Rapid Determination of Strawberry Flavour Integrity using Static Headspace-Selected Ion Flow Tube Mass Spectrometry

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Automated, direct headspace analysis using selected ion flow tube mass spectrometry (SIFT-MS) provides rapid and economic screening of flavour mixes. By coupling the SIFT-MS analysis with multivariate statistical analysis, rapid determination of ingredient flavour quality can be achieved without needing to resort to expert data interpretation. In this paper, classification of different batches of various strawberry flavour mix types of unknown, proprietory compositions is achieved with analysis times of less than one minute per sample. This approach has quality control applications for the food industry.

Introduction

Unintended use of an out-of-specification flavour mix in foods, beverages or nutritional formulations can lead to reduced consumer confidence and product losses. However, traditional screening using a trained sensory panel is expensive. Viable instrumental alternatives for determining acceptance are few due to various practical limitations:

- The gold-standard VOC analysis methods - gas chromatography (GC), gas chromatography-olfactometry (GC-O), and liquid chromatography (LC) - are slow and can struggle with the diversity of compounds in flavours. They also require significant sample preparation, including derivatisation for the short-chain aldehydes and organic acids.

- Electronic noses are subject to significant drift, susceptible to contamination and false positive readings (e.g. from ethanol residues), and cannot identify individual flavour components.

- Traditional direct mass spectrometric (DMS) methods are too harsh or not selective enough to give unique spectral fingerprints.
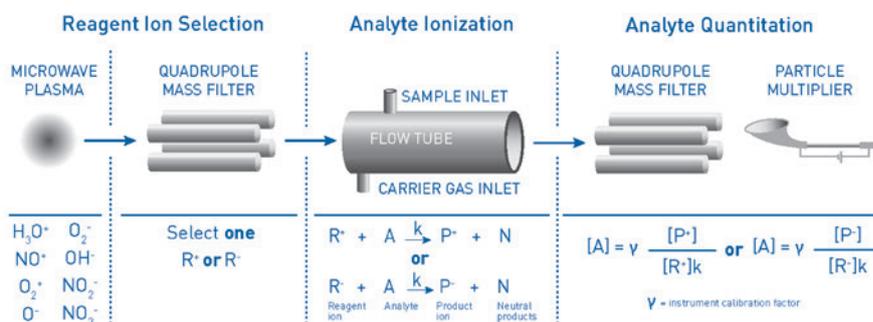
Selected ion flow tube mass spectrometry

Figure 1. Schematic diagram of SIFT-MS – a direct chemical-ionisation analytical technique.

(SIFT-MS), on the other hand, is a direct mass spectrometry (DMS) technique that eliminates chromatography and applies very soft chemical ionisation. In doing so, SIFT-MS can selectively fingerprint samples of proprietary composition – without identification of flavour compounds – in less than one minute.

Recently, rapid geographical classification of Mediterranean olive oils [1] and Moroccan Argan oils [2] has been achieved using untargeted SIFT-MS analysis combined with multivariate statistical analysis. In this paper, a similar approach is applied to classify various commercial strawberry flavour mixes for intra-mix (i.e. batch) and inter-mix variations.

Method

1. The SIFT-MS technique

SIFT-MS [3,4,5] uses soft chemical ionisation (CI) to rapidly quantify VOCs to low parts-per-trillion concentrations (by volume, pptV). The SIFT-MS technique is represented schematically in Figure 1. Eight individually selectable reagent ions (H_3O^+, NO^+, O_2^+, O^-, OH^-, O_2^-, NO_2^- and NO_3^-) are generated in a microwave discharge through moist or dry air. These eight reagent ions react with VOCs and other trace analytes in well-controlled ion-molecule reactions, but they do not react with the major components of air (N_2, O_2 and Ar). This enables real-time analysis of air samples at trace and ultra-trace levels

Table 1. Example NO⁺ reaction chemistry for several potential components of the flavour mixes [7].

