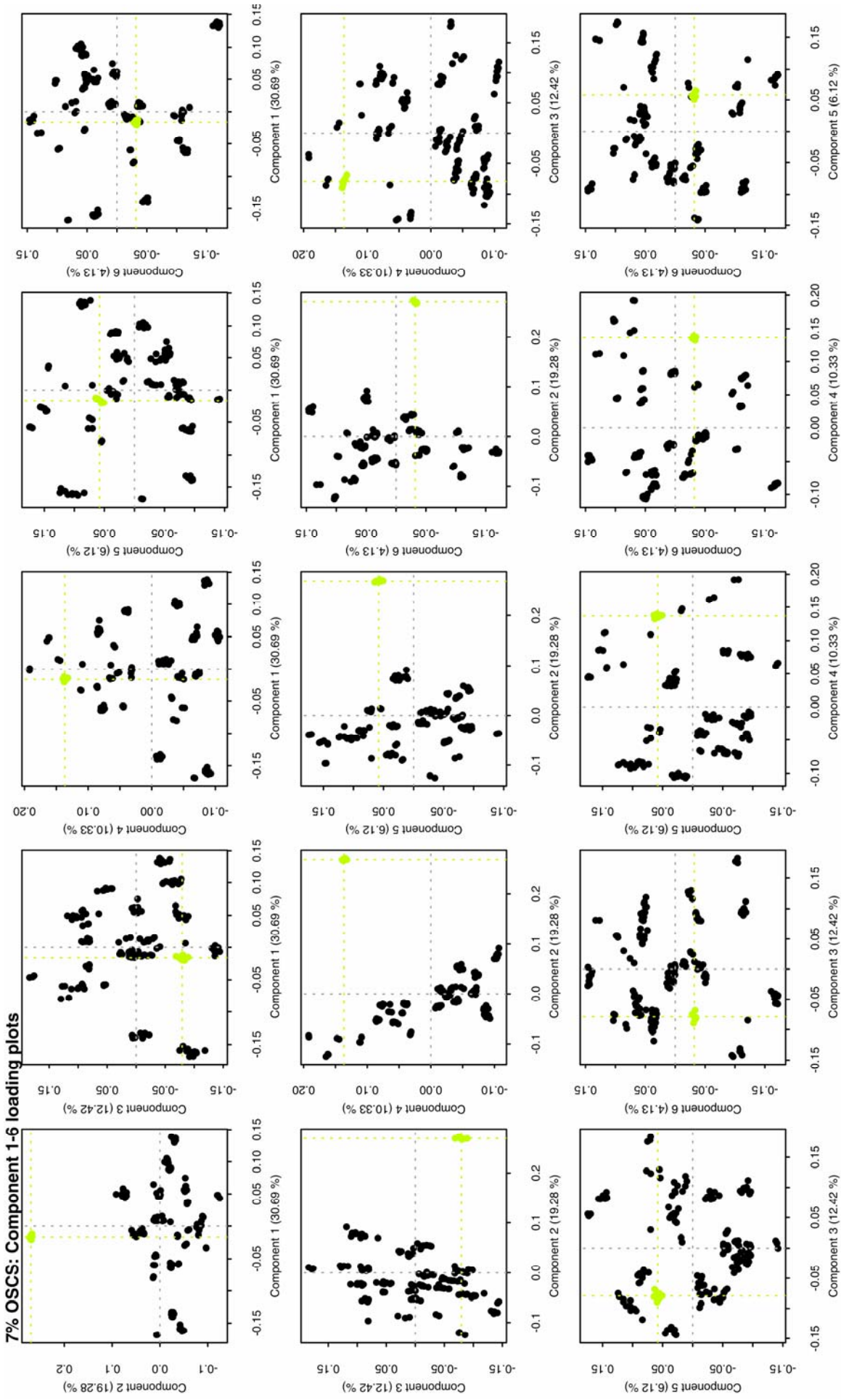

Figure 8 Combinatorial loading plots (Component 1-6) for the PCA of 7 % OSCS contaminated heparin and a heparin library.

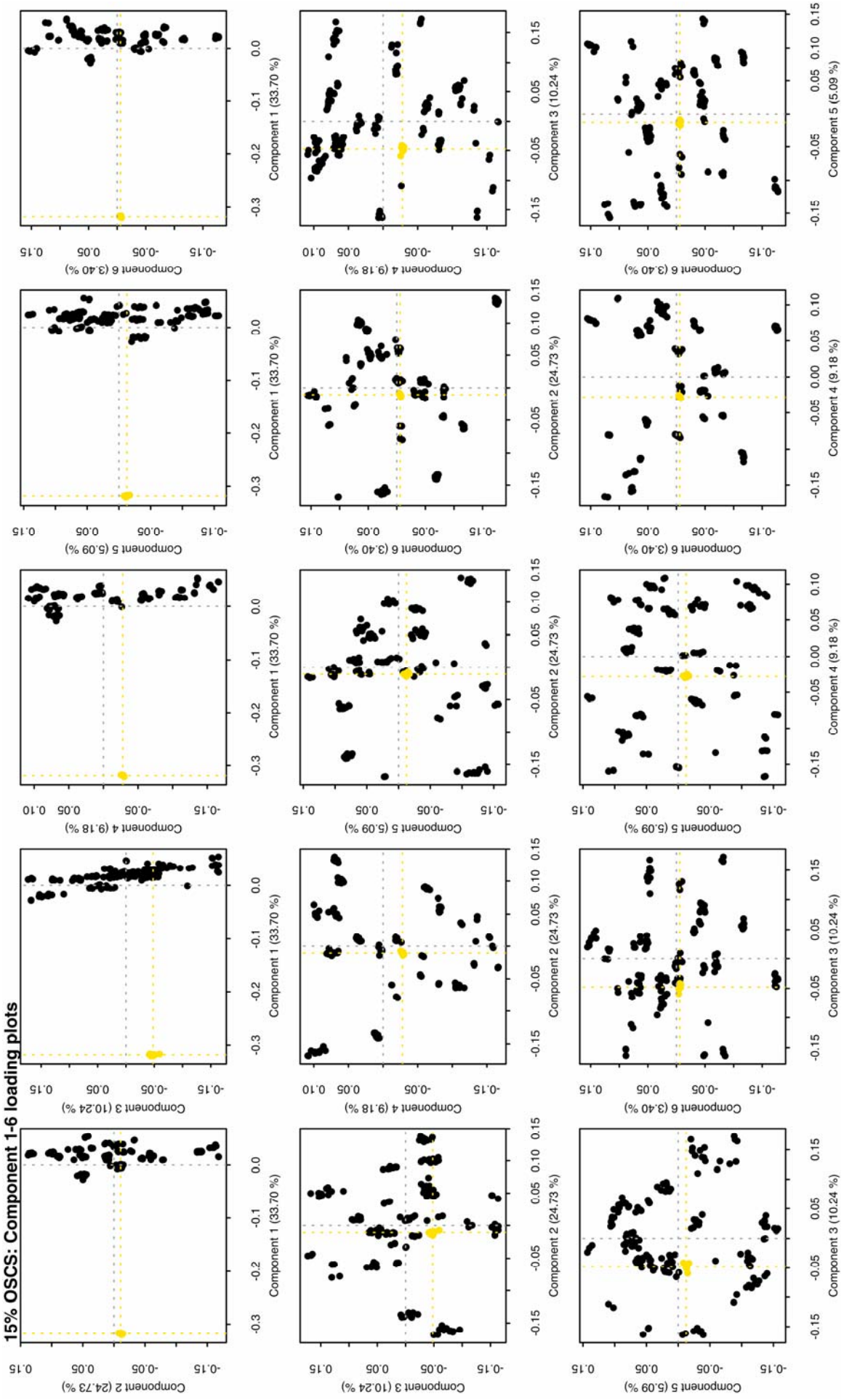

Figure 9 Combinatorial loading plots (Component 1-6) for the PCA of 15 % OSCS contaminated heparin and a heparin library.

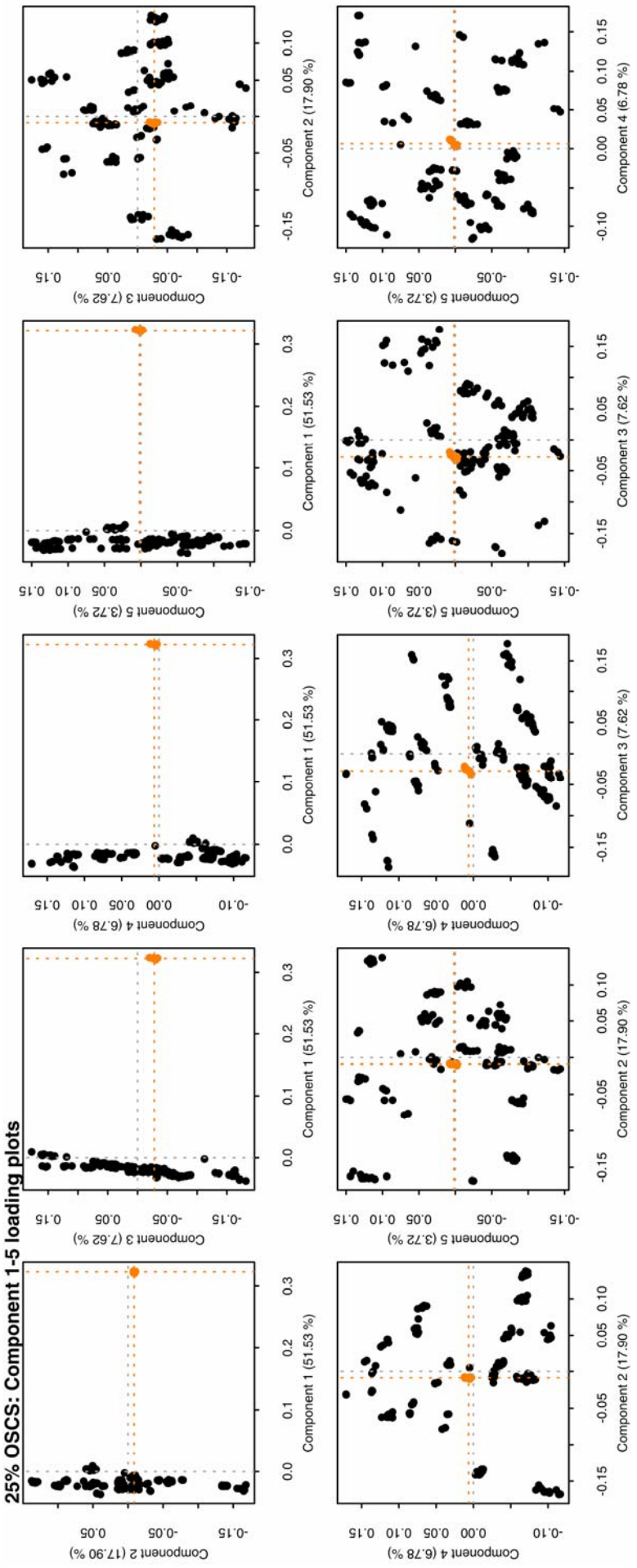

Figure 10 Combinatorial loading plots (Component 1-5) for the PCA of 25 % OSCS contaminated heparin and a heparin library

S4-2: OSCS sample one by one versus Heparin Library [N-Acetyl Region] Dataset was normalised to average spectral area, mean centered and autoscaled before PCA.

