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Plasma Pencil Atmospheric Mass Spectrometry Detection of Positive Ions from Micronutrients Emitted from Surfaces

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Abstract

Analysis and detection of micronutrients is important for the reduction of the global burden of malnutrition-related disease. A relatively new technique, plasma pen atmospheric mass spectrometry (PPAMS) was applied in a comprehensive evaluation for rapid, simultaneous detection of the key micronutrients: zinc, iron, folate, vitamin A, and iodine. PPAMS was performed through the coupling of a low-temperature plasma pen to an atmospheric mass spectrometer (MS). The effectiveness of the PPAMS system was demonstrated through the generation of characteristic mass spectra and tandem mass spectra on neat micronutrient powders suspended on double sided tape. The analytical performance and ability to qualitatively separate out the nutrients from a complex biological solution and each other was then assessed through the application of PPAMS on a sample matrix of micronutrients in porcine plasma in which nutrient concentration is varied from high blood level concentrations (HBLCs) to low blood level concentrations (LBLCs). A multivariate software model, principal components analysis (PCA) was then used to qualitatively separate the fragments obtained by nutrient type. The resulting PCA scores plots of the positive ion spectra from each mixed sample showed excellent separation of HBLCs and LBLCs of single nutrients at the 95% confidence level.¹ The associated PCA loadings plots showed that key loadings could be attributed to the expected micronutrient fragments. The PPAMS technique was successfully demonstrated and compared with traditional MS techniques: time-of-flight secondary ion mass spectrometry (ToF-SIMS) and electrospray ionization mass spectrometry (ESI-MS). Separation of the nutrients at concentrations relevant for human blood-based nutrient detection was possible in both ESI-MS and PPAMS. However, only PPAMS could detect the nutrients at physiological concentrations from porcine plasma. ToF-SIMS could detect the nutrients from plasma solution but required 5× to 1000× higher concentrations of folate, vitamin A, and iodine to achieve adequate separation of the micronutrients via PCA.

INTRODUCTION

Micronutrient deficiencies persist as one of the major contributors to the global burden of disease. For this reason, interest in the measurement of certain key micronutrients in humans and food is intensifying. Conventional serum micronutrient concentration measurements are slow, complex and the cost for materials can run between \$5–10/measurement (cost of ELISA kits or autoanalyzer methods²) making them cost-prohibitive for large studies of

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SUPPORTING INFORMATION AVAILABLE

Additional analytical and spectral characterization data from the ToF-SIMS and ESI-MS experiments and an expanded description of the PPAMS device are included in the electronic supporting information file.

multiple analytes. Rapid, efficient micronutrient detection technology demands rapid sampling time, high sensitivity, analytical accuracy, and instrument portability. A device possessing all of these features could have a dramatic impact on global health by facilitating population-wide nutritional studies. However, to our knowledge there is currently no one technology that fulfills all of these requirements.

Mass spectrometry (MS) and MS-based methods are recognized as being among the most sensitive general purpose analytical methods with multiple features advantageous for the rapid and specific trace identification of specific organic chemical compounds. MS instrumentation is selective, broadly applicable and has high specificity.^{3–5} However, it is only with the fairly recent development of ambient MS ionization methods^{5–9} that MS could be applied without significant sample manipulation, which had previously limited the technique to the laboratory environment. Since its introduction, direct ambient ionization has yielded more than a dozen different ambient desorption ionization methodologies¹⁰ that have been applied to a wide variety of compounds such as peptides,^{11–14} proteins,^{9,12,14–17} explosives,^{8,18–24} and pharmaceuticals.^{8,9,15,25–28} Among this set, plasma pencil atmospheric mass spectrometry (PPAMS) is a technique that employs a low-temperature plasma probe (LTP-probe) for desorbing and ionizing species of interest from liquid or solid samples (see supporting information Figure S-1).^{13,26,28–30}