Flavour compound	Reaction(s) of NO⁺ with the compound*	Mechanism name
Furaneol	$C_6H_8O_3 + NO^+ \rightarrow C_6H_8O_3^+$ (m/z 128) + NO [95%] $C_6H_8O_3 + NO^+ + N_2 \rightarrow C_6H_8O_3.NO^+$ (m/z 158) + N_2 [5%]	Electron transfer Association**
Methyl cinnamate	$C_{10}H_{10}O_2 + NO^+ \rightarrow C_{10}H_{10}O_2^+$ (m/z 162) + NO [100%]	Electron transfer
Methyl hexanoate	$C_7H_{14}O_2 + NO^+ \rightarrow C_6H_{11}O^+$ (m/z 99) + NO [70%]	Hydride abstraction
	$C_7H_{14}O_2 + NO^+ + N_2 \rightarrow C_7H_{14}O_2.NO^+$ (m/z 160) + N_2 [30%]	Association**
4-Decanolide	$C_{10}H_{18}O_2 + NO^+ \rightarrow C_{10}H_{18}O_2 .NO^+$ (m/z 200) + NO + N_2 [100%]	Association**

* Mass-to-charge ratio of the product ion is shown in parenthesis; the percentage of product formed in a given reaction path is shown in square brackets.

** Nitrogen (or helium) carrier gas mediates formation of this product. The 'third body' carries some excess kinetic energy away enabling binding of $C6H8O_3.NO^+$.

without pre-concentration, and results compare well with gas chromatography mass spectrometry (GC-MS) [6].

Rapid switching between reagent ions provides high selectivity, because the multiple reaction mechanisms can provide additional independent measurements of each analyte. The multiple reagent ions also help to remove uncertainty from isobaric overlaps in mixtures containing multiple analytes.

In this study, full mass scan analyses (SCAN) were carried out using a Voice200*ultra* SIFT-MS instrument (Syft Technologies, Christchurch, New Zealand). Only the NO⁺ reagent ion was utilised due to this ion being the least affected by the relatively high ethanol residues in the flavour mixes (the rate coefficient for reaction of NO+ with ethanol is somewhat slower than H3O+ and O2+).

The NO⁺ reagent ion also has two other significant benefits for flavour analysis:

- NO⁺ reacts *via* multiple reaction mechanisms (association, hydride abstraction, electron transfer (ET) and dissociative ET), the relevance of which depends on the molecule's ionisation energy and chemical functionality. This maximises selectivity both for conventional targeted analysis and for the fingerprinting approach applied here.

- NO⁺ is highly immune to moisture variations.

Since the flavour mixes analysed in this study are proprietory formulations, identities of specific components in the spectra were not provided. However, Latrasse [7] reviews of compounds that are likely to be present, which include alcohols, aldehydes, esters, furanone derivatives. Table 1 provides some examples of the reaction chemistry for several compounds identified in the flavour mixes.

2. Automated SIFT-MS analysis

In SIFT-MS, direct sample analysis facilitates high-throughput headspace analysis, because the rate-limiting chromatographic analysis is eliminated. In contrast to automated chromatographic techniques, which require rapid injection to achieve good peak shapes and temporal separation, SIFT-MS requires steady sample injection for the duration of the analysis. In SIFT-MS, sample injection and analysis occur simultaneously.

Automated headspace analysis was carried out using a SIFT-MS instrument coupled with a GERSTEL multi-purpose sampler (MPS; GERSTEL, Mülheim an der Ruhr, Germany). Samples were first incubated in a GERSTEL agitator prior to sampling of the headspace and subsequent injection into the SIFT-MS instrument through a GERSTEL septumless sampling head. A make-up gas flow (high-purity N_2) was also introduced through the sampling head, maintaining the standard sample gas flow (nominally 25 cm³ min⁻¹) into the SIFT-MS instrument.

The GERSTEL MPS2 autosampler was controlled using GERSTEL's Maestro software. In addition to controlling the injection into the SIFT-MS instrument, the Maestro software's PrepAhead function allows for optimal scheduling of pre-injection preparation steps, such as syringe flush or incubation. This ensures that the highest sample throughput is achieved – a feature that is more important for SIFT-MS than for chromatographic methods.

3. Samples and analysis conditions

Table 2 summarises the powdered strawberry flavour samples supplied for analysis. For each flavour mix, five replicate samples (10 ± 1 mg) were weighed into 20 mL headspace vials and incubated at 50°C for 15 minutes. The headspace was sampled with a 2.5 mL headspace syringe and injected at a flow-rate of 10 μL s⁻¹ into the SIFT-MS instrument's inlet together with the make-up gas, giving a total flow rate of ca. 420 μL s⁻¹. A blank was analysed between each set of replicates and subsequently subtracted from the following group. Flavour mixes and blanks were analysed in less than one minute per sample.