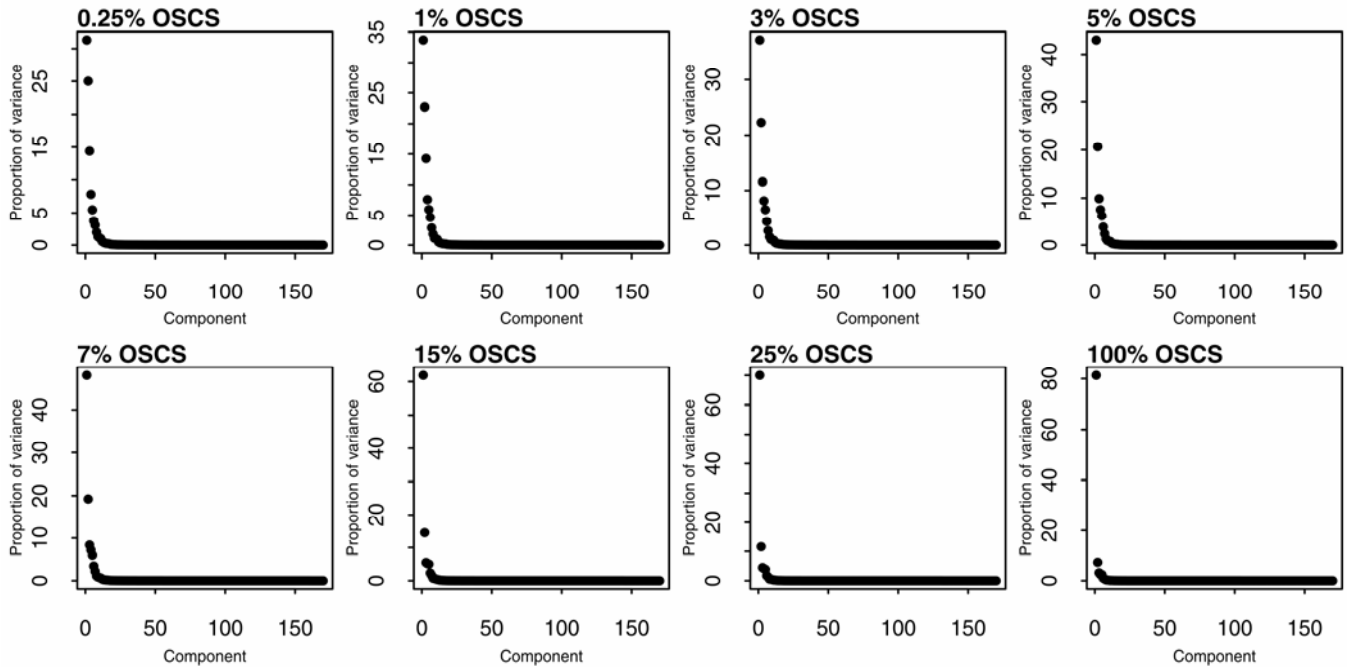

Figure 11. Scree plots derived from the PCA analysis of the OSCS samples with the heparin library, analysis was performed on the N-acetyl region only.

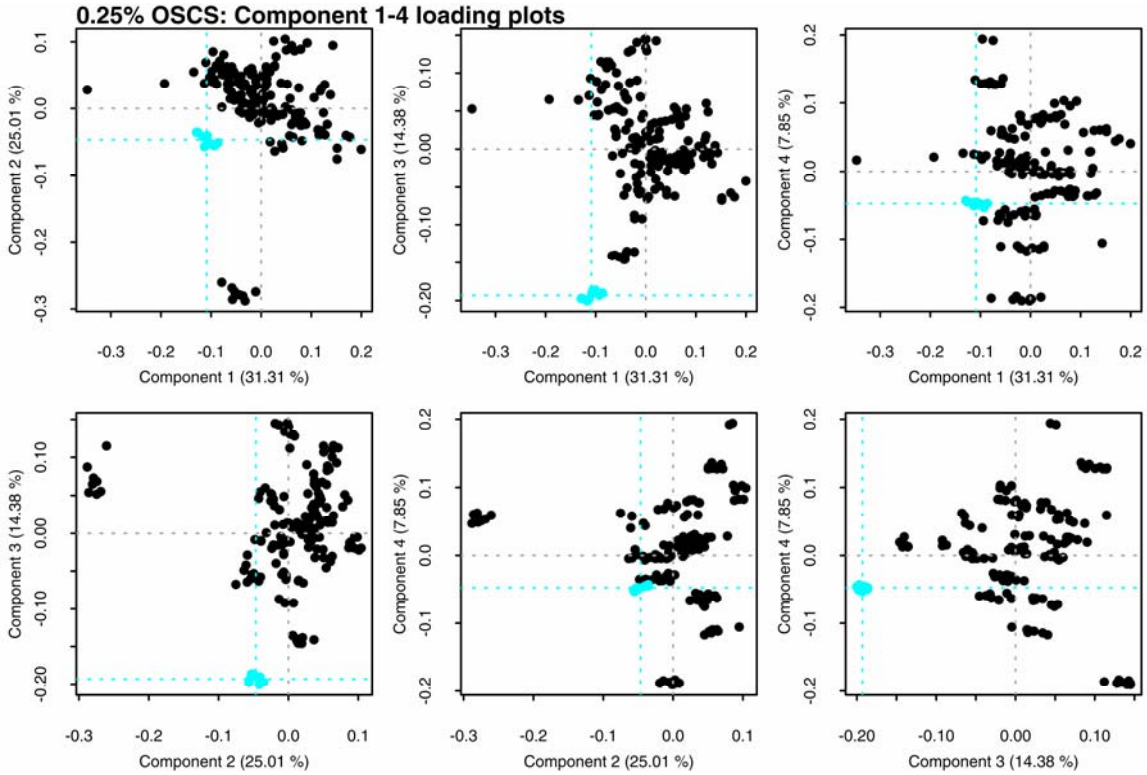

Figure 12. Combinatorial loading plots (components 1-4) derived from the PCA analysis of the OSCS samples with the heparin library, analysis was performed on the N-acetyl region only.

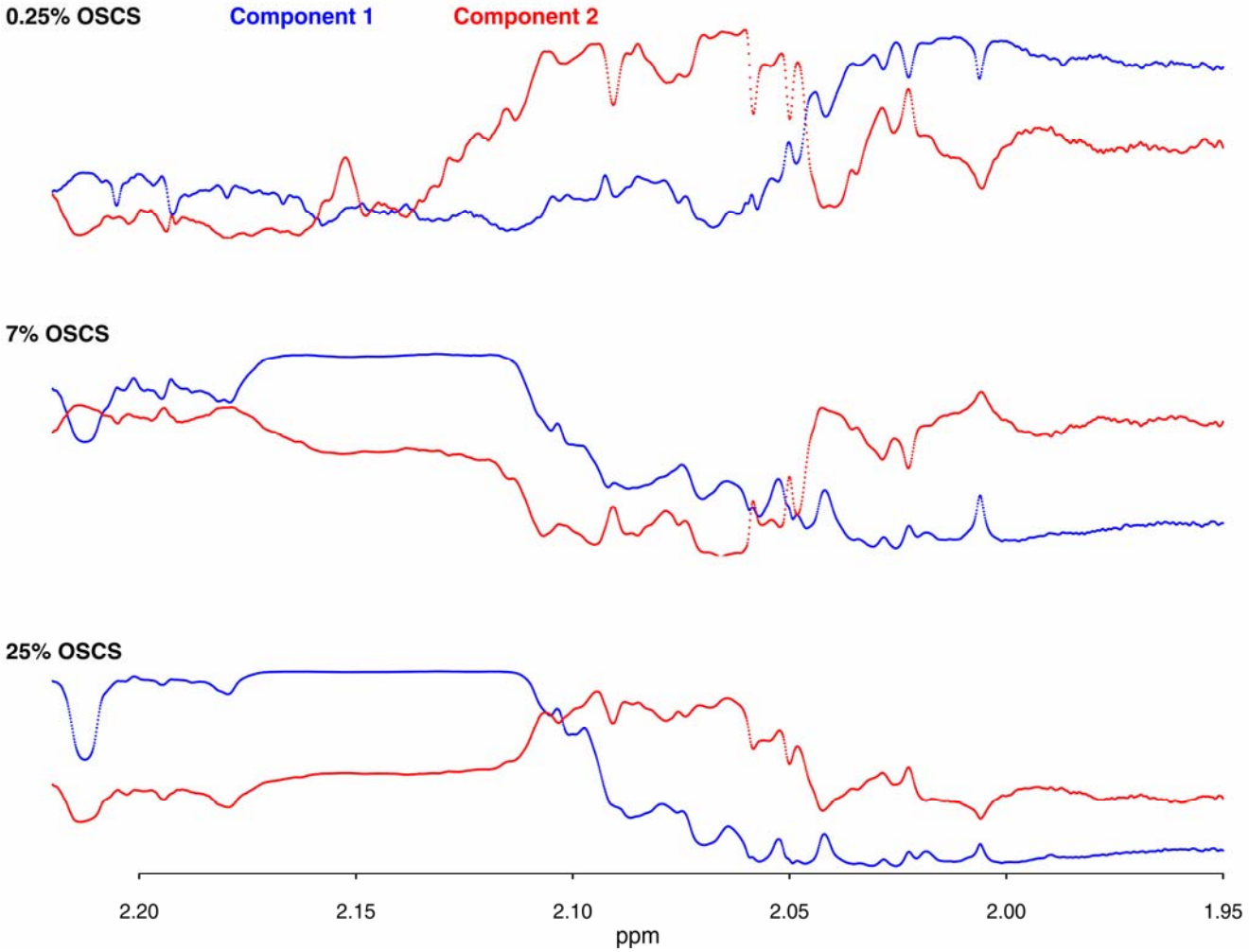

Figure 13. Component score plots derived from the PCA analysis of the OSCS samples with the heparin library, analysis was performed on the N-acetyl region only.

S5: OSDxS and OSAS PCA Dataset was normalised to average spectral area, mean centered and Pareto scaled before PCA.

