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OptiMS: An Accessible Program for Automating Mass Spectrometry Parameter Optimization and Configuration

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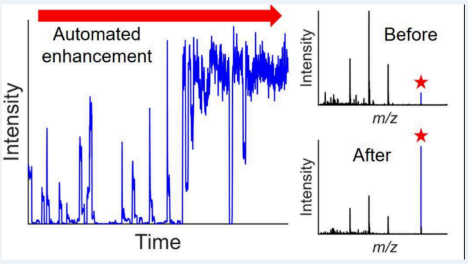

ABSTRACT: Mass spectrometers have an enormous number of user-changeable parameters that drastically affect the observed mass spectrum. Using optimal parameters can significantly improve mass spectrometric data by increasing signal stability and signal-to-noise ratio, which decreases the limit of detection, thus revealing previously unobservable species. However, ascertaining optimal parameters is time-consuming, tedious, and made further challenging by the fact that parameters can act dependently on each other. Consequently, suboptimal parameters are frequently used during characterization, reducing the quality of results. OptiMS, an open-source, cross-platform program, was developed to simplify, accelerate, and more accurately determine optimal mass spectrometer parameters for a given system. It addresses common difficulties associated with existing software such as slow performance, high costs, and limited functionality. OptiMS efficacy was demonstrated through its application to multiple systems, quickly and successfully optimizing instrument parameters unassisted to maximize a user-defined metric, such as the intensity of a particular analyte. Additionally, among other features, OptiMS allows running of a sequence of predefined parameter configurations, reducing the workload of users wishing to obtain mass spectra under multiple sets of conditions.

■ INTRODUCTION

Mass spectrometry (MS) is widely used for chemical sample analysis, due to its high sensitivity, specificity, and ability to provide a large amount of structural information.[1−4] However, one of the greatest issues associated with MS is quantification, particularly when compared to spectroscopic techniques, such as nuclear magnetic resonance (NMR) spectroscopy.[5−7] This is due to various complications, including but not limited to variation in ionization efficiency, ion stability, and ion suppression or enhancement. These complications are analyte specific, making it challenging to predict the exact behavior of species.[5−15] Such quantification is made further complicated by the physical differences between different instruments.[16,17]

Mass spectrometers have a large number of user-changeable parameters with the quantity and functions of these parameters varying between spectrometer models. The values of these parameters can drastically affect the observed spectrum due to differences in the response of different analytes.[18] Therefore, determining optimal parameters for a particular system and set of analytes can increase signal stability and signal-to-noise ratio, and decrease the limit of detection, revealing previously unobservable species.[19,20] As such, using optimal parameters can significantly improve the results of experiments characterized using MS.[18,21] However, determining these parameters is difficult because the physical instrumental changes and the effect on the analytes of these parameters are often not well understood or explained by spectrometer manuals. As a result, it is difficult for operators to quickly and accurately predict appropriate parameter changes to improve the data quality.

User-driven optimization is often performed online by trial and error, one factor at a time (OFAT). This involves the user adjusting a parameter, analyzing changes in the spectrum, and repeating this cycle until they are satisfied that they have achieved optimization. However, ascertaining multiple optimal parameters manually is time-consuming, tedious, and made further challenging by the fact that parameters often act dependently on each other, i.e., optimizing one parameter may deoptimize another.[22,23] Furthermore, OFAT optimization risks obtaining a local optimum but not the global optimum. Consequently, suboptimal parameters are frequently used during characterization, reducing quality of results and in turn, scientific knowledge.[24]

In contrast to OFAT, more sophisticated methods, such as design of experiments (DOE) or Bayesian optimization (BO), use a multivariate approach and sample across the defined search space.[25−30] While both techniques can be used for optimization, DOE is primarily used to systematically sample the defined search space to determine the effect of input variables on output variables, whereas BO is primarily an optimization method. Consequently BO has a high chance of

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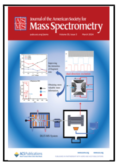

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reaching the global optimum more rapidly than DOE, which is especially advantageous when each evaluation is expensive.[28,30] While both approaches are slower than OFAT, their multivariate nature enhances the likelihood of identifying the global optimum.[25−30] The complex algorithms involved in such optimization processes necessitate digital assistance.