Described here is a study on the application of PPAMS for the detection of five key micronutrients: Vitamin A (in the form of retinol), iron, zinc, folate, and iodine (bound in thyroxine). For this study, the PPAMS LTP-probe was coupled to an ion trap MS and its sensitivity and specificity was assessed for each of the micronutrients individually, as well as in a physiologically-based model for blood plasma. Key ion fragments were obtained from neat micronutrient powders that aided in the characterization of the nutrients in methanol, bovine serum albumin (BSA) and porcine blood plasma matrices. The ion fragments obtained were in excellent agreement with corroborating experiments conducted with time-of-flight secondary ion mass spectrometry (ToF-SIMS) and electrospray ionization mass spectrometry (ESI-MS) experiments. Furthermore, PPAMS data were obtained on porcine blood plasma solutions in which micronutrients were doped to levels modeling artificially healthy and unhealthy individuals. Experiments aimed at identifying and separating out the individual micronutrients were conducted through the use of the multivariate statistical modeling method, principal components analysis (PCA), on the spectra resulting from the physiological models.

EXPERIMENTAL SECTION

Chemicals and Reagents

The micronutrients investigated are listed in supporting information, Figure S-2, along with their molecular weights and formulas. These nutrients consisting of folic acid (FA, $C_{19}H_{19}N_7O_6$), retinol (Ret, $C_{20}H_{30}O$, analog for vitamin A), thyroxine (Thyr, iodine bound to a physiologic carrier, $C_{15}H_{11}I_4NO_4$), iron (Fe, prepared from $FeCl_2$ salt), and zinc (Zn, prepared from $ZnCl_2$ salt) were acquired as dry crystalline powders from Sigma-Aldrich Chemical Co. (St. Louis, MI) and used as received. In the case of folic acid and retinol (which are not water soluble), stock solutions were prepared by dissolving the powders in dimethylsulfoxide (DMSO, Sigma-Aldrich, Milwaukee, WI) and ethanol (EtOH, Mallinckrodt Baker Inc., Phillipsburg, NJ), respectively. The final concentrations were 0.5 mg/mL FA:DMSO, and 0.65 mg/mL Ret: EtOH. The nutrients could then be further diluted to their desired concentrations with aqueous solvents. Deionized/distilled water (dH_2O) was obtained from a Barnstead/Thermolyne deionizer unit (Nanopure, 18M Ω -cm resistivity, Dubaque, IA). Bovine serum albumin (BSA, A-7638, Sigma, St. Louis, MO) was purchased

and used as an initial analog for blood. Porcine plasma (PL26009, Innovative Research, Novi, Mi) was used as our blood model for PPAMS testing.

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)

ToF-SIMS data was obtained with a TOF.SIMS 5–100 time-of-flight spectrometer (ION-TOF, Münster, Germany) as described in supplemental information. As positive spectra produced the strongest data trends, only positive ion ToF-SIMS data was presented in this study. The resulting spectra were analyzed with the Surface Lab 6 software package from ION-TOF. Peak lists were constructed starting with a base of protein-related peaks adapted from Brown et. al.,^{31,32} and were supplemented with nutrient-related peaks. Nutrient-related peaks were verified either by: (1) the presence of the peak in the nutrient sample and absence of the peak in the control sample or (2) a peak whose intensity was proportional to nutrient concentration.

Electrospray Ionization Mass Spectroscopy (ESI-MS)

To verify that the MS to be utilized in the future PPAMS experiments could measure the micronutrients in a physiologically relevant range, ESI-MS was performed. Positive ion electrospray MS and MS/MS spectra were obtained on a Bruker-Esquire LC-ion trap MS (Bruker/Hewlett-Packard, Billerica, MA). Samples were infused by flow injection at 1.5 $\mu\text{L}/\text{min}$ via a syringe pump (Cole Parmer Model 74900) and ionized in a standard orthogonal Bruker ionizer. MS equipment settings are available in supporting documentation. Mass spectra were obtained by ejecting trapped ions in the range of m/z 50–1100 for all samples. Approximately 100 scans were accumulated and averaged to provide the spectra used for quantification. Mass assignments were determined from spectra using Bruker data analysis software.

Plasma Pencil Atmospheric Mass Spectrometry (PPAMS)

Experiments were performed on a Bruker-Esquire LC-ion trap MS (Billerica, MA). As with the ESI-MS, data was acquired and analyzed with the associated Bruker software. PPAMS was performed in the positive and negative-ion mode on pure micronutrient stock powders. As positive mode yielded the best data, only positive ion PPAMS data are presented in this study. The spectrometer was programmed to collect spectra for a maximum ion trap injection time of 200 ms with 2 microscans per spectrum. The scans were averaged over 30 seconds of acquisition time. An in-house low-temperature plasma probe (LTP-probe) was constructed as described elsewhere^{26–30} for the generation of an atmospheric plasma at low temperatures ($\sim 30^\circ\text{C}$). This instrument enables the analysis of samples without visibly noticeable sample decomposition or destruction. A detailed description of the LTP-probe can be found in supporting information.