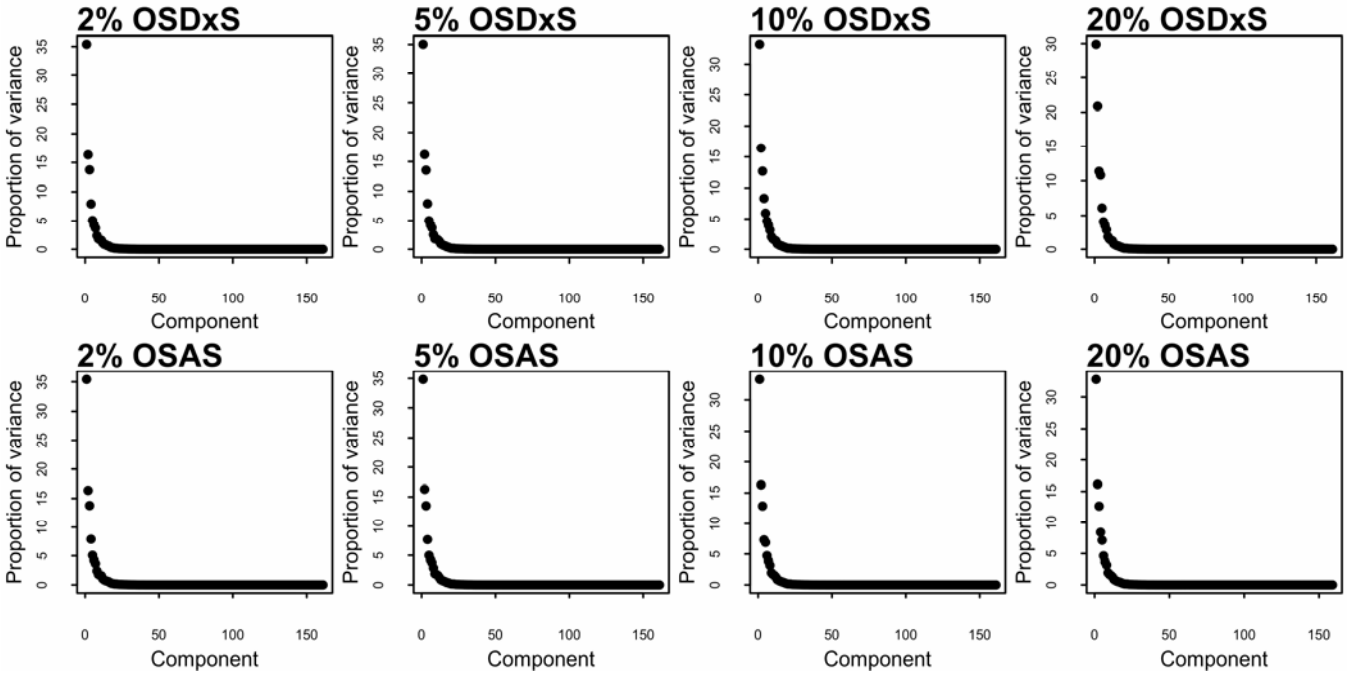

Figure 1. Scree plots for OSDxS and OSAS analysis

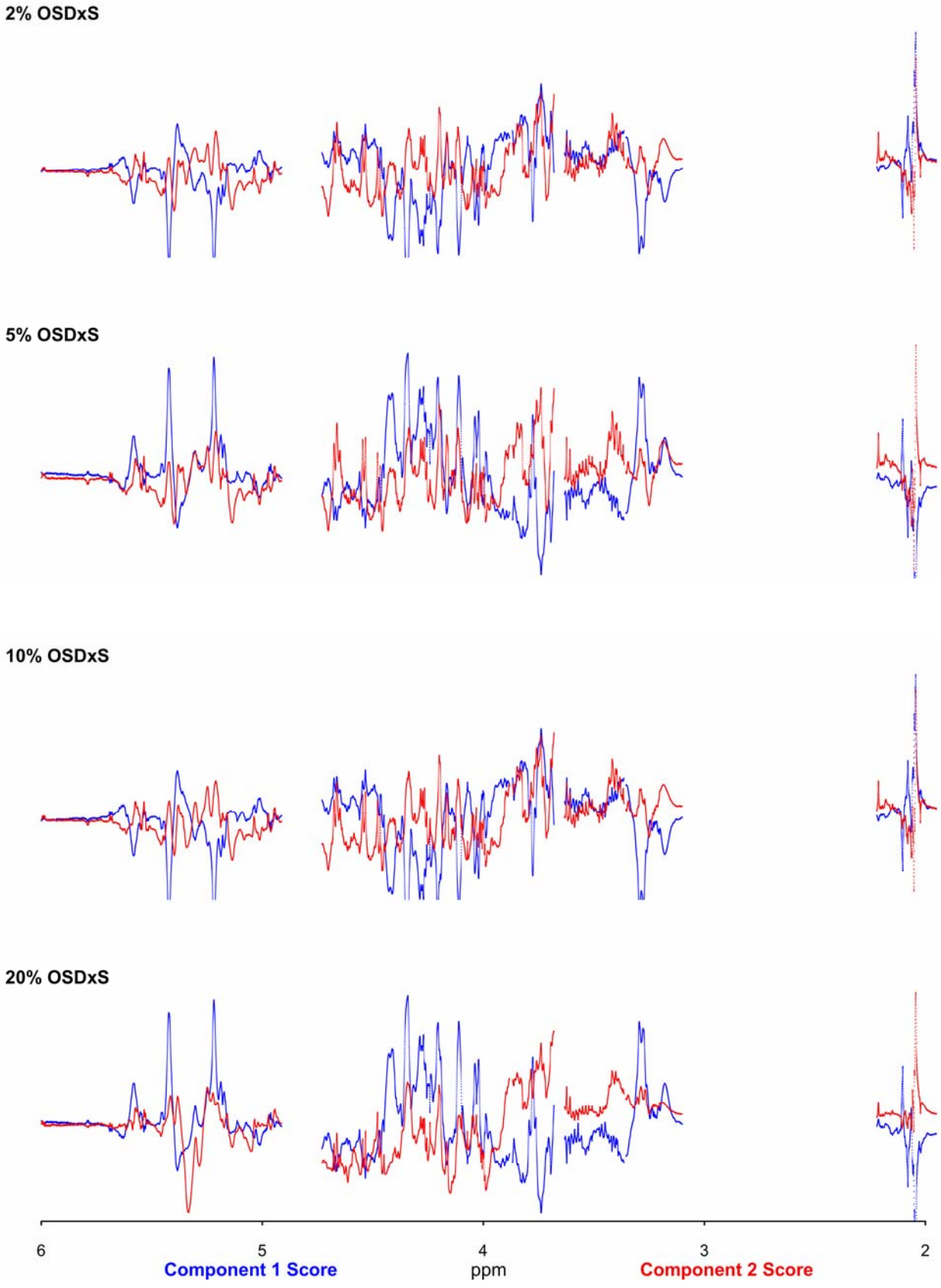

Figure 2. Score plots for OSDxS analysis

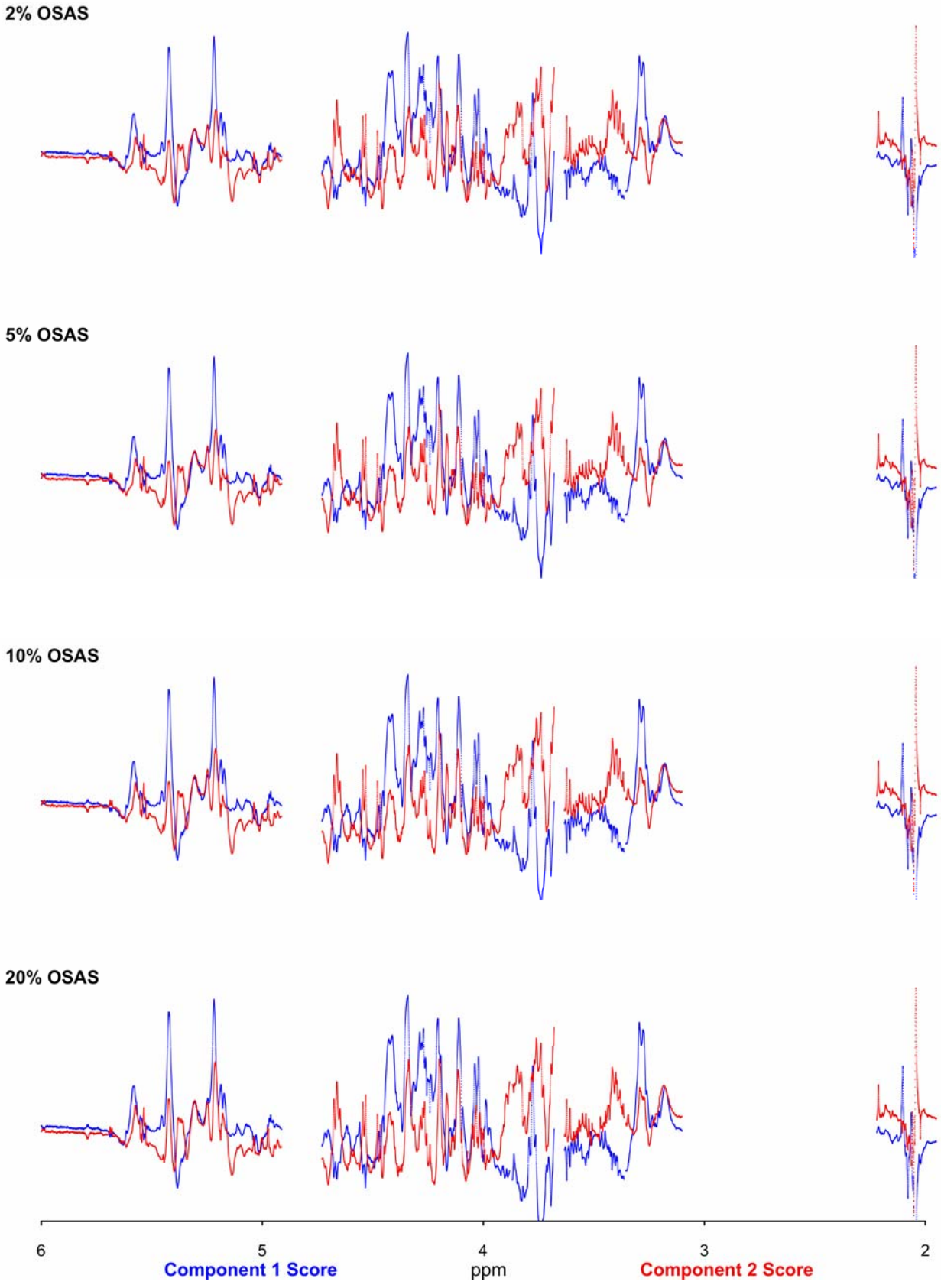

Figure 3. Score plots for OSAS analysis

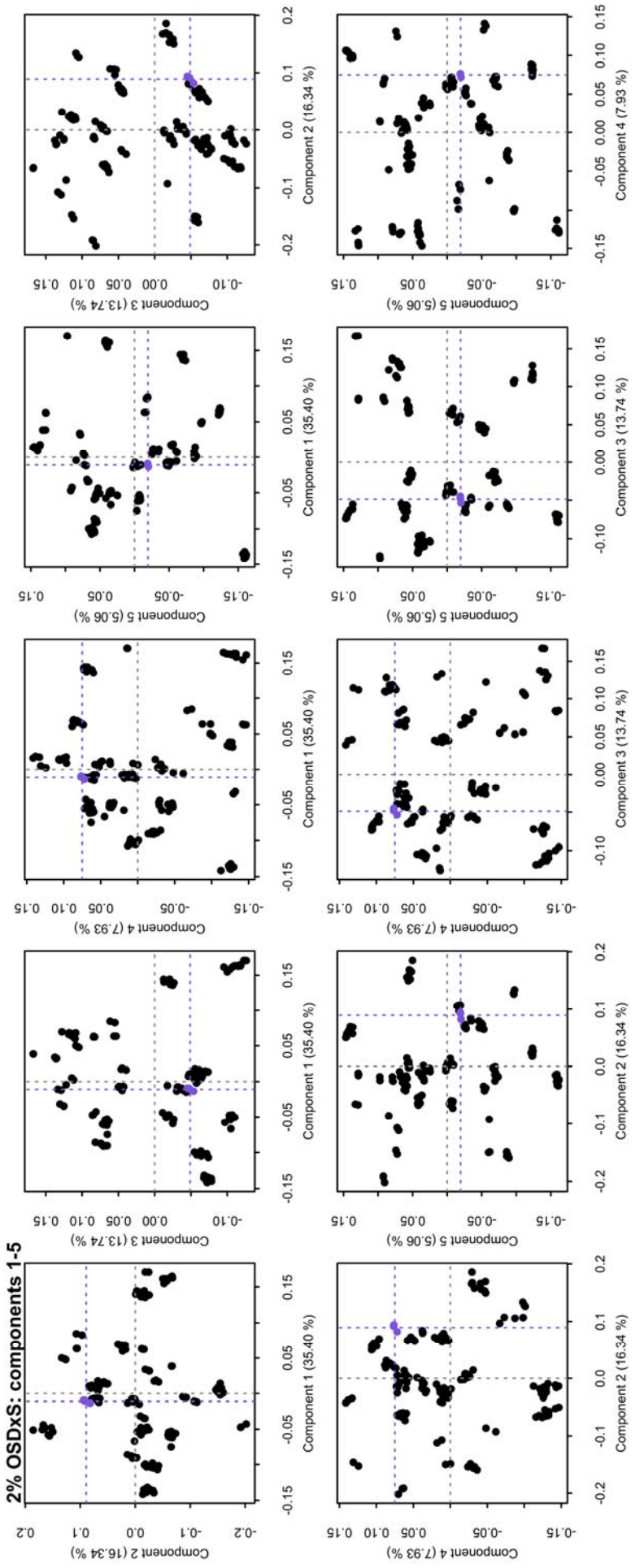

Figure 4 Combinatorial loading plots (Component 1-5) for the PCA of 2 % OSDxS contaminated heparin and a heparin library.