Most instruments are controlled by software, or have software available, which can optimize direct-infusion (DI) parameters. However, such software can be prohibitively expensive, have limited, subpar, or no optimization functionality (particularly for older instruments), or be unadaptable.[31] For example, while Agilent G1732AA MSD Security ChemStation offers a variety of optimization tools and options, Waters MassLynx 4.1 restricts automated optimization to manufacturer-selected compounds.[32,33] Elsewhere, nonvendor software packages have been developed to aid parameter optimization in gas chromatography-MS (GC-MS) and liquid chromatography-MS (LC-MS).[34−37] However, such software requires time-consuming conversion of raw data files in order to calculate the system response to parameter alteration. While acceptable for slower analytical techniques such as GC-MS and LC-MS, this file conversion would significantly impede the efficiency of DIMS parameter optimization. Furthermore, some of these programs have subpar optimization and pose accessibility challenges due to being closed access, no longer operational, or written in less commonly used languages.

OptiMS, an open-source, cross-platform application programming interface (API), was developed in Python to automate mass spectrometer parameter optimization with limits, metrics, and the optimization method stipulated by the user. Additionally, OptiMS allows the running of a sequence of predefined parameter configurations, reducing the workload of users wishing to obtain mass spectra under multiple sets of parameters. This work outlines the key components and functions of the OptiMS code. It is then implemented in a variety of scenarios to demonstrate its efficacy. To the best of our knowledge, there exists no other open-source, cross-platform, rapid, online DIMS parameter optimization software. OptiMS therefore offers an adaptable alternative for MS parameter optimization and configuration, where the available instrument software options are unaffordable or lack the necessary functionality.

■ METHODS

OptiMS was designed to be open access and cross platform, such that it could be used with an array of mass spectrometers and software. It was originally developed for two Waters electrospray ionization (ESI) mass spectrometers and their corresponding MassLynx software: the Synapt G2-*Si* time-of-flight (TOF) spectrometer and the Tandem Quadrupole Detector (TQD). It has subsequently been tested on a Thermo LCQ Classic spectrometer and its Xcalibur software. All of these instruments were running on Windows operating systems. As for these instruments, spectrometers used in conjunction with OptiMS must be able to store chromatographic data and have their parameters changed during acquisition.

Creating cross-platform code is complicated by the fact that each vendor typically encodes their backend log and data files in a vendor-specific, proprietary, closed format, with access restricted to a particular operating system.[31,38] This makes it difficult to make a universal, cross-platform, nonvendor licensed software. However, the general frontend graphical user interface (GUI) design of MS software is much more similar between instruments, typically consisting of a tune window where parameters can be adjusted and a readout window where data is visualized. Therefore, it was decided that the most universally applicable cross-platform code would manipulate the frontend of MS software, i.e., would utilize API functionality. Indeed, many researchers use existing API software for interfacing with MS software, such as AutoHotKey.[39,40]

To navigate the different parameters and GUIs utilized by each spectrometer, the Pythonic OptiMS code automates mouse and keyboard movements to interact with fields in the tune window. Such front-end manipulation can be used for automated parameter configuration as required. Prior to running the code, the user defines: the location of fields; parameter configurations and values; and the number of scans following transitions between them and subsequent stabilization. The code is then executed while the sample is continuously infused. The resulting parameter configurations and the time at which they were set are recorded by the code and exported in the stipulated format.

For automated parameter optimization, the same code was used as described above to interact with the instrument software. In addition, to determine the effect of adjusting parameters, the resulting m/z data had to be rapidly interpolated into the code. As previously mentioned, limited access to the proprietary, vendor-specific data files made this challenging. However, many vendor software packages, particularly those used with TOF and quadrupole instruments, allows quick and easy retrieval of m/z and chromatographic data from the spectrometer GUI, with typical extraction being in standard .txt or .csv data format. The exact menus and buttons used for such data extraction vary depending on the software. As such, the open-access code was designed such that a new user could adapt it to their system, providing that their instrument software supported the simple online extraction of chromatographic data. This differs from most existing APIs, which cannot rapidly interpolate characterization data. Alternatively, the raw data files could be converted into a format suitable for assimilation into the code, similarly to as mentioned previously.[34−36]

This strategy of front-end manipulation for automated parameter configuration and interpolation of chromatographic data allowed for optimization. In addition to the parameters stipulated by the user above, the optimization method, chromatographic data for interpolation, and optimization metric, i.e., the metric that is maximized during optimization, are defined by the user prior to running the code. Additionally, the chromatograms of the desired peaks and their m/z ranges requiring optimization are made visible on the GUI prior to code initiation. This allows the user full control over the optimization of particular species. As previously, the code is executed while the sample is continuously infused. Following a transition from a previous parameter configuration and subsequent stabilization, chromatographic data from a user-defined number of scans are interpolated, and from this, the code selects a new parameter configuration for further optimization. The resulting parameter configurations, the time at which they occurred, the chromatogram intensities, the resulting metric values, and errors in the averaged region are all logged and exported as above.