Principal Component Analysis (PCA)

A multivariate analysis technique, PCA,²⁶ which captures the linear combination of peaks that describe the primary sources of variance in a given dataset (known as principal components, PCs) was employed to analyze the resulting spectral data using a Matlab (The MathWorks, Inc., Natick, MA) program written in-house.¹ For ToF-SIMS data, initially, a complete peak set was created for data analysis which included all peaks whose intensities were > 100 counts for $m/z < 100$, > 50 counts for m/z between 100–200, and > 5 counts for $m/z > 200$. Then, to further analyze the data, the peak list was reduced to include only the protein and nutrient peaks as described in the ToF-SIMS section. For all other data, depending on the experimental protocol, either the entire spectra or chosen peak sets were normalized to the sum of the selected peaks to account for fluctuations in yield between spectra, while attempting to reduce the influence of background noise on the analysis. PCA

was performed using the NESAC/BIO MVA Toolbox (Seattle, WA) for MATLAB (the MathWorks, Inc., Natick, MA). All spectra were mean-centered before running PCA. Further data treatment prior to PCA is described as needed below.

RESULTS AND DISCUSSION

Time-of-Flight Secondary Ion Mass Spectrometry with Principal Component Analysis of Spectral Features of Micronutrients

To establish that the PPAMS system could detect micronutrients at appropriate physiological concentrations, we prepared standard mixtures of the five micronutrients of interest and analyzed those mixtures using ToF-SIMS. A sample preparation protocol was developed for these corroborative experiments with standard concentrations of the micronutrients dissolved in a 1 mg/ml bovine serum albumin (BSA) dH₂O solution. The standard concentrations were based on adding 100% of the recommended daily allowance (RDA) of each nutrient in 1 cup of protein solution to simulate nutrient detection in a food source. The final RDA concentrations used were 1.7 ppm FA, 3.8 ppm Ret, 625 ppb Thyr, 75 ppm Fe and 46 ppm Zn. A secondary preparation protocol was developed based off of the concentrations of nutrients expected in the blood of an adult human. These samples were based on the HBLCs expected in human blood and were also prepared in a 1 mg/ml BSA/dH₂O solution. The final HBLCs used were 50 ppb FA, 650 ppb Ret, 105 ppb Thyr, 2 ppm Fe and 20 ppm Zn. A 10 μ L droplet of each solution was pipetted onto a 12 mm diameter, clean glass coverslip and allowed to dry overnight in a vacuum desiccator prior to analysis.

ToF-SIMS experiments were performed in both positive- and negative-ion modes. As noted in the methods section, this study focuses on the positive ion results. ToF-SIMS is a highly sensitive surface analysis technique yielding information about the chemistry of the outermost 1–2 nm of a sample. Each spectrum contains hundreds to thousands of peaks often challenging our ability to visually discern trends in the data. To facilitate data analysis, mathematical algorithms such as PCA are commonly applied to visualize and identify groupings of peaks responsible for the greatest variance between samples. The PCA algorithm leads to two primary matrices referred to as *scores* and *loadings*.³³ The scores plots show relationships between samples in the new axis system, and the loadings plots relate the original variables (i.e. *m/z* peaks in the case of ToF-SIMS) to the new variables (i.e. axes) named principal components (PCs).

The PCA results in these experiments were generally encouraging. All of the nutrients were found to be detectable from the BSA solution over a certain concentration range. The metal ion nutrients (Fe and Zn) were readily detectable at the listed HBLCs and could be easily separated from the BSA controls using PCA. Representative ToF-SIMS positive ion scores and loadings plots (Figure S-3 thru S-6), and a more in-depth results discussion are included in this article's supporting information.

