Anal. Chem. **1995,** *67,* 3788–3792

Exact Mass Determination for Elemental Analysis of Ions Produced by Matrix-Assisted Laser Desorption

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The exact masses of bastadins, cyclic peptides from marine sponges *Ianthella basta*, are determined using matrix-assisted laser desorption ionization (MALDI) coupled to a Fourier transform mass spectrometer. Two known compounds were mixed with the unknown to serve as internal calibrants. The mass of the calibrants bracketed the mass of the unknown compound. With this method, exact masses were obtained to within 5 ppm for single determinations, and less than 3 ppm for multiple determinations, allowing the derivation of elemental composition. This method is viable for routinely obtaining the exact masses of new compounds with MALDI.

One of the most important features of mass spectrometry (MS) is the capability for providing exact masses, thereby allowing the determination of elemental composition. The analyses can be performed with high sensitivity and more importantly when the compound contains several impurities. For this reason, mass spectral data are often allowed in place of elemental composition as a requirement for the characterization of new compounds. Accurate mass determination is typically performed with mutiple sector instruments using a known ionic species in the spectrum (internal calibration) as a reference point.[1]

The recent development of matrix-assisted laser desorption ionization (MALDI) has provided greater versatility in MS analyses by allowing formation of large ionic species from thermally labile compounds.[2–5] An impressive mass range has been demonstrated surpassing 10^6 daltons. Although MALDI produces very little fragmentation, favoring instead strong signals of quasimolecular ions, accurate mass determination remains a difficult task. Several recent attempts have shown the progress in assigning exact masses using a variety of MS techniques. Beavis and Chait showed mass accuracy of 0.01% with MALDI-time-of-flight (TOF) MS.[5] This has been improved to less than 50 ppm using careful sample preparation and a reflectron/time of light.[6] MALDI-sector mass spectrometry of CsI clusters has yielded mass assignments within 0.015% of actual values.[7,8] MALDI-quadrupole ion trap mass spectrometry (ITMS) was used to obtain mass accuracy of less than 0.05%.[9] Unfortunately, all of these examples are still well above the 5 ppm (0.0005%) necessary for the identification and the subsequent publication of new compounds.

Wilkins has shown that MALDI-Fourier transform mass spectrometry is a viable method for obtaining high mass and high resolution of MALDI-produced ions.[10,11] With an external source MALDI-FTMS, McIver has shown deviation of less than 10 ppm between known and determined mass with external calibration and by including the trapping plate potential in the mass calibration equation.[12,13] In this regard, FTMS may provide the most immediate and accessible method for obtaining exact masses of MALDI-produced ions.

In this report we illustrate a two-calibrant technique for obtaining the exact masses of a group of compounds called bastadins with an external source MALDI-FTMS. We are not aware of other examples where the exact mass of an unknown compound was determined from MALDI within the acceptable limit for the characterization (by elemental composition) of new compounds. The compounds are cyclic peptides isolated from marine sponges. They are particularly difficult to analyze with MS because the compounds contain several bromine atoms that contribute to significant isotopic peaks to make the lightest isotopmer, the most important for exact mass determination, also the least abundant.