4. Multivariate statistical analysis

The SIFT-MS SCAN data (NO⁺ reagent ion only) were post-processed using multivariate statistical analysis to determine the ability of SIFT-MS to discriminate between the flavour mixes.
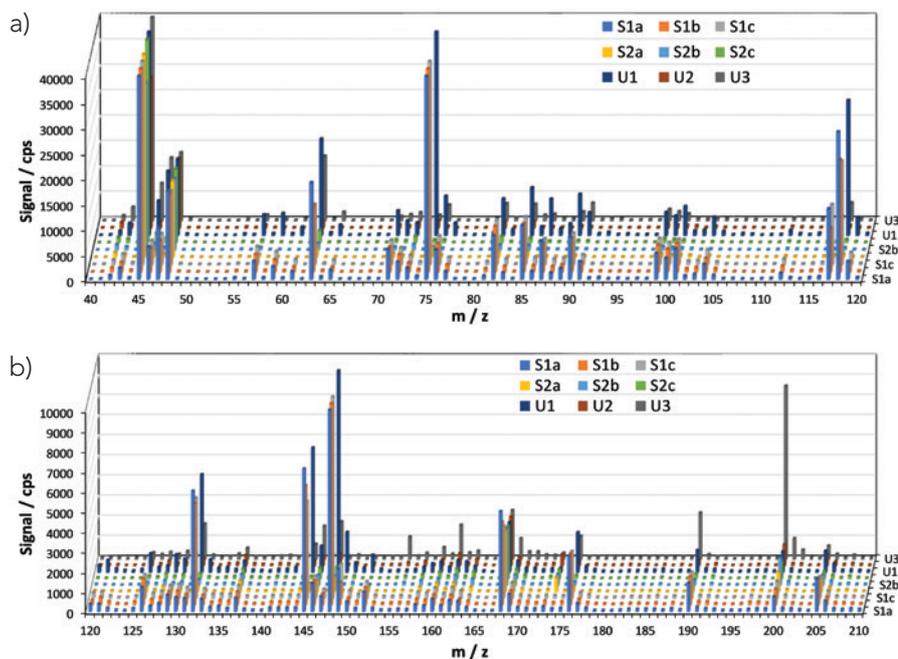
The multivariate statistical methodology

Table 2. Strawberry flavour mix samples supplied for analysis, identification codes and abbreviations used in this article.

Sample type/name	Batch	Abbreviation for figures	Labelling of replicates (5) in class projection plots
Flavour standard 1 ('S1')	Batch A	S1a	S1a-1 to S1a-5
	Batch B	S1b	S1b-1 to S1b-5
	Batch C	S1c	S1c-1 to S1c-5
Flavour standard 2 ('S2')	Batch A	S2a	S2a-1 to S2a-5
	Batch B	S2b	S2b-1 to S2b-5
	Batch C	S2c	S2c-1 to S2c-5
Unknown 1 ('U1')		U1	U1-1 to U1-5
Unknown 2 ('U2')		U2	U2-1 to U2-5
Unknown 3 ('U3')		U3	U3-1 to U3-5

Figure 2. SIFT-MS scan data obtained with the NO+ reagent ion for the averaged replicates of each flavour mix batch: (a) low m/z region and (b) higher m/z region.
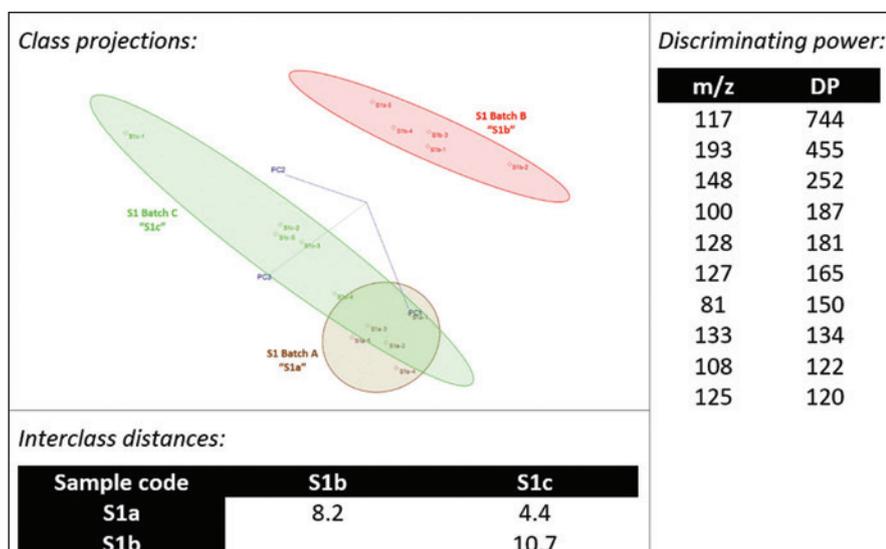

Figure 3. Evaluation of the variability of different batches (A, B, and C) of flavour standard 1 ('S1') using SIFT-MS in scan mode coupled with SIMCA multivariate statistical analysis. Class projections, interclass distances, and the top 10 variables (m/z) for discrimination of the samples are shown.

separability indicated by these distances is not apparent in the three-dimensional class projection plot.