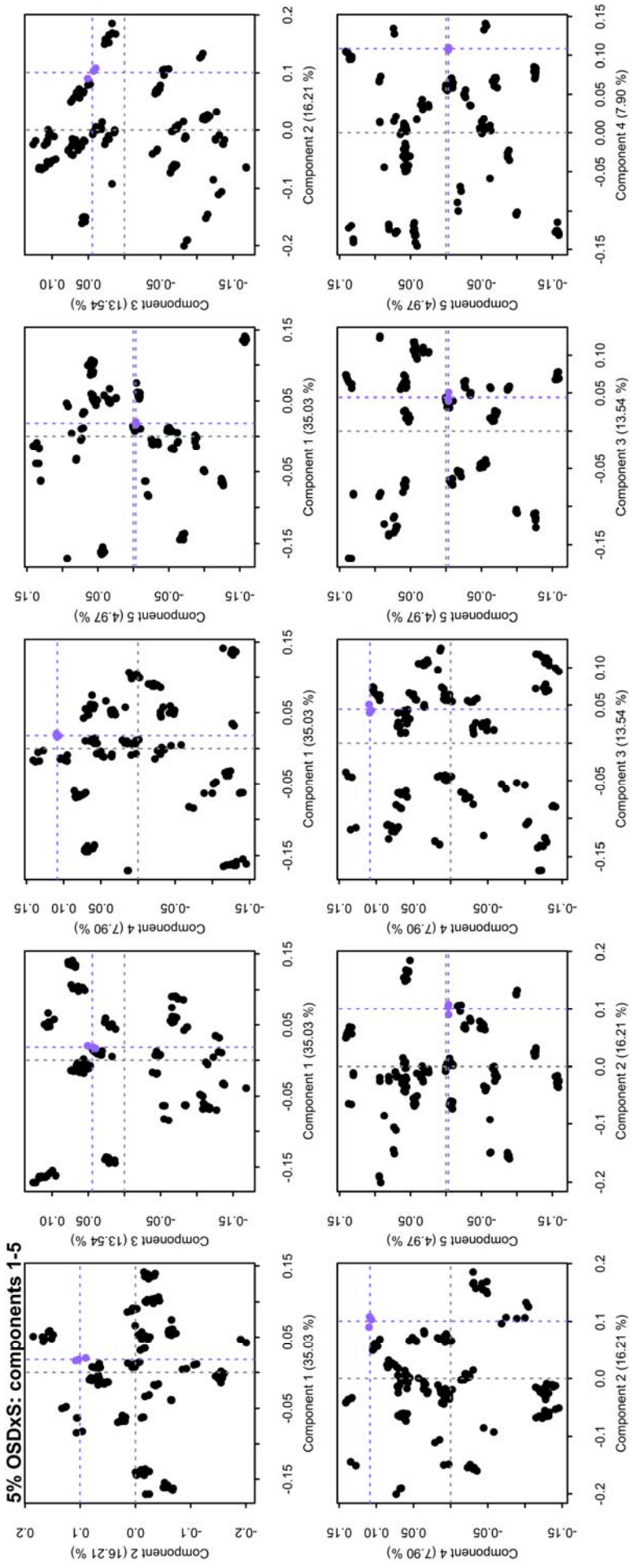

Figure 5 Combinatorial loading plots (Component 1-5) for the PCA of 5 % OSDxS contaminated heparin and a heparin library.

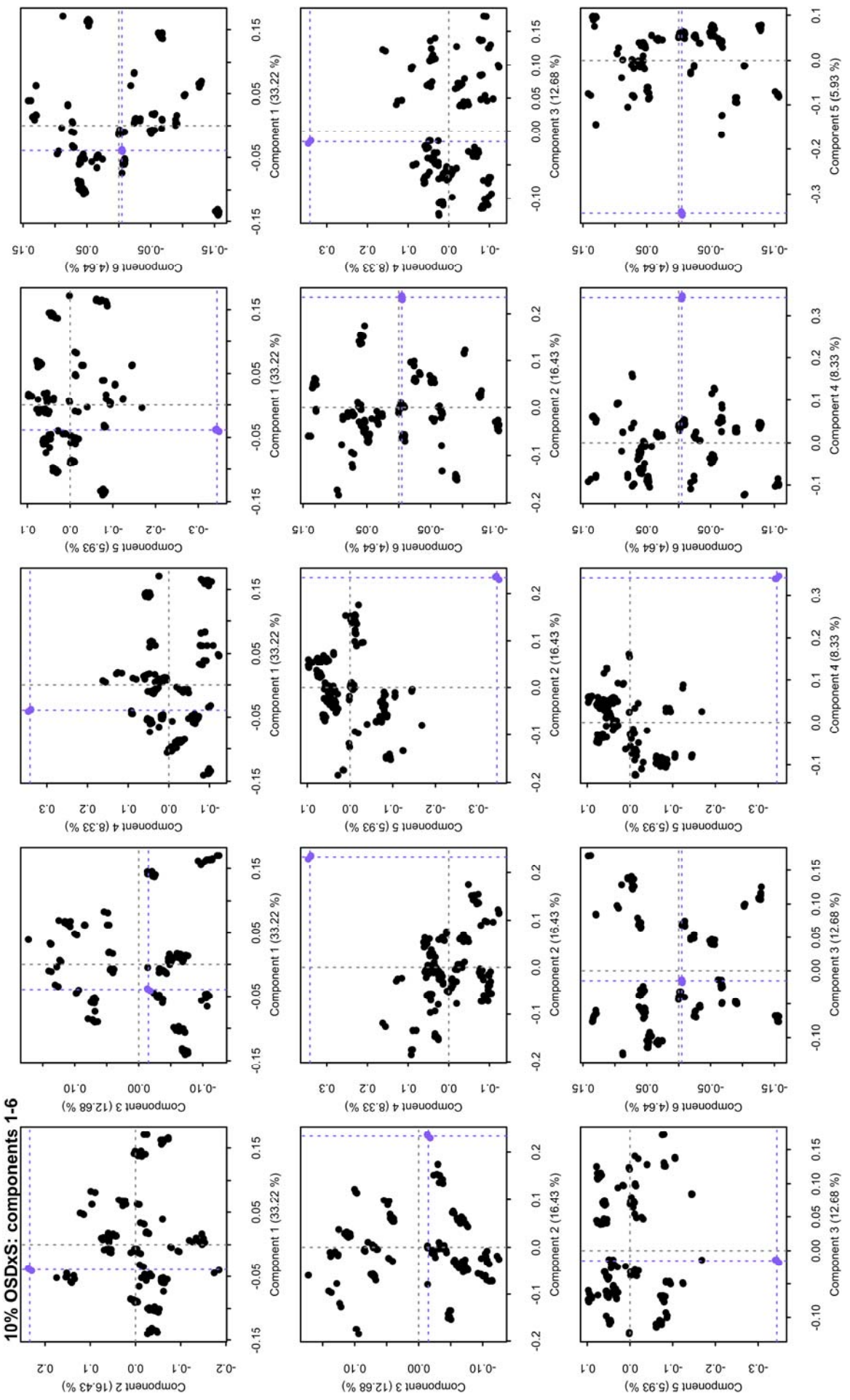

Figure 6 Combinatorial loading plots (Component 1-6) for the PCA of 10 % OSDxS contaminated heparin and a heparin library.