The optimization methods available in OptiMS include OFAT, exhaustive, and BO, to best fit an array of user needs.

As mentioned previously, OFAT optimization utilizes a small number of e evaluations and hence is fast, as optimization runtime ∼ e × (stabilization delay + number of scans × scan time). This could be useful when some optimization is required, but time is a severely limiting constraint, such as for analytes that rapidly decompose in solution. However, it risks failing to find the global optimum.[25] Exhaustive optimization tests every possible parameter configuration within the defined limits, which guarantees the finding of the global optimum. However, a p number of input parameters each with a l number of levels (i.e., values) yields $e = l^p$; thus, sampling many levels of multiple parameters quickly becomes prohibitively time-consuming.[25] The BO method, which in OptiMS uses Gaussian processes (GP), is a machine learning algorithm which takes a multivariate approach with limited evaluations across the defined search space.[28−30] This optimization method balances a high possibility of converging to the optimal conditions with a minimal number of evaluations; a compromise between the OFAT and exhaustive methods.

BO-GP aims to find a desired optimum of an objective function with as few evaluations as possible by rapidly adapting to changes in output variables.[28−30] First, random parameter configurations are performed within the user-defined parameter limits. This provides information about the behavior of the chosen metric across the multidimensional search space. This algorithmic element has similarities to DOE but is less systematic. A probabilistic surrogate model is constructed from this, in which parameter values are assumed to follow a multivariate Gaussian distribution. An acquisition function selects the next point for evaluation by balancing the exploitation of promising search space regions with the exploration of unknown regions. The surrogate model is updated after each point of evaluation, and the process is repeated until reaching the designated number of honing configurations, with the surrogate model becoming increasingly accurate with each iteration.

When the BO method is used, the number of random and honing configurations performed should be chosen to suit the optimization requirements of the user. Many resources are available describing factors users should consider when determining the appropriate number of evaluations.[25,28,30] Nevertheless, some broad guidance is given here. Input parameters believed to cause a linear or second-order response can be evaluated by using a minimum of two or three levels. Therefore, the minimum number of random evaluations required to screen the general effects of second-order parameters, has $e = 3^p$, similar to a full factorial design.[25] If e becomes sufficiently large that optimization would become prohibitively time-consuming, a fractional number of random evaluations may be more appropriate, similar to a fractional factorial design. This assumes that high order interactions (three or higher) are negligible.[25] For the f fractionality, $e = 3^{p-f}$. Lower f, and hence higher e, increases the chance of finding the global optimum but also increases optimization runtime and therefore, f should be chosen to satisfy the requirements of the user. In this article, the number of random and honing configurations was always kept equal. Work is ongoing to develop algorithms that can determine the completion of optimization and initiate termination to minimize runtime.

The OptiMS code is freely available at https://github.com/peterjhw07/OptiMS, and further details regarding program adaptation, optimization methods, method metrics, and other parameters are described in its documentation.

RESULTS AND DISCUSSION

Parameter Optimization. The primary purpose of OptiMS was to automatically optimize spectrometer parameters to maximize a desired metric, such as the intensity of a particular species. An example signal maximization for perfluorooctanesulfonic acid (PFOS) is shown (Figure 1).