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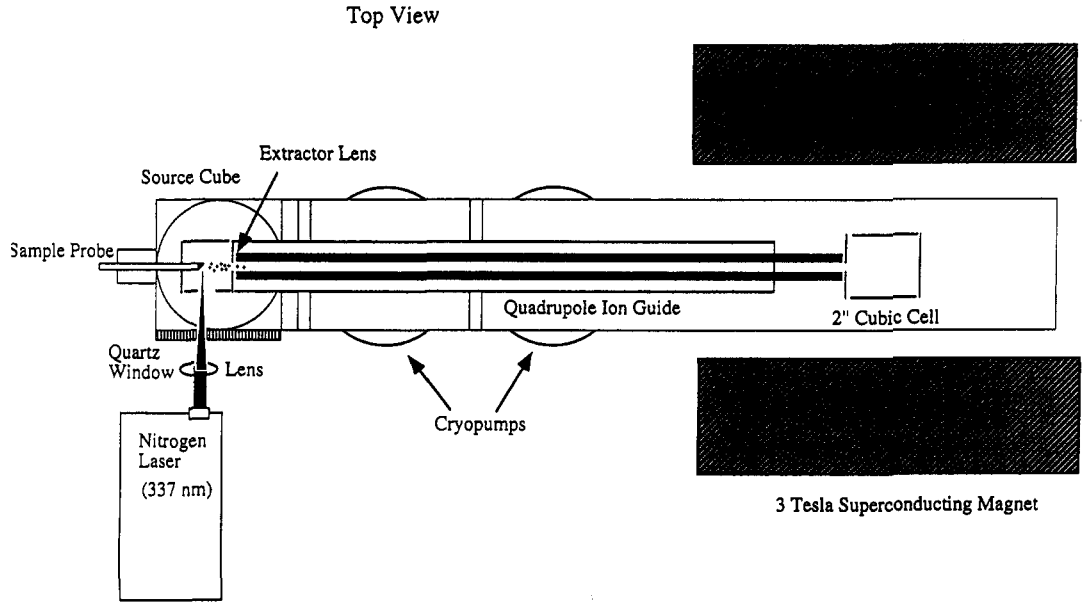


Figure 1. Top view of FTMS instrument with MALDI source. A nitrogen laser (with 337 nm wavelength) is aimed at a sample probe in the source region of the instrument. The ions are formed and guided into the analyzer cell using a single-stage quadrupole rod assembly. The analyzer cell rests in the homogeneous region of a 3 T superconducting magnet.

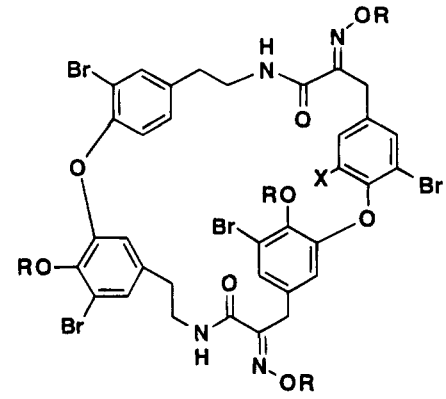
Exact mass determination with this particular FTMS is notable for several reasons. To our knowledge, it is the first determination of the exact mass of an unknown compound. It is performed with a relatively low field (3 T) superconducting magnet which has average homogeneity; the magnet does not contain shim coils for adjustment. It has not also been possible to obtain exact masses of MALDI-produced ions.

EXPERIMENTAL SECTION

The experiments were performed on an external source FTMS instrument described earlier.[14,15] The MALDI source was fabricated using an existing LSIMS source. The ionization source was modified by removing a stainless steel mesh which functioned as part of a side electrode (Figure 1). Removal of the mesh did not significantly appear to affect ion intensities. A Laser Science Inc. nitrogen laser with a maximum 230 μJ/pulse was mounted directly on the instrument and pointed toward the ion source. The beam was attenuated with a single quartz disk placed directly in the path of the beam and a quartz window that allowed the beam access to the source. The total attenuation by the window and the quartz disk totaled between 10 and 20% of the maximum output.

As discussed previously by McIver and co-workers, the timing of the pulse sequences is important for the MALDI experiments.[12,13] The experiment is started by a 1.0 ms pulse of N_2 gas. With the reservoir at 22 Torr, the maximum pressure obtained in the analyzer cell was in the low 10^{-5} Torr. Simultaneously, the quadrupole rods were activated for ion transmission. The laser is pulsed 50 ms after the pulsed gas. The front trapping plate is set initially to 0 V; at 0.5 ms after the laser pulse, the front trapping plate is rapidly raised to 10 V. The rear trapping plate is maintained at 10 V at the beginning of the experiment. Both trapping plates are maintained at 10 V for 10 s to allow ions to translationally cool by collisions with the N_2 gas. After the cooling period, the trapping plates were dropped to a final voltage of 1.0 V, unless specified. The analyzer pressure during the detection period is typically 2×10^{-8} Torr. These are conditions that have been established by Li et al.[12,13]

The bastadins were obtained from samples of the marine sponge *Ianthella basta* and were purified by HPLC as described elsewhere.[16,17] Since they are derived from one basic structure, their molecular formulas fall within a limited number of combinations. This facilitated the assignments of the molecular formulas for given exact masses.