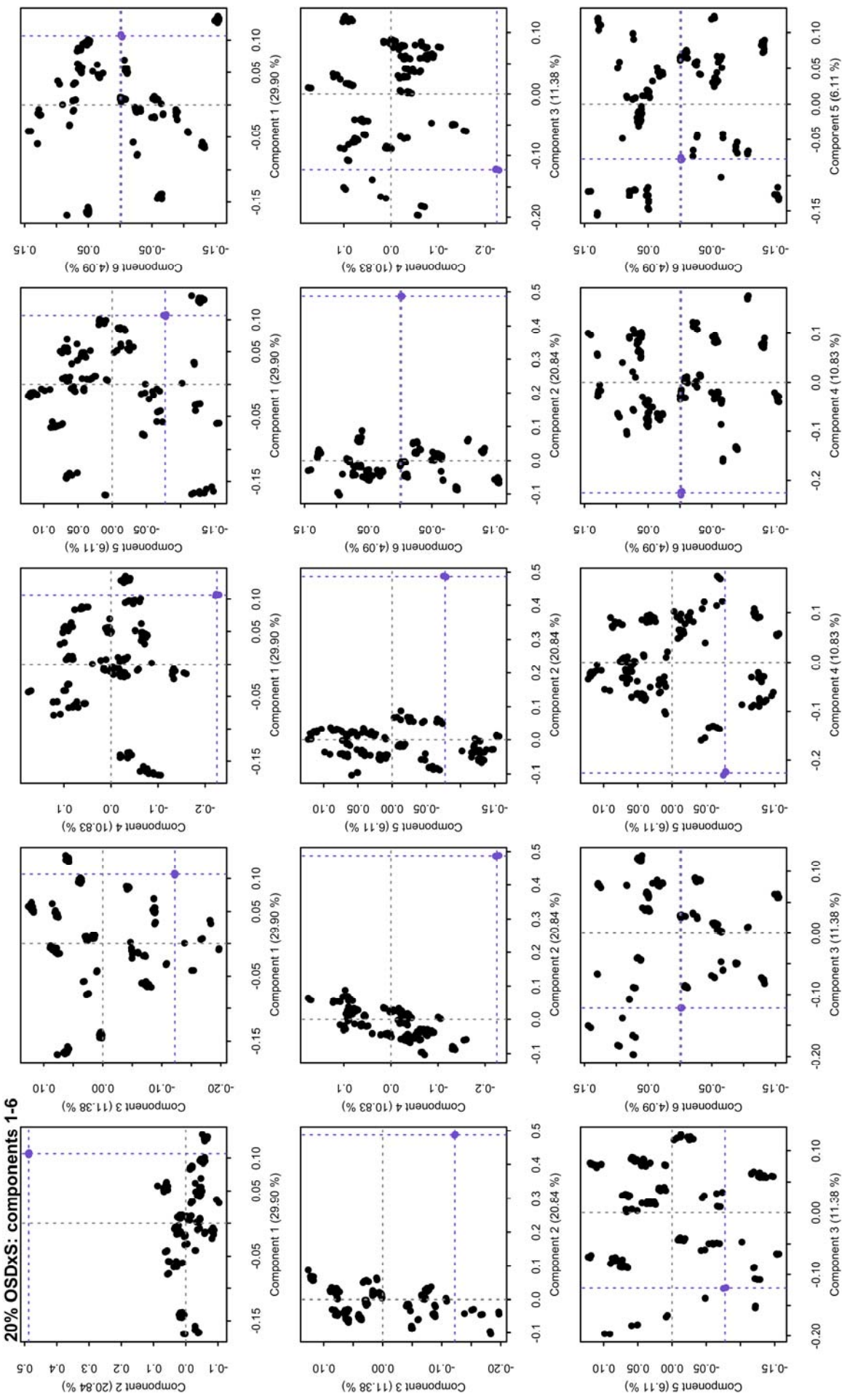

Figure 7 Combinatorial loading plots (Component 1-6) for the PCA of 20 % OSDxS contaminated heparin and a heparin library.

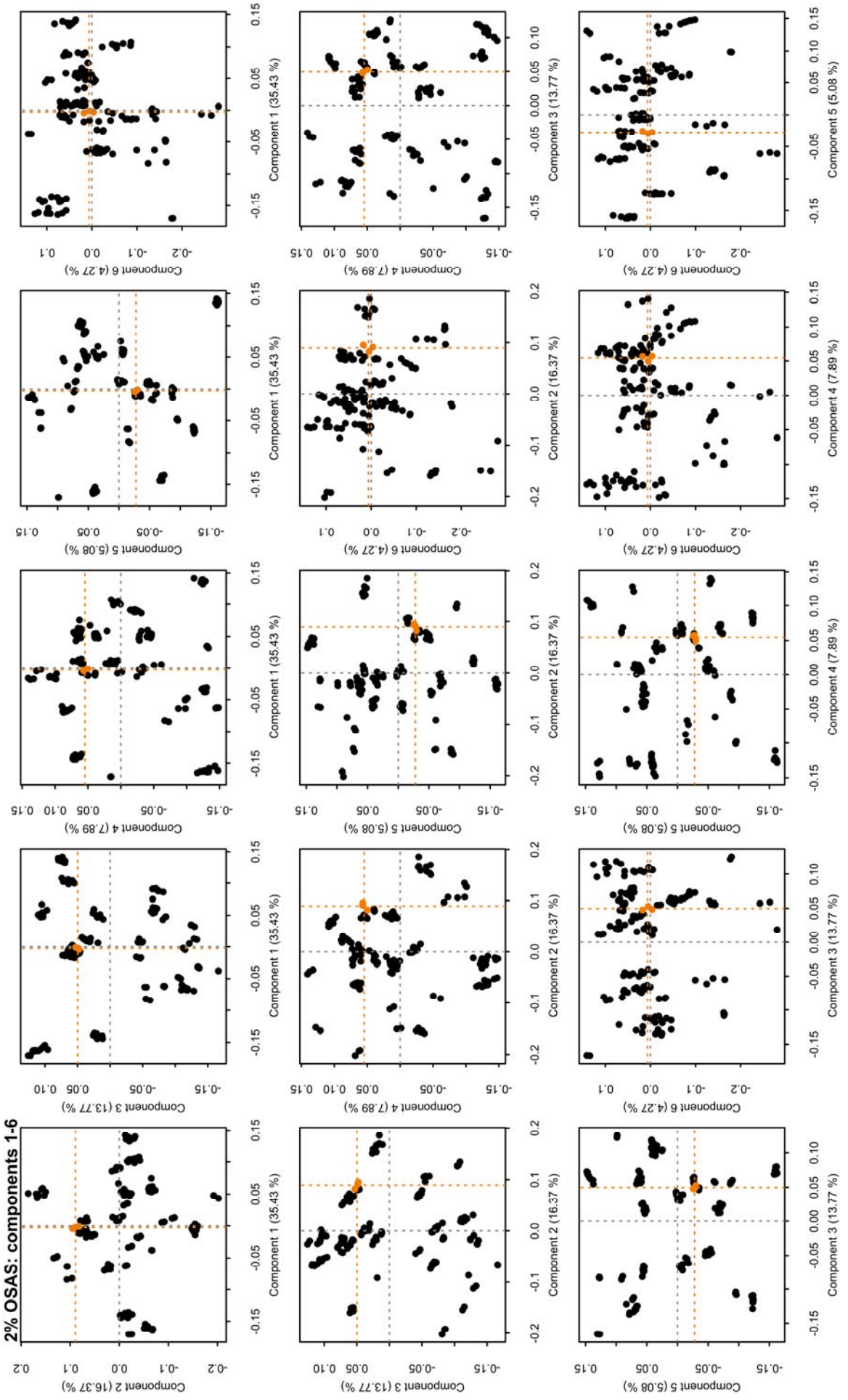

Figure 8 Combinatorial loading plots (Component 1-6) for the PCA of 2 % OSAS contaminated heparin and a heparin library.

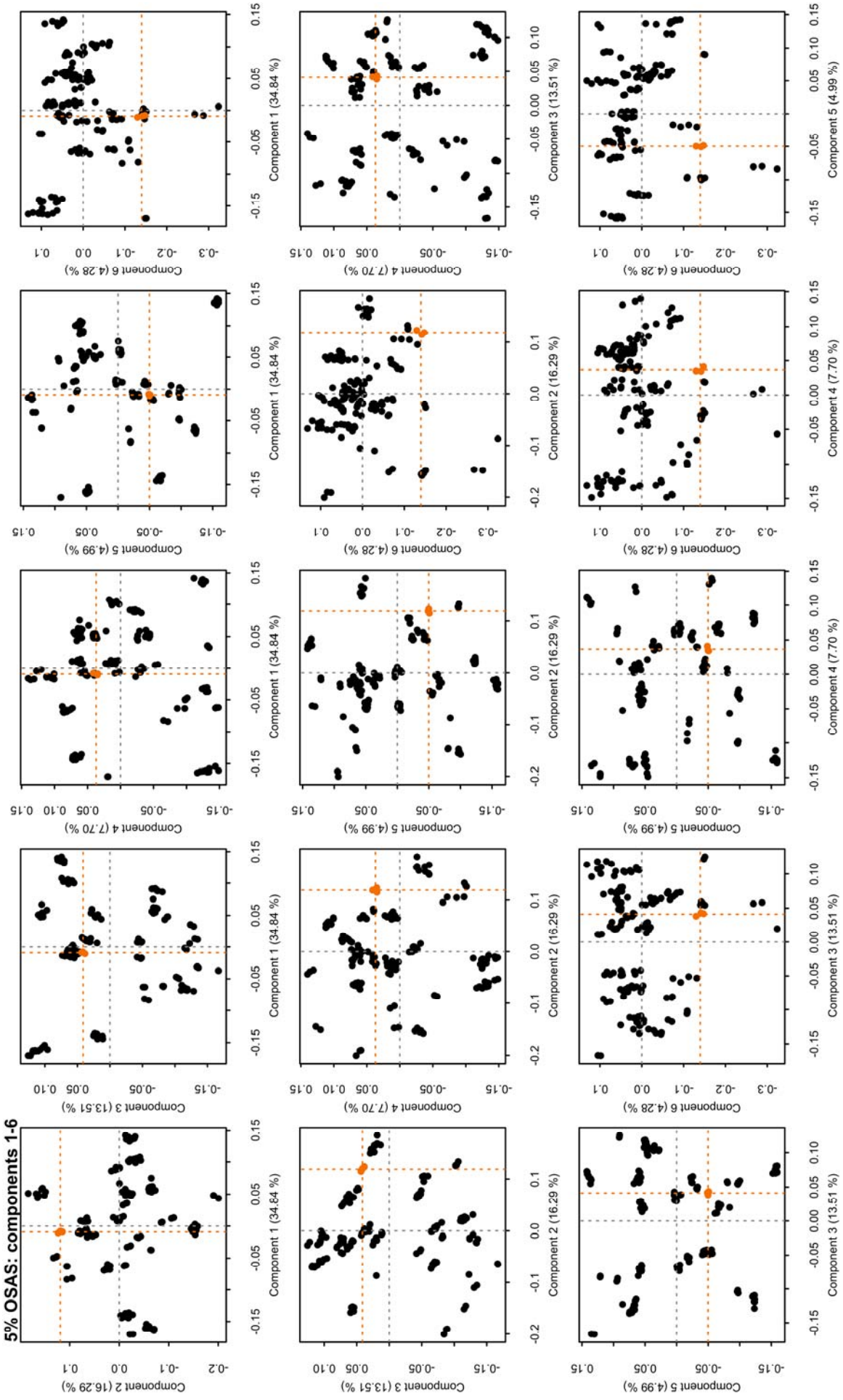

Figure 9 Combinatorial loading plots (Component 1-6) for the PCA of 5 % OSAS contaminated heparin and a heparin library.