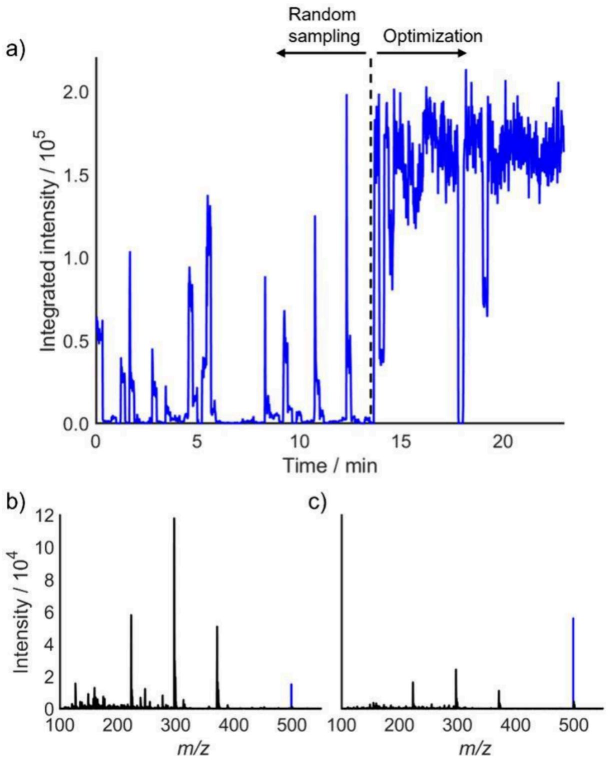

Figure 1. Automated Bayesian optimization of MS parameters for maximization of perfluorooctanesulfonic acid (PFOS) signal using OptiMS. (a) Chromatogram of [PFOS-H]$^-$ signal (m/z ∼ 498.93, blue) during optimization including random sampling (left) and subsequent optimization (right). Mass spectra (b) before optimization (∼0 min) and (c) after optimization (∼23 min) showed more than a 3-fold increase in [PFOS-H]$^-$ intensity (m/z ∼ 498.93, blue) following optimization.

This was undertaken as part of an investigation into a PFOS decomposition system, characterized by ESI-MS. The limit of detection of PFOS needed to be minimized such that PFOS decomposition could be observed over as wide a concentration range as possible.

The intensity of the PFOS corresponding peak was maximized using BO (Figure 1). Six parameters were optimized with starting values: capillary voltage = 2.5 kV; sampling cone = 40 V; source offset = 80 V; nebulizer pressure = 3.0 bar; trap collision energy = 2.0 V; and transfer collision energy = 0.0 V (see the Supporting Information for fixed parameters). OptiMS successfully optimized these parameters, showing a significant increase in the PFOS corresponding peak intensity (Figure 1). BO behavior, including random sampling of the multidimensional search space (Figure 1a, left) and subsequent optimization, including intermittent exploration of unknown regions causing sharp decreases in intensity (Figure 1a, right), was clearly observed.