ESI-MS and PCA Analysis of HBLC Nutrients

An initial experiment was performed on the Bruker-Esquire LC-ion trap MS to characterize nutrient fragmentation, and to verify that the HBLCs were within the detection limits for the spectrometer. Mixed solutions of nutrients were prepared at 1 $\times$ HBLC for four nutrients and at 10 $\times$ HBLC for the remaining nutrient in methanol for each of the five nutrient types. Similar to the ToF-SIMS samples, the final HBLCs used were 50 ppb FA, 650 ppb Ret, 105 ppb Thyr, 2 ppm Fe and 20 ppm Zn. Samples were infused by flow injection at 1.5 μ L/min and analyzed via ESI-MS. The mixed nutrient mass spectra were then cross-compared to a control solution spectrum taken on a solution of all five nutrients at HBLCs. Unlike ToF-SIMS and PPAMS, methanol was chosen over a BSA or porcine plasma solution for the

dilutions due to the signal saturation caused by high salt content in BSA or plasma (data not shown).

Figure 1 shows the positive-ion ESI-MS spectra of the mixed micronutrient samples prepared in methanol. Most of the peaks are present in all spectra. Certain peaks show an increase in intensity in the spectra in which an excess of a single micronutrient is added (Figure 1(a) through (e)). PCA was run for each individual spectrum (Figure 1(a) through (e)) versus the control spectrum Figure 1(f) to obtain the nutrient ion peaks responsible for differentiating the peaks from the control group. A few representative peaks are clearly visible and have been labeled in Figure 1. The identities of each of the labeled peaks (discussed in the Figure 1 caption) were confirmed through MS/MS spectra taken during subsequent scans (data not shown). The complexity and number of peaks present in these spectra complicate the analysis of nutrient concentration. This process becomes even more challenging upon the addition of protein and salt solutions. Multivariate techniques assist in performing this analysis by reducing multiple variables to a single variable best expressing the greatest degree of variance.

PCA of the positive ion ESI-MS data from Figure 1 readily distinguishes between the solutions with the excess micronutrient. The scores plot for the first two PCs is shown in Figure 2(a). The first two PCs account for 95% of the total variance in the data set. PC 1, which captures 70% of the variance, displays a loose positive correlation with an increase in the sum of the concentrations of the added nutrients (i.e. separation in PC 1 is seen to develop from an increase in the total nutrient content in the sample).¹ The corresponding loadings plot for PC 1 is shown in Figure 2(b). Each loading peak is marked by colored dots that indicate the peak's contributing nutrients (black for the control sample, red for FA, olive for Ret, blue for Fe, orange for Zn, and magenta for Thyr). In comparing Figure 2(a) and 2 (b), we can clearly see more Zn and Fe peaks contributing to the positive loadings, as well as more Thyr and FA peaks contributing to the negative loadings. Visually, the addition of excess Zn (the micronutrient with the highest HBLC) appears to account for the separation demonstrated in PC 2. This trend continues with excess Fe correlating to the separation in PC 3. Additional PCs were also seen to separate the nutrients with lower blood concentrations (see Figures S-3 and S-4). It is noted that while this correlation appears to be strong for this particular PCA plot, the PCA scores represent a multivariate combination of several peaks that are up and down regulated depending on fragmentation patterns. With the addition of a physiological buffer solution and proteins the scores may not yield as linear a correlation between the abundance of the individual micronutrients.

PPAMS

Following the ESI-MS results, a test was conducted with the PPAMS coupled with the Bruker-Esquire LC-ion trap MS to determine if the LTP-probe was capable of ionizing the nutrients. Pure powders of the individual nutrients were suspended on double stick tape and analyzed. Then, a solution of all five nutrients at HBLC was prepared in methanol, as previously described, dried onto a glass surface, and analyzed. As shown in Figure 3(a), mass spectra were acquired from the control surfaces with a good signal to noise ratio. Several key fragments were observed for each of the nutrients. The peaks shown in Figure 3(a) were first observed in the PPAMS (and MS/MS) data of the raw nutrient powders suspended on tape (data not shown). As an example, single PPAMS/MS data acquired from each of the nutrient powders are presented in Figure 3(b)–(e). MS/MS data were collected for a number of fragments-of-interest for each of the nutrient powders. Representative spectra are shown in Figure 3.