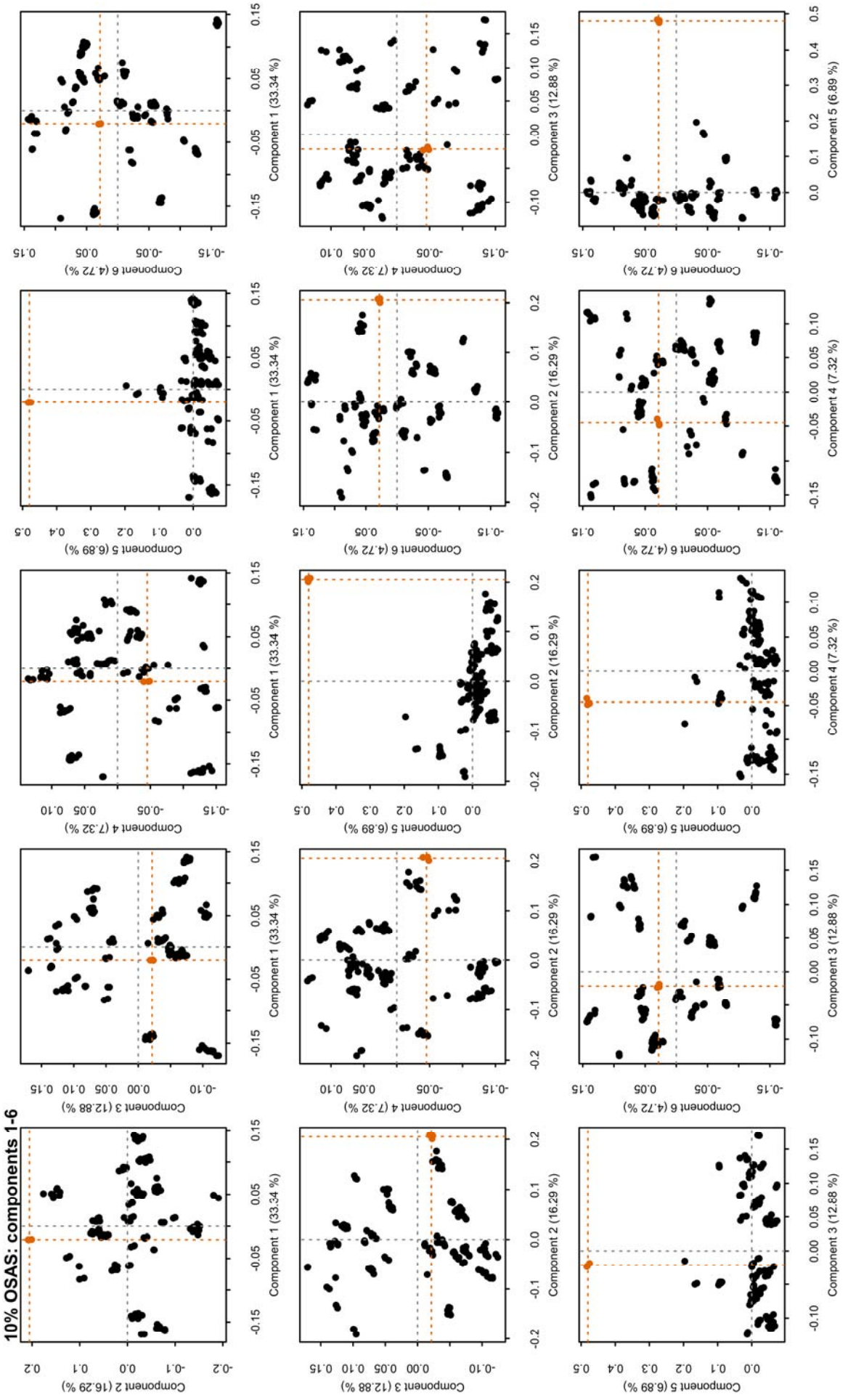

Figure 10 Combinatorial loading plots (Component 1-6) for the PCA of 10 % OSAS contaminated heparin and a heparin library.

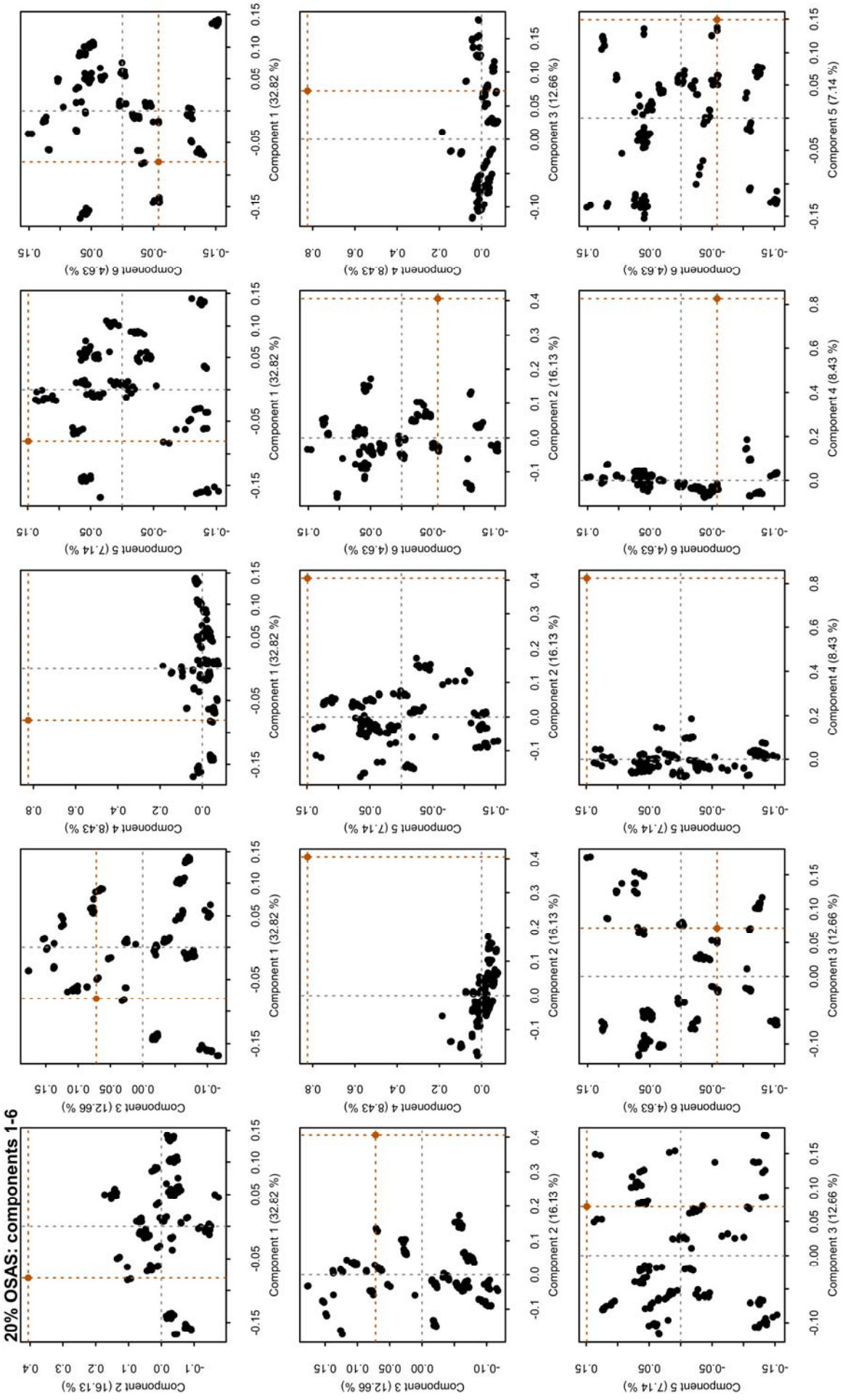

Figure 11 Combinatorial loading plots (Component 1-6) for the PCA of 20 % OSAS contaminated heparin and a heparin library.

S6: All OSCS versus Heparin Library Dataset was normalised to average spectral area and mean centered before PCA.

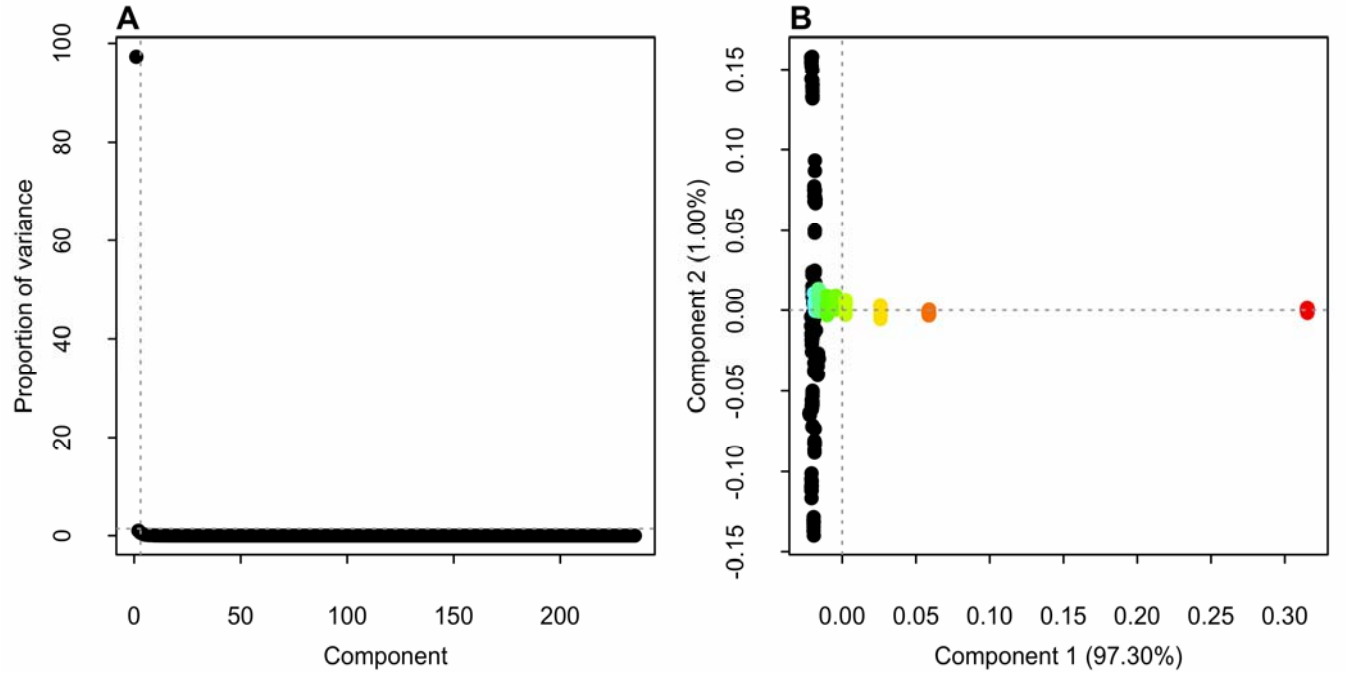

Figure 1. A. Scree plot and Loading plot of the PCA of All OSCS samples and the Heparin Library.