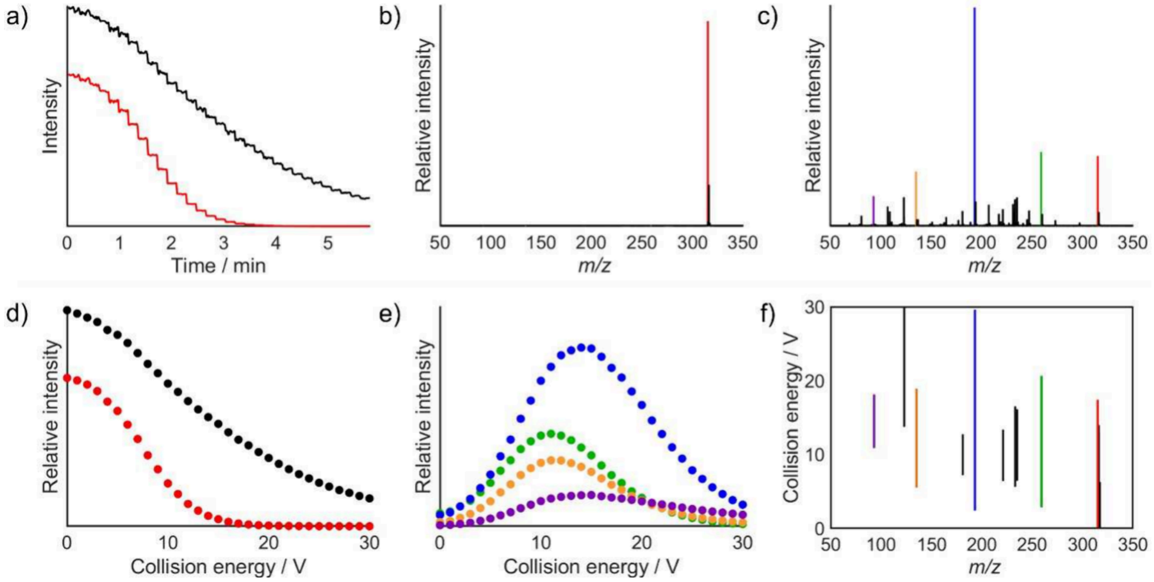

Figure 2. Cannabidiol (CBD) fragmentation experiment, where collision energy was ramped automatically (0−30 V, 1 V increments) by OptiMS software. (a) Monitored chromatograms of the total ion current (black) and the peak corresponding to CBD (red). Mass spectra at (b) 0 V and (c) 15 V, with some of the most abundant fragments highlighted (see the SI for structures and *m/z* values). (d) Extrapolated results of the original OptiMS output. (e) Subsequently extrapolated results by OptiMS of key fragments observed in mass spectra (c). (f) EDESI-MS plot showing the regions in which peaks, including CBD (red) and key fragments observed in mass spectra (c), were of high intensity (≥1% maximum CBD intensity).

Following automated optimization, intensity of the PFOS corresponding peak increased more than 3-fold and became significantly larger than the background peaks in the mass spectrum, which themselves decreased in intensity. Unassisted optimization was completed in <25 min, significantly reducing user workload, and was likely faster and more accurate than a user-driven OFAT approach could achieve. Optimal parameters were determined to be: capillary voltage = 2.0 kV; sampling cone = 0 V; source offset = 2 V; nebulizer pressure = 3.1 bar; trap collision energy = 5.8 V; and transfer collision energy = 0.0 V. This significant increase in intensity lowered the limit of detection, thus allowing PFOS decomposition to be monitored across a wider concentration range.

Additionally, OptiMS has been used for parameter optimization in many other systems described herein and in the Supporting Information (SI), utilizing different spectrometers in both positive and negative ionization modes. Within these systems, OptiMS achieved intensity increases of between ∼10% and >250% for desired peaks, demonstrating its efficacy at parameter optimization for intensity maximization. Furthermore, in one such system, OptiMS yielded notably different optimal parameters for two neighboring peaks (<2 Da), exhibiting its capability to selectively optimize the signal of closely separated analytes.

Although the primary aim of OptiMS was automated parameter optimization, it can also be used to execute specific parameter configurations as required. This is already possible with other APIs, but OptiMS offers the added benefit of being able to rapidly extrapolate data for simpler processing upon experiment completion. To this end, OptiMS has been used for parameter manipulation across a variety of experiments, for example, in a tandem MS experiment, where fragment distribution was screened across a range of discrete collision energy values.

Screening Parameter Effects. Tandem MS was explored as a method to characterize products resulting from reactions of cannabidiol (CBD). For this, the fragmentation patterns of CBD at different collision energies were investigated. The resulting mass spectra were then analyzed to determine the optimal fragmentation pattern for product characterization. First, OptiMS was used to optimize parameters to maximize the intensity of the CBD corresponding peak (see the SI). Second, using the optimized parameter values, OptiMS was used to exhaustively ramp transfer collision energies from 0 to 30 V in 1 V increments and record the total ion current (TIC) and CBD corresponding peak intensities (Figure 2a). The output results were then extrapolated (Figure 2d). Next, mass spectra were analyzed to determine the key fragment ion identities (Figure 2b,c), and their intensities subsequently outputted and extrapolated (Figure 2e). These key fragments have previously been reported in literature.[41,42] OptiMS was additionally used to collect mass spectra at each collision energy, allowing an energy-dependent ESI-MS (EDESI-MS) plot[43−45] to be created (Figure 2f).

CBD corresponding peak intensity decreased as collision energy increased, as expected. Generally, higher *m/z* fragment intensities peaked at lower collision energies while lower *m/z* fragment intensities peaked at higher collision energies, as expected. OptiMS facilitated rapid data collection and analysis for determining the effect of changing spectrometer parameters for use in experiments; in this case, the optimal collision energy for product characterization from CBD reactions and EDESI-MS data.