RESULTS

Accurate mass determinations in FTMS require that the frequencies of the ions be stable. If external calibrants are to be used, then the frequencies must remain constant for a long period of time. With internal calibrants, the frequencies must be stable during the detection period (typically less than a few seconds). Several factors can affect the stability of the ion's cyclotron frequency. The most important include the homogeneity of the magnetic and the electric field within the analyzer cell. With a 6

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Table 1. Exact Mass (*m/z*) Determination of a Known Compound, α-Cyclodextrin, Using the Sodiated Parent and the Fragment of Gramicidin S as Internal Calibrants

theor mass	determination 1 (dev, ppm)	determination 2 (dev, ppm)	av of determns	dev (ppm)
995.3067	995.3033 (−3.4)	995.3122 (5.5)	995.3078	1.1

[a] Reference compounds: gramicidin S (MH^+ *m/z* 1141.7138 and MNa^+ *m/z* 1163.6957) + fragment (*m/z* 717.4214 and 571.3608).

T magnet and low trapping potentials (less than 1.0 V), Li et al. showed that use of an external reference provides a maximum deviation of less than 10 ppm between the observed and actual masses.[12,13] An external reference is naturally advantageous. It decreases the number of necessary manipulations and minimizes the inadvertent introduction of impurities. However, many FTMS instruments have magnets with lower and in many cases less homogeneous magnetic fields. The superconducting magnet in this laboratory, for example, has a field strength of only 3 T. Although this field strength and the available homogeneity provide adequate mass accuracy, less than 20 ppm is routinely attainable with external calibration; it does not fulfill the rigorous requirement for publications of new compounds (5 ppm or less). In these cases, internal calibration is necessary to obtain more accurate masses.

Selection of a calibrant depends primarily on the desired mass range. The compounds were chosen to bracket the mass of the sample as closely as possible. Rigorous experiments have not yet been performed to determine the largest possible mass range between the two calibrants that would still provide acceptable results. The two calibrants need not be structurally similar to the unknown compound, although a calibrant that exhibits good sensitivity to MALDI is desirable. When fragment ions from one calibrant is abundant, the quasimolecular parent and a strong fragment ion signal may suffice for calibration. This is illustrated with the determination of a known compound, a cyclic hexasaccharide α-cyclodextrin, whose exact mass is measured by using the quasimolecular peak (MH^+, *m/z* 1141.7138 and MNa^+, *m/z* 1163.6957) and fragments (*m/z* 717.4214 and 571.3608) of the gramicidin S calibrant (Table 1). The average of two determinations produce a value (*m/z* 995.3078) that varies by only 1.1 ppm from the theoretical mass (*m/z* 995.3067). The largest single deviation is 5.5 ppm for the lowest mass isotopmer. The major problem with using fragments stems from the unpredictability of MALDI for producing fragment ions. The use of two compounds that bracket the expected mass is more desirable.