As representative of the Zn powder PPAMS/MS, Figure 3(b) shows the results for m/z 119 ($\text{ZnCl}^+ + \text{H}_2 + \text{H}_2\text{O}$). The PPAMS/MS data is characterized by the typical adducts m/z 64

(Zn^+), m/z 99 (ZnCl^+), and m/z 101 ($\text{ZnCl}^+ + \text{H}_2$). The peak at m/z 129 dominated the original Fe PPAMS spectrum (data not shown), and the resulting PPAMS/MS spectrum is shown in Figure 3(c). This peak was attributed to the Fe complex ($\text{CHNFe} + \text{N}_2 + \text{H}_2\text{O}$) based upon the presence of m/z 57 (FeH^+), 71 (FeNH^+), 83 (CHNFe), and 111 ($\text{CHNFe} + \text{N}_2$) in the MS/MS data.

In preliminary tests on neat Ret solutions and powders with both PPAMS and desorption electrospray ionization (DESI), Ret was observed to display significant fragmentation under the ambient conditions used. Subsequent tests determined that the fragmentation mechanism appeared to be primarily through pi-bond ozonolysis resulting in an aldehyde (or ketone)-terminated ion. This fragmentation was previously observed to occur in unsaturated fatty acids and esters.³⁴ The Ret molecule contains four locations where this cleavage can occur resulting in four fragments with corresponding m/z values of 153, 193, 219, and 259 referenced as fragments A, B, C, and D, respectively. The PPAMS/MS spectrum taken on the full Ret peak shown in Figure 3(d) displays evidence of these four fragments through further potential fragmentations (water and/or ethylene loss) as well as epoxidation of the four starting fragments. The peaks present in the displayed spectrum are m/z 155 (Frag. A + H_2), m/z 199 (Frag. C + $\text{O} - \text{CH}_2 - \text{OH}$), m/z 256 (Frag. D + $\text{O} - \text{H}_2\text{O}$), and m/z 271 ($\text{M}^+ - \text{H}_2\text{O}$).

Determination of the PPAMS/MS peak at 389 (shown in Figure 3(e)) as a FA fragment was accomplished through the identification of the ion present at m/z 297 as the larger fragment produced by cleavage at the peptide bond or ($\text{C}_{12}\text{H}_{13}\text{N}_2\text{O}_5 + \text{O}_2$). The peaks at m/z 167 and m/z 149 were assigned to the cleavage of the second peptide bond removing ($\text{C}_5\text{O}_4\text{H}_7$) and an additional water molecule, respectively. As representative of the Thyr powder PPAMS/MS, Figure 3(f) shows m/z 363 consisting of one of the ring structures present in the full thyroxine molecule. The Thyr spectrum also showed expected fragments at m/z 345 ($\text{M}_{363}^+ - \text{H}_2\text{O}$), m/z 247 ($\text{M}_{363}^+ + \text{O} - \text{I} - \text{H}_2\text{O} + \text{CH}$) and m/z 232 ($\text{M}_{363}^+ - \text{H}_2\text{O} - \text{I} + \text{CH}_2$).

The efficacy of using PPAMS for ambient sampling of blood plasma with little or no sample preparation is demonstrated using a series of model solutions. Samples of the five micronutrients were prepared for PPAMS in a 10% porcine plasma solution prepared in isotonic citrate-phosphate buffered saline (cPBSz) containing sodium azide (0.01 M sodium citrate, 0.01 M sodium phosphate, 0.12 M sodium chloride, 0.02% (w/v) sodium azide, and is adjusted to a pH 7.4 with sodium hydroxide).³⁵ The citrate is added for use both as a buffer and as a calcium chelator to inhibit the calcium-dependant proteases common to blood and blood products.^{35,36} The azide inhibits the growth of organisms that require oxidative phosphorylation to grow.^{35,36} Solutions were based off of HBLCs and LBLCs. HBLC samples were doped at the following levels: 50 ppb FA, 625 ppb VitA, 105 ppb Thyr, 2 ppm Iron (prepared from FeCl_2 salt, Fe), and 20 ppm Zinc (prepared from ZnCl_2 salt, Zn). LBLC samples were doped at: 5 ppb FA, 288 ppb Ret, 46 ppb Thyr, 0.5 ppm Fe, and 10 ppm Zn. Control samples included plain glass, plain 10% porcine plasma solution, all 5 nutrients at LBLC in 10% porcine plasma, and all 5 nutrients at HBLC in 10% porcine plasma.