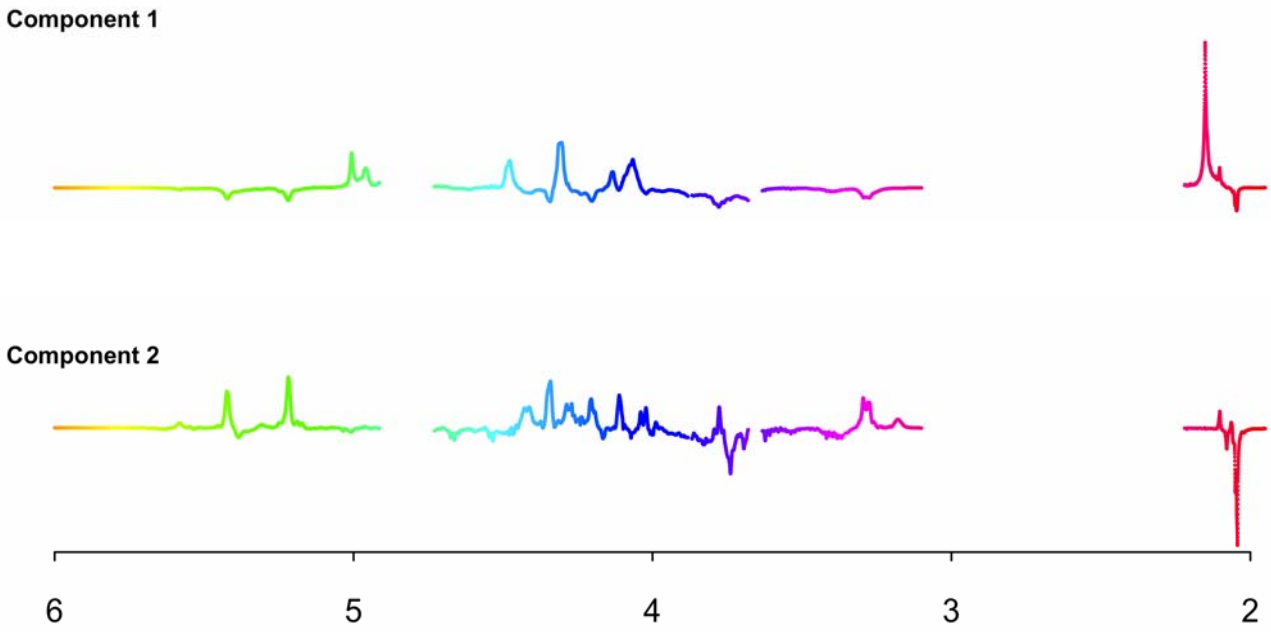

Figure 2. Component Score plots of the PCA of All OSCS samples and the Heparin Multiple Library.

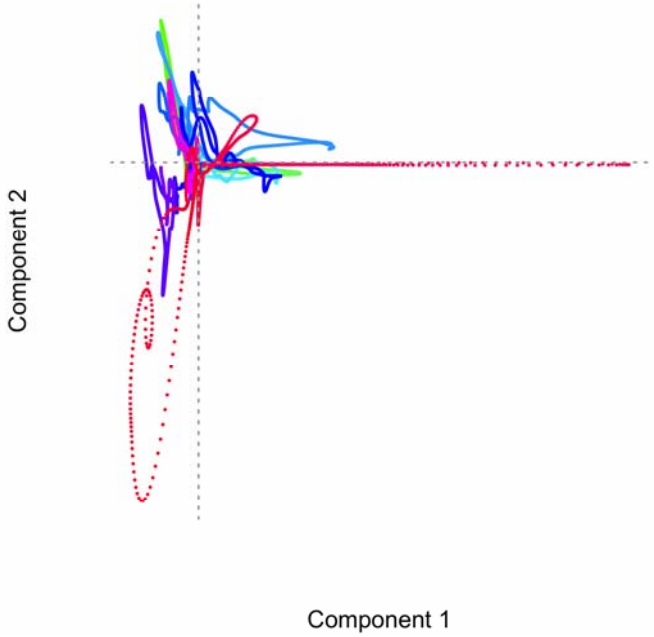

Figure 3. Component 1 Score versus Component 2 Score of the PCA of All OSCS samples and the Heparin Multiple Library.

S7: DS versus Heparin Library Dataset mean centered before PCA.

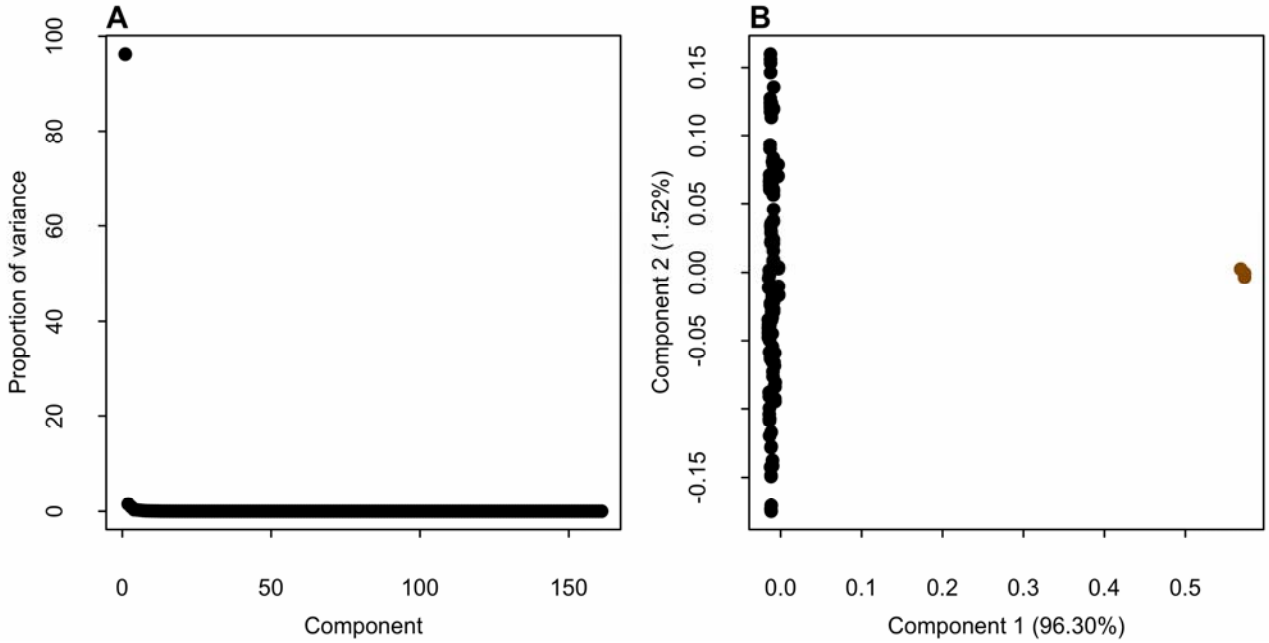

Figure 1. Scree plot (A) and Loading plot (B) for the PCA of DS (DS samples are brown in the loading plot) and heparin multiple library.

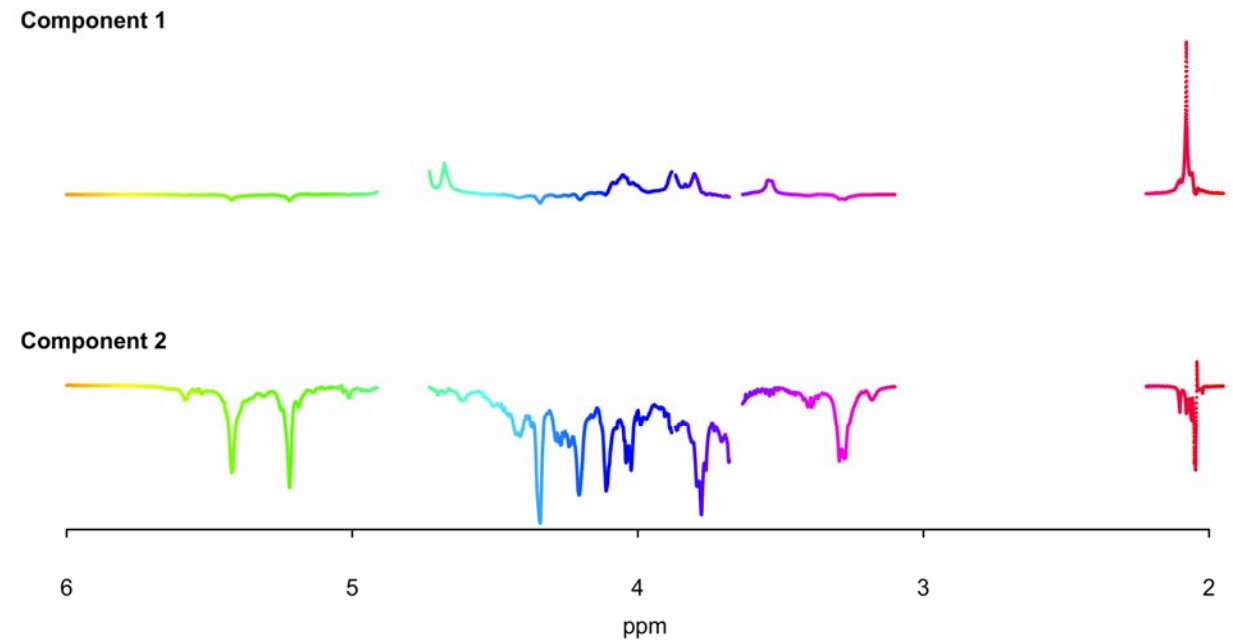

Figure 2. Component score plots for the PCA of DS and heparin multiple library.

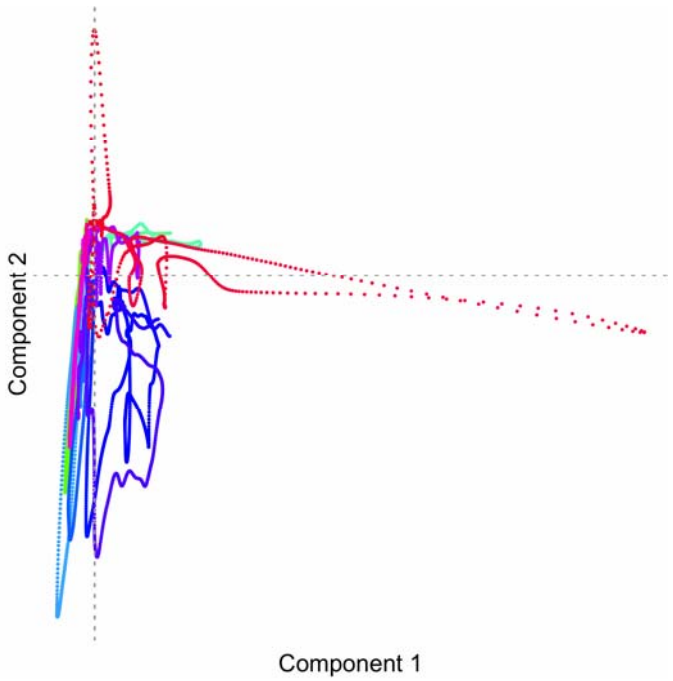

Figure 3. Component 1 Score v Component 2 Score for the PCA of DS and heparin multiple library.