The mass spectrum of a mixture is shown (Figure 2) derived from ~1 pmol of a bastadin, which we designate as unknown 1, and 0.1 pmol each of the two calibrant compounds maltopentaose (a linear oligosaccharide, left arrow) and gramicidin S (a cyclic decapeptide, right arrow). Although the mass spectrum was obtained in the low-resolution broad-band mode, the isotopic envelope contains easily resolvable peaks with a corresponding resolution of 5000 (fwhh, inset). The large number of Br atoms produces a complicated quasimolecular ion region. However, with the resolution and the high S/N, the isotopic pattern is less of a hindrance and is useful for determining the number of Br atoms. The isotopic pattern corresponds to the presence of four bromine

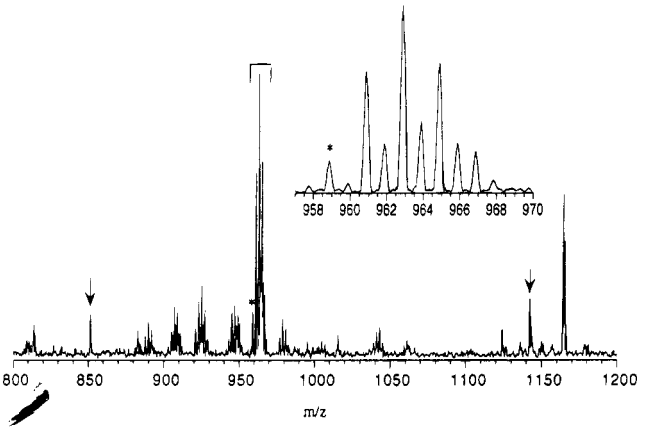

Figure 2. MALDI-FTMS of unknown 1 (given the name bastadin-20). The labeled peaks are the lowest mass isotopmer of the calibrant compounds maltopentaose (sodiated, left arrow) and gramicidin S (protonated, right arrow). Both protonated and sodiated gramicidin S are observed, and either one could be used for calibration. The lowest mass isotopmer (asterisk) and the pseudomolecular ion region are shown (inset). The ionic bastadin species correspond to sodium adducts.

atoms on the bastadin ring. The high S/N of the spectrum also facilitates the assignment of the lightest isotopmer (asterisk). The identification of this peak is key in providing accurate masses because it has the least interference from isobaric peaks of other isotopmers. In the fast atom bombardment (FAB) spectrum, the matrix background often makes this peak less prominent.

The exact mass of unknown 1 has been independently verified using standard methods that employ a double-sector instrument. In this procedure, fast atom bombardment was used in a vacuum generator ZAB-2F and the quasimolecular ion was peak matched, by overlaying the signals, to a known reference signal. In MALDI-FTMS, the quasimolecular ion cluster corresponds to the sodiated species. The cyclic peptides appear to strongly coordinate alkali metal ions. All the bastadins we have analyzed to date appear as sodium-coordinated species as do the two quasimolecular ions of the calibrants (arrow).

With the known masses of sodiated gramicidin S and maltopentaose, the mass of the sodiated bastadin (unknown 1) is determined to be (*m/z* 958.8588). This value deviates by only 5.2 ppm from the exact mass of the lowest isotopmer for a compound with the corresponding molecular formula $C_{34}H_{28}Br_4N_4O_8Na$ (*m/z* 958.8538). The discrepancy lies within the acceptable limit for the identification and publication of new compounds.

In the past, only a single accurate mass determination was necessary for new compounds. More recently, multiple determinations have been encouraged. With multiple analyses, FTMS gives numbers with even better agreement. Unknown 2 is a compound obtained by permethylating unknown 1 (diazomethane, 4 °C, 24 h). The permethylation reaction allows the determination of the number of hydroxyl groups on the ring. The sodiated parent is observed to have a *m/z* 1015 (Figure 3). The summary of three determinations is tabulated (Table 2). An average value of the exact mass was obtained corresponding to *m/z* 1014.9143. The closest molecular formula for unknown 2 is $C_{38}H_{36}Br_4N_4O_8Na$, which has a theoretical mass of *m/z* 1014.9164 producing a deviation of only 2.1 ± 1.5 ppm. The mass change from unknown 1 to unknown 2 corresponds to the addition of four methyl groups. The analysis was performed over a period of several days,