3. *Discriminating power:* This parameter helps variables to be identified that provide the most discrimination between the classes. A variable with larger discriminating power has greater influence on separating the classes than one with a small discriminating power. There does not appear to be a set threshold value above which a discriminating power is considered 'good', because these values vary strongly with interclass distance.

Results and Discussion

The SIFT-MS SCAN data (obtained using the NO+ reagent ion) for the strawberry flavour mixes are shown in Figure 2. The data in Figure 2 are the mean of the five replicate analyses, whereas the individual replicates are utilised for the subsequent statistical analyses in which these scans are utilised as 'flavour fingerprints'.

 1. Evaluation of the ability to discriminate different batches of flavour standards

SIFT-MS scan data obtained using the NO+ reagent ion can be used to rapidly screen different flavour mix batches for acceptability. Figures 3 and 4 show the results obtained for flavour standards 1 (S1) and 2 (S2), respectively, following multivariate statistical analysis with the SIMCA algorithm. The analysis reveals that the different batches of S1 are significantly less consistent than those of S2, both visually in the class projections and quantitatively from the interclass distance metric (Figures 3 and 4).

2. Evaluation of the ability to discriminate between standards

Figure 5 shows an evaluation of the ability of SIFT-MS to discriminate between the different flavour mixes. For this statistical analysis, the three batches for each of flavour standard 1 and 2 are grouped together into their parent classes. The separation obtained is very large, confirming the visual differences observable in the scan spectra (Figure 2). Chemically, these differences arise from different compositions of the flavour mixes that then give rise to different product ion profiles in the SIFT-MS mass spectra. The discriminating powers indicate the product ion m/z that discriminate these mixes most effectively. For example, it appears that 4-decanolide and methyl cinnamate are significant in this instance.

3. Evaluation of ability to classify unknowns

utilised was Soft Independent Modelling by Class Analogy (SIMCA), which was developed by Wold in the 1970s [8]. SIMCA applies principal component analysis (PCA) to the whole dataset and to each of the classes with the end goal of creating a model that discriminates each class from the others. The Infometrix® Inc. (Bothell, WA) implementation of the SIMCA algorithm in the Pirouette software package was employed here.

Prior to analysis using the Pirouette software package, SIFT-MS SCAN data were normalised (giving a sum of unity for all masses in the range), had the blank subtracted, and had masses with normalised signals less than 0.000005 removed.

Three types of output from the SIMCA analysis are presented in this report:

1. *Class projections:* These three-dimensional plots show how each sample falls with respect to the three most important principal components derived from PCA on the entire data set. Each user-defined class shows the sample with the same color and a 'cloud' representing the calculated space in which all samples of the class are expected to lie. Better class separations lead to more confident assignment of unknown samples to a predefined class, if a suitable one exists.