S8: Bovine lung heparin (2nd international standard) versus Heparin Library Dataset was mean centered before PCA.

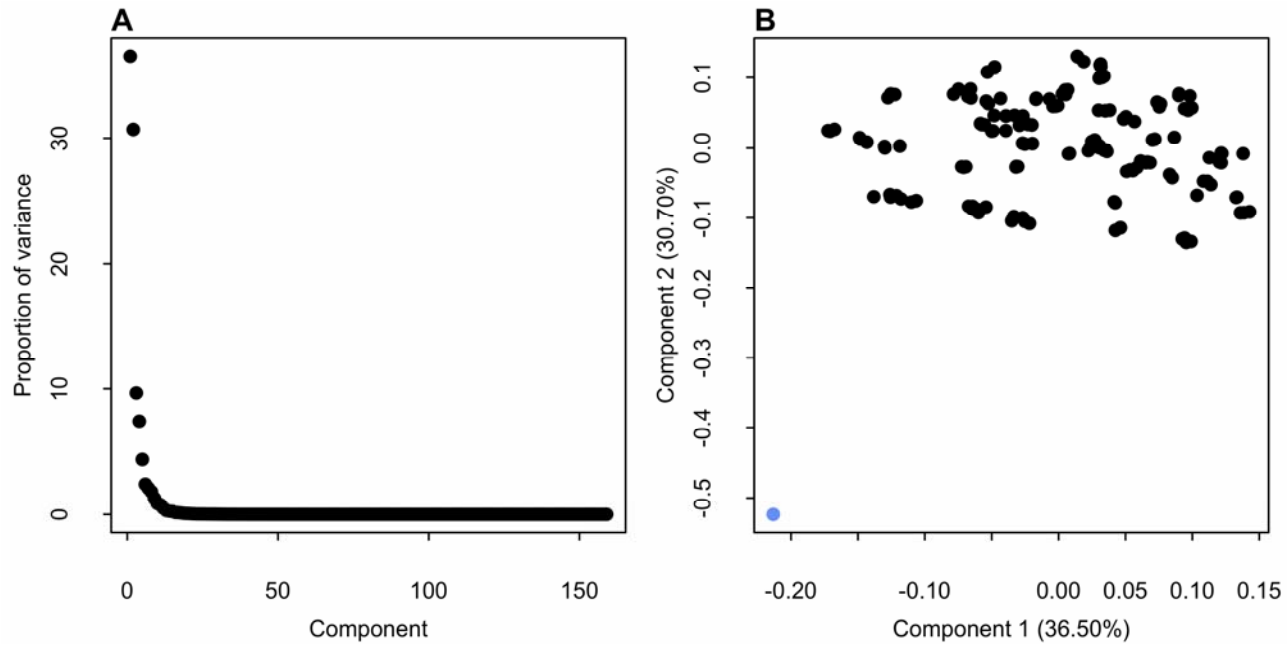

Figure 1. Scree plot (A) and Loading plot (B) for the PCA of bovine heparin (the bovine heparin sample is cornflower blue in the loading plot) and heparin multiple library.

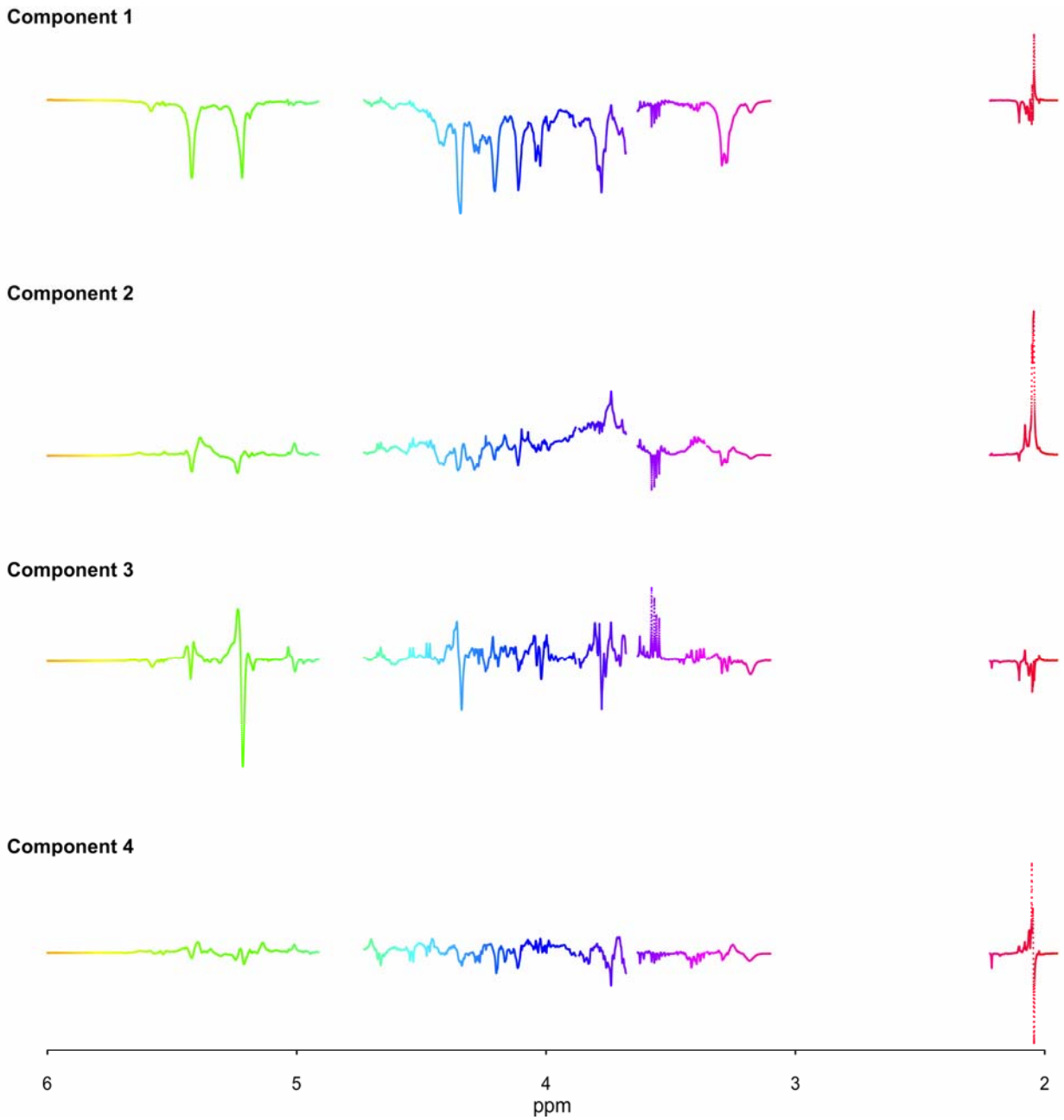

Figure 2. Component Score plots for the PCA of bovine heparin and heparin multiple library.

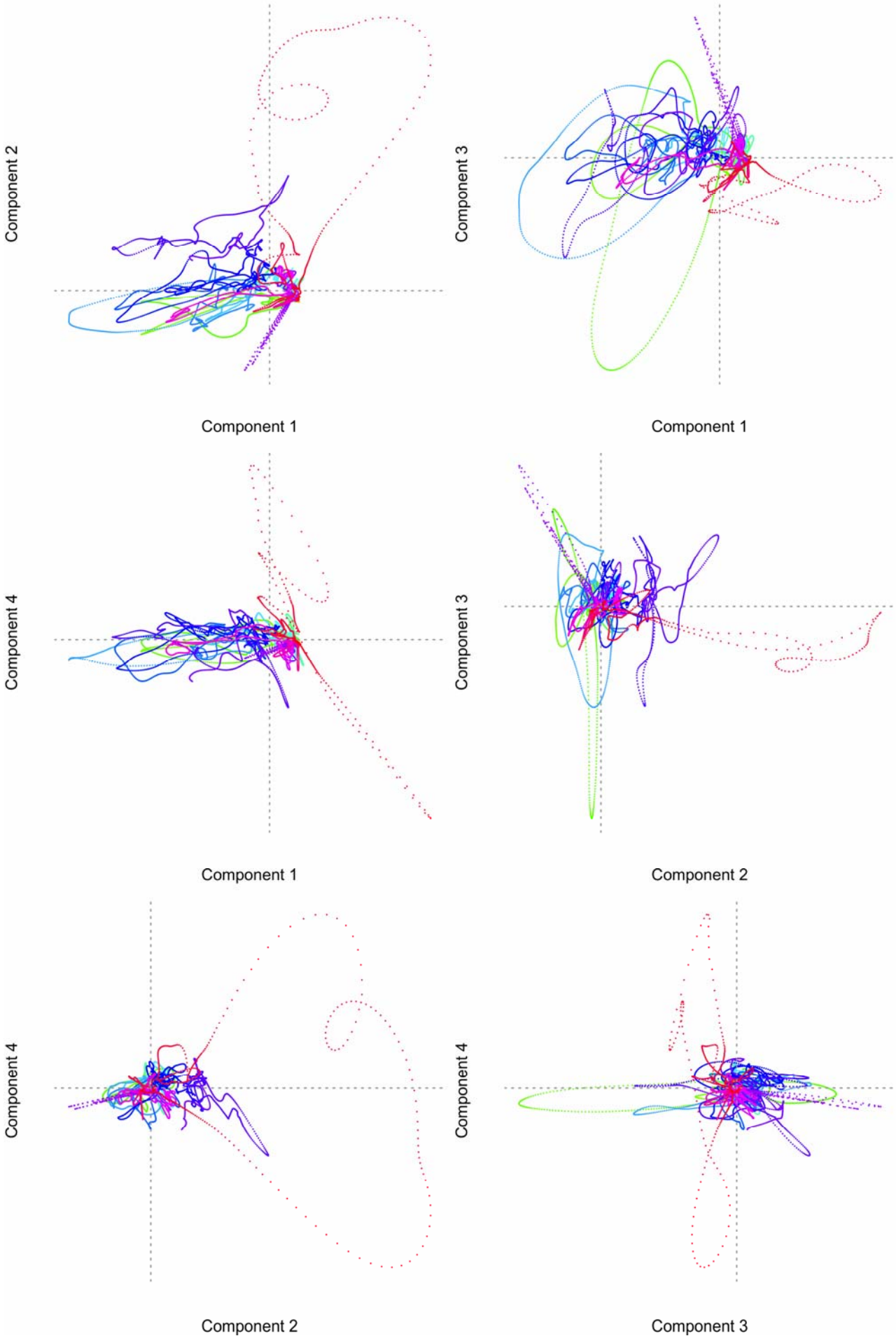

Figure 3. Component Score v Component Score plots for Component 1-4 the PCA of bovine heparin and heparin multiple library.