Additionally, OptiMS was used for automated systematic exploration of the effects of source and desolvation temperatures on analyte signal intensity in a DOE-like manner. While these temperatures are generally known to be able to affect the degree of evaporation, desolvation, ionization, fragmentation, and thermal decomposition during analysis, their precise effect on specific analytes is poorly documented. This lack of data is partly due to the significantly longer time required for the heating and cooling associated with changing such temper-

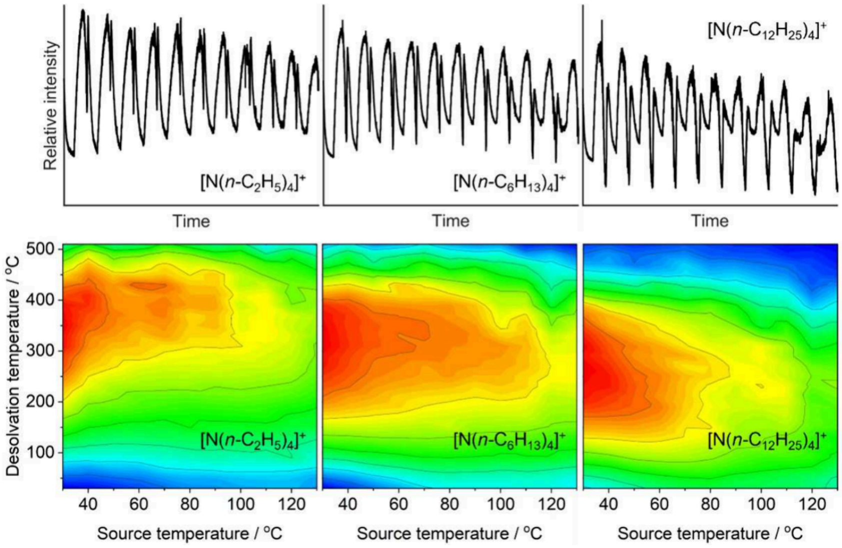

Figure 3. Chromatograms (top) of peaks corresponding to ammonium cations in an equimolar solution of $[N(n\text{-}C_2H_5)_4]^+Cl^-$, $[N(n\text{-}C_6H_{13})_4]^+Cl^-$, and $[N(n\text{-}C_{12}H_{25})_4]^+Cl^-$. The source temperature (30−130 °C, 10 °C increments) and desolvation temperature (30−510 °C, 20 °C increments) were ramped (desolvation temperature ramped across each source temperature) automatically by OptiMS. This yielded corresponding contour maps (bottom, low-high intensity represented by blue-red).

atures compared to most other parameters. This discourages investigation of temperature effects due to the required experiments being time-consuming, laborious, and tedious. This knowledge gap, combined with the significant time required for temperature adjustment, makes it challenging to intelligently optimize the source and desolvation temperatures for specific substrates. As such, the effect of these temperatures on substrates deserved exploration. Fortunately, OptiMS could facilitate this investigation by automated parameter manipulation.

This investigation was carried out using Synapt G2-*Si* which has adjustable source and desolvation temperatures. An equimolar solution of three ammonium salts, $[N(n\text{-}C_2H_5)_4]^+Cl^-$, $[N(n\text{-}C_6H_{13})_4]^+Cl^-$, and $[N(n\text{-}C_{12}H_{25})_4]^+Cl^-$, was used for the analysis in positive ion mode. The structural similarity of these ammonium cations made it relatively simple to compare the effects of the temperatures on each. First, parameters were optimized using OptiMS to maximize the sum of peaks corresponding to the three ammonium salts (at source temperature = 100 °C, desolvation temperature = 250 °C, see the SI). Notably, the intensity of the $[N(n\text{-}C_2H_5)_4]^+$ corresponding peak was at least an order of magnitude lower than for the other two ammonium cations for nearly all the tested parameter configurations, highlighting the complications associated with quantitative MS.

Second, using the optimized parameter values as fixed parameters, predefined specific parameter configurations were used to screen the effects of various source and desolvation temperatures on the mass spectra of the equimolar ammonium salt solution. Source temperature was ramped from 30 to 130 °C in 10 °C increments, with the range chosen to straddle the solvent boiling point (~80 °C in this example), and desolvation temperature was ramped from 30 to 510 °C in 20 °C increments, screening the full instrument desolvation temperature range. Each combination of source and desolvation temperature values within these limits was investigated, resulting in 275 measurements. Because the desolvation temperature heated and cooled significantly quicker than the source temperature, it was ramped across each source temperature value (~30 s for stabilization) and let cool to its lowest value while the source temperature was raised to the next value (~10 min). The results showed a variety of behavior for each analyte (Figure 3).