2. *Interclass distances:* These are a measure of the separation between classes. A value of three (3) is usually considered acceptable for class separation [9]. Sometimes the class
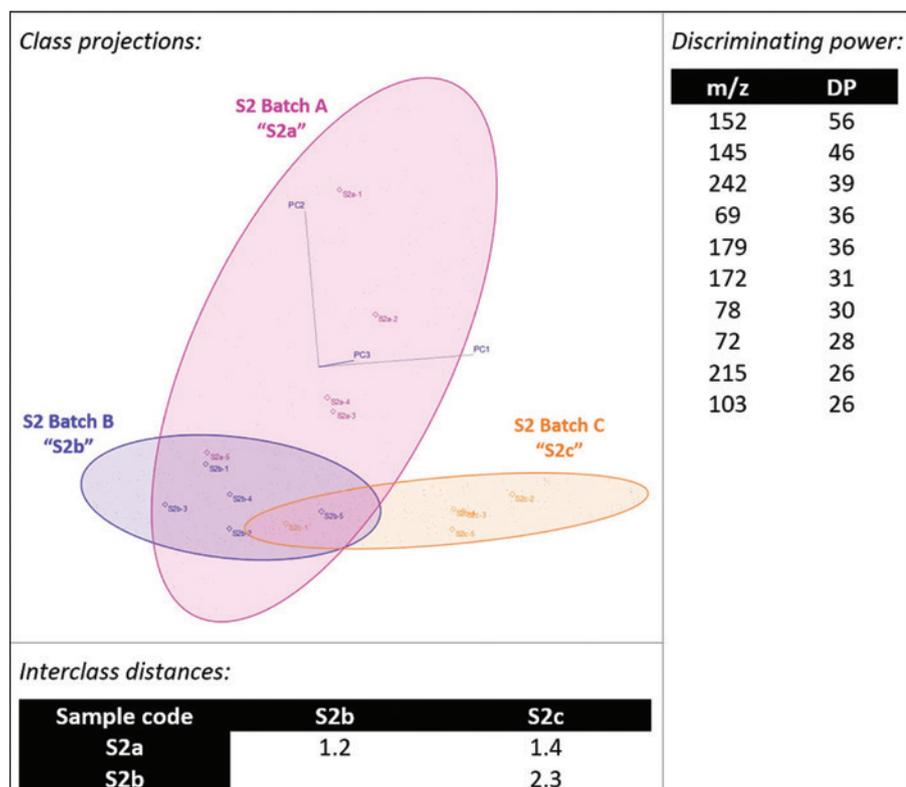
Figure 4. Evaluation of the variability of different batches (A, B, and C) of flavour standard 2 ('S2') using SIFT-MS in scan mode coupled with SIMCA multivariate statistical analysis. Class projections, interclass distances, and the top 10 variables (m/z) for discrimination of the samples are shown.
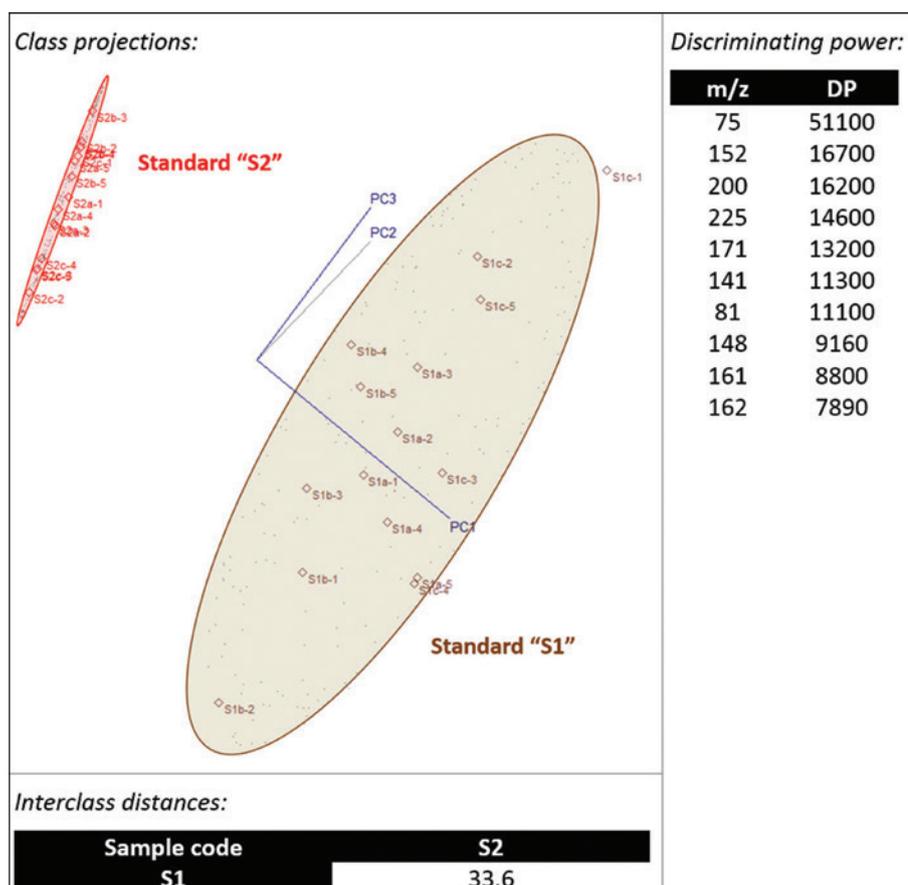

Figure 5. Evaluation of the ability of SIFT-MS coupled with SIMCA multivariate statistical analysis to discriminate between flavour standards 1 and 2 (S1 and S2). Class projections, interclass distances, and the top 10 variables (m/z) for discrimination of the samples are shown.