Several different sample groups were tested, all in 10% porcine plasma solutions. The first test had samples doped with single nutrients at 10 $\times$ HBLC, which was completed to determine peaks that may be indicative of specific nutrients. Next, samples were tested with a 1 $\times$ HBLC for four nutrients, and 10 $\times$ of the remaining single nutrient. The next experiment was completed to mimic a “relatively healthy” individual, with one nutrient at LBLC, and the other four at HBLC. Finally, a “relatively unhealthy” individual was tested, with one nutrient at HBLC and the other four at LBLC. 10 μl of each sample solution was deposited onto 12mm clean glass cover slips, and placed in a desiccator overnight prior to analysis.

Using the LTP to ionize the samples and the ion trap MS for detection, the mass range of 50–1000m/z was scanned in the positive mode. Unsupervised PCA was performed on the resulting spectra to determine if the nutrients could be separated at both the LBLCs and HBLCs from the complex solutions. In the “relatively healthy” sample, with 4 nutrients at HBLC and 1 nutrient at LBLC, the data can be completely separated using the PC1 vs. PC2 sketch (Figure 4(a)). In the “relatively unhealthy” sample, with 4 nutrients at LBLC and 1 nutrient at HBLC, the data is mostly separable to 95% confidence (Figure 4(c)). While some of the 95% confidence ellipses do overlap, very few of the actual data points overlap. As expected, the scores did not yield a linear correlation between the abundance of the individual micronutrients with the addition of the buffer and protein solutions. However, the nutrients were separable at both high and low blood plasma concentrations and the PCA scores shifted based upon nutrient concentration. In addition, many of the peaks that were dominant in the loadings plot for the “healthy” plasma model were also present in the loadings plot for the “unhealthy” model (Figure 4(b) and (d)). We also performed additional analysis on this data by combining the relatively healthy and unhealthy data sets (data not shown). While the data did not completely separate using PC1 and PC2, the data grouped in anticipated manners. As expected, we observed that several of the samples with low nutrient concentrations contained significant overlap. However, even the overlapping confidences ellipses were completely separated using additional PCs. This gives us confidence that a multivariate regression model will be able to quantitatively separate the different nutrients.

CONCLUSIONS

A feasibility study on the application of PPAMS for the detection and eventual quantification of five key micronutrients has been completed. Analysis of the micronutrients was performed quickly under ambient conditions from each surface tested and confirmatory PPAMS/MS was performed. Subsequent analysis with the multivariate PCA technique yielded simultaneous separation of the nutrients by type and quantity at both the lower and upper physiological blood nutrient levels. If needed, increased selectivity can be obtained by employing PPAMS/MS. Ongoing work is focused on the formation of a multivariate regression software model to apply quantitative information to the spectra obtained from the PPAMS technique on human blood plasma samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Positive ion ESI-MS data of mixed micronutrient samples prepared in methanol. Solutions are multi-component mixtures consisting of one nutrient at a 10-fold concentration of its HBL concentration and the remaining four nutrients at a $1\times$ HBL concentration (4 NutrHBL). ESI-MS product ion mode spectra of the $M+$ protons of the mixtures are shown. Changes in each spectrum versus the control 5 NutrHBL spectrum (f) were assumed to be due to the presence of the excess nutrient. The major ions believed to be from the fragmentation of each nutrient are labeled: (a) The majority of Thyr's major fragments are above m/z 300. Only m/z 271 ($C_6H_5O_2ICl^+$) is visible in the 80–300 range shown; (b) m/z 101 ($ZnCl^+ + H_2$), 133 ($ZnCl^+ + O_2 + H_2$), 143 ($ZnCl^+ + C_2H_2O + H_2$), 172 ($ZnCl_2^+ + HCl + H_2$), 228 ($ZnCl_2 + FeCl^+ + H_2$), 268 ($2ZnCl_2$) and 291 ($2ZnCl_2 + H_2O + H_2 + H^+$) were attributed to Zn; (c) m/z 91 ($FeCl^+$), 109 ($FeCl^+ + H_2O$), 228 ($ZnCl_2 + FeCl^+ + H_2$), and 289 ($2FeCl_2 + 2H_2O + H^+$) represented Fe; (d) m/z 165 ($C_{11}H_{17}O^+$), 181 ($C_{11}H_{17}O_2^+$), 251 ($C_{15}H_{23}O^+ + O_2$), 269 ($C_{15}H_{23}O^+ + O_2 + H_2O$), and 291 ($C_{18}H_{27}O_3^+$) indicated Ret; and (e) m/z 177 ($C_7H_7N_5O^+$), 193 ($C_7H_9N_6O^+$), 253 ($C_{12}H_{13}NO_5^+$), and 290 ($C_{13}H_{11}N_6O_2 + Na^+$) represented FA. ESI-MS/MS product ion spectra confirmed the identification of the labeled peaks (data not shown).