For all ammonium cations, the maximum intensity was observed at a 30 °C source temperature (Figure 3, bottom). This was significantly lower than the typically chosen starting point for source temperature, i.e., the solvent boiling point (~80 °C in this example). This result was surprising, as it was expected that a higher source temperature would increase evaporation and desolvation, thus maximizing intensities. In contrast, maximum intensity was observed at desolvation temperatures of 350, 310, and 270 °C for $[N(n\text{-}C_2H_5)_4]^+$, $[N(n\text{-}C_6H_{13})_4]^+$, and $[N(n\text{-}C_{12}H_{25})_4]^+$, respectively. This phenomenon appears to be related to a rise in fragmentation and/or thermal decomposition of $[N(n\text{-}C_{12}H_{25})_4]^+$ as the desolvation temperature increased (Figure 4).

While maximum intensity was observed for all ammonium cations at a 30 °C source temperature, it should be noted that maximum intensity was observed at higher source temperatures for lower than optimal desolvation temperatures. This behavior was clearly observed in the contour plots, where a maximum ridge extends from the optimal source and desolvation temperatures to higher source temperature and lower desolvation temperature (Figure 3, bottom). It was hypothesized that this was due to a higher source temperature being required for increased evaporation and desolvation to compensate for the decreased desolvation at lower desolvation temperature.

These experiments illustrate the effectiveness of OptiMS for examining the impact of MS parameters on analytes in mass spectra. Without OptiMS, conducting these experiments would be highly laborious and time-intensive, placing a significant burden on experimentalists or discouraging such investigations.

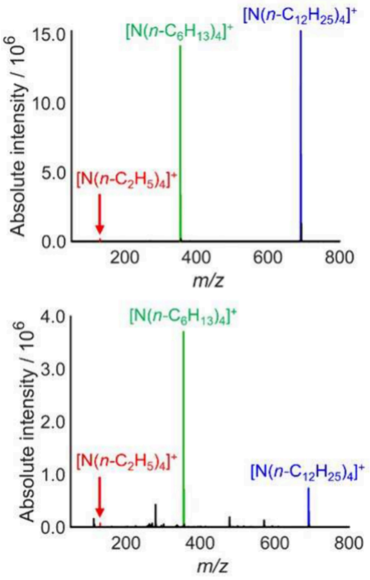

Figure 4. Mass spectra showing peaks corresponding to ammonium cations ($[N(n\text{-}C_2H_5)_4]^+$ - red, $[N(n\text{-}C_6H_{13})_4]^+$ - green, $[N(n\text{-}C_{12}H_{25})_4]^+$ - blue), recorded at source and desolvation temperatures of 30 and 270 °C (top, maximum $[N(n\text{-}C_{12}H_{25})_4]^+$) and 130 and 510 °C (bottom, minimum $[N(n\text{-}C_{12}H_{25})_4]^+$). Other peaks were also clearly observable under harsher conditions, likely caused by fragmentation (bottom).

CONCLUSIONS

OptiMS, an open-source, cross-platform API, was developed to automate mass spectrometer parameter adjustment and optimization. This improves and accelerates collection and analysis of experimental MS data and parameter optimization while also reducing user workload. Although some other software packages are available that may perform similar functions, OptiMS can serve as an alternative in cases when the existing software is slow, unaffordable, has limited functionality, does not exist, or is no longer supported. Efficacy was demonstrated through automated parameter optimization in a variety of chemical systems and performing multiple experiments involving the automatic adjustment of parameters such as ramping collision voltage (EDESI-MS) and investigating the effects of instrument temperatures.

OptiMS was designed with the intention of enabling users to adapt the code to their specific systems. Ideally, OptiMS would include presets for various instruments and software. Therefore, feedback regarding the adaptations made by users to facilitate the interfacing of OptiMS with their instruments and software would be gratefully received. Furthermore, the current capability of OptiMS to optimize and extrapolate peak intensities is limited to instruments with software which allows for simple online importation of *m*/*z* data from the GUI. Therefore, further development is required to allow OptiMS to be compatible with a broader range of spectrometers.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jasms.3c00354.

materials and methods, specific experimental details, an example OptiMS output, additional results (particularly automated parameter optimizations) and supplementary citations[46] (PDF)

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Notes

The authors declare no competing financial interest.

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