Three unidentified flavour mixes were supplied for evaluation using SIFT-MS. To identify the mix group to which they belong (if any), each of the 'unknown' samples (U1, U2, and U3) was added as a new class in the SIMCA analysis. The results obtained are summarised in Figure 6. Based on the interclass distances determined (and confirmed visually in the class projection plot):

• Unknown 1 (U1) is another batch of flavour standard 1 (S1). The interclass distance between U1 and S1 is greater than 3, but as shown in Figure 3, the three batches (identified as S1a, S1b, and S1c) are all readily differentiable based on their volatile profiles. That is, there is a lot of variability in the batches of the S1 mix.

• Unknown 2 (U2) is identified as another batch of flavour standard 2 (S2), because the interclass distance is very small.

• Unknown 3 (U3) is extremely different from all other flavour mixes, as indicated by large interclass distances with all other samples. It represents a completely different (i.e. a third) flavour mix.

Assignments of unknown samples U1 and U2 to S1 and S2, respectively, were confirmed by adding them to the S1 and S2 data sets and reprocessing with SIMCA. Further confirmation of these assignments was provided by the customer. They likewise observed significant variation in S1 batches using gas chromatographic analysis and attributed it to degradation of the flavour mixes.

Conclusions

This study demonstrates that untargeted SIFT-MS analysis coupled with multivariate statistical analysis can rapidly screen strawberry flavour mixes to ensure that they fall within the required specification prior to their use in foods, beverages, and nutritional formulations. Automated static headspace-SIFT-MS analyses samples in less than one minute using a fingerprinting approach (full scan mode).

The combined instrumental and statistical approach utilised here has potential to facilitate enhanced quality control through rapid, economical screening of food ingredients.
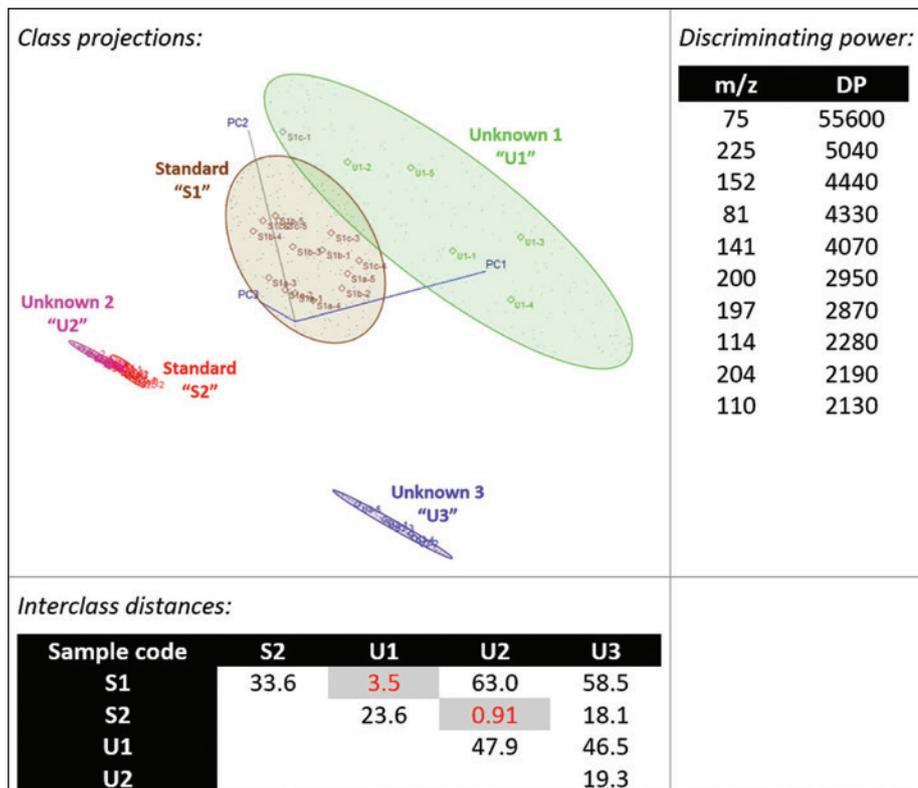
Acknowledgments

Figure 6. Evaluation of the ability of SIFT-MS coupled with SIMCA multivariate statistical analysis to classify unidentified samples (U1, U2, and U3) as standard S1 or S2, or not at all. Class projections, interclass distances, and the top 10 variables (m/z) for discrimination of the samples are shown.

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