Figure 2.

The PCA results for the ESI-MS positive ion spectra shown in Figure 1 are presented as scores (a) and loadings (b) plots. (a) The scores plot displays an excellent separation of each of the micronutrients present in the HBL mixed solutions. Ellipses drawn around each of the groups represent the 95% confidence limit for that group on PCs 1 and 2.¹ (b) The loadings associated with PC 1, capturing 75% of the system variance, show how the original ESI-MS peaks relate to the location of the spectra on the scores plot. Colored dots indicate the nutrient associated with a given peak as determined through a plot of the raw nutrient mass peaks at each mass number. Colors and symbols: (–, black) 5 NutrHBL; (○, red) 4 NutrHBL + 10× FA; (*, olive) 4 NutrHBL + 10× Ret; (X, blue) 4 NutrHBL + 10× Fe; (∇, orange) 4 NutrHBL + 10× Zn; and (+, magenta) 4 NutrHBL + 10× Thyr (n=3).

Figure 3.

(a) Raw positive ion PPAMS data of a mixed micronutrient sample of all five micronutrients at HBL concentration in methanol spotted and dried on a glass disk. While numerous MS/MS spectra were taken on each sample, a single characteristic peak and accompanying PPAMS/MS has been included for each micronutrient. The PPAMS/MS product ion positive-ion mode spectra taken on raw single nutrient powders fixed on double stick tape included: (b) MS/MS of m/z 119 ($\text{ZnCl}^+ + \text{H}_2 + \text{H}_2\text{O}$); (c) MS/MS of m/z 129 ($\text{CHNFe}^+ + \text{N}_2 + \text{H}_2\text{O}$); (d) MS/MS of m/z 287 Ret ($\text{M} + \text{H}^+$); (e) MS/MS of m/z 389, a fragment from FA ($\text{C}_{12}\text{H}_{13}\text{N}_2\text{O}_5^+ + \text{O}_2 + 2\text{N}_2 + 2\text{H}_2\text{O}$); (f) MS/MS of m/z 363, a single ring from Thyr ($\text{C}_6\text{H}_5\text{I}_2\text{O}_2^+$).

Figure 4.

(a) Scores plot from PCA of the PPAMS positive ion spectra of a set of solutions modeling a relatively “healthy” individual in which four of the nutrients are at HBL concentrations and only one is at LBL concentrations as indicated. Symbols: (–) 5 NutrLBL; (○) 4 NutrHBL + FALBL; (*) 4 NutrHBL + RetLBL; (X) 4 NutrHBL + FeLBL; (∇) 4 NutrHBL + ZnLBL; and (+) 4 NutrHBL + ThyrLBL. (b) Loadings plot for PC 1 (41%) from PCA of positive ion spectra for the “Healthy” blood model. Peaks of interest have been labeled and the nutrient(s) associated with them were determined through plots of the raw spectra for each mass. (c) Scores plot from the positive ion spectra of the inverse set of samples modeling a relatively “unhealthy” individual in which four of the nutrients are at LBL concentrations and only one is at HBL concentration as indicated. (d) Loadings plot for PC 1 (46%) for the “unhealthy” blood model. All solutions were formed in a 10% porcine plasma solution in cPBSz buffer. Symbols: (–) 5 NutrHBL; (○) 4 NutrLBL + FAHBL; (*) 4 NutrLBL + RetHBL; (X) 4 NutrLBL + FeHBL; (∇) 4 NutrLBL + ZnHBL; and (+) 4 NutrLBL + ThyrHBL.