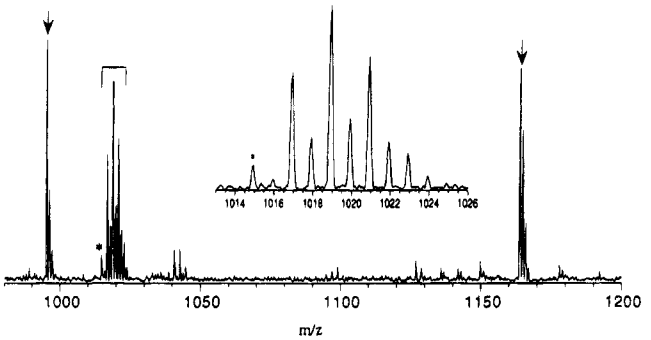

Figure 3. MALDI-FTMS of unknown 2 (bastadin-20 tetramethyl ether). The calibrants used are α-cyclodextrin (sodiated, left arrow) and gramicidin S (sodiated, right arrow). The lowest mass isotopmer (asterisk) and the quasimolecular ion region are shown (inset). The most abundant quasimolecular ions are typically sodium adducts.

Table 2. Exact Mass (*m/z*) Determination of a Bastadin, Unknown 2, Obtained by Permethylation of Unknown 1[a]

theor mass	absol dev (ppm) of each determn 1	2	3	av of determns	dev[b] (ppm)
1014.9164	−3.4	−0.4	−2.4	1014.9143	2.1 ± 1.5
1016.9144	2.6	3.5	9.4	1016.9197	5.2 ± 3.7
1018.9124	2.6	2.6	5.5	1018.9160	3.5 ± 1.7
1020.9103	2.6	5.6	7.5	1020.9157	5.3 ± 2.5
1022.9083	1.7	−3.2	−1.3	1022.9073	1.0 ± 2.3

[a] Reference compounds: α-cyclodextrin (MNa^+, *m/z* 995.3067) + gramicidin S (MNa^+, *m/z* 1163.6957). [b] Deviation of average values from theoretical mass.

illustrating good instrumental stability. With the given molecular formula, the deviation of the determined mass from the exact mass for the entire pseudomolecular region is provided (Table 2). There is excellent reproducibility when the internal standard is used. The largest deviations are observed in determination 3 for the heavier isotopmers having *m/z* 1017 and 1021. As mentioned, the higher masses may contain isobaric species of other naturally occurring isotopes such as ^{13}C and ^{18}O. For this reason, it is better to chose the lightest isotopmer for exact mass determinations. The heavier isotopmers, however, are useful for confirmation of the assigned molecular formula.

Based on the above results and the NMR data,[17] unknown 1 was shown to be a new compound and given the name bastadin-20, where X = H and R = H (see structure). Unknown 2 is the tetramethyl derivative of bastadin-20 where X = H and R = CH_3.

A third compound, unknown 3, was also separated by HPLC from extracts of *Ianthella basta*. The average value obtained from the MALDI is *m/z* 1036.7666 corresponding to a molecular formula of $C_{34}H_{27}Br_5N_4O_8Na$ (Table 3). The calculated mass for the given formula corresponds to *m/z* 1036.7642 for the lightest isotopmer and lies only 2.3 ppm from the experimentally determined value. Most of the values for each isotopmer in the two determinations vary by less than 5 ppm from the calculated values except for the heaviest isotopmer, which is 7.7 ppm away from its calculated value. The determined mass was key to identifying this compound. Based on this value and the NMR of unknown 3,[16] it is identified as the previously characterized bastadin-19 where X = Br and R = H.

In all the spectra, a cluster of peaks 16 u larger than the sodiated quasimolecular peaks is observed (See, for example,

Table 3. Exact Mass Determination of a Bastadin, Unknown 3

theor mass	absol deviation (ppm) of each determn 1	2	av of two determns	dev[b] (ppm)
1036.7642	1.2	3.4	1036.7666	2.3
1038.7623	8.1	1.1	1038.7671	4.6
1040.7603	1.9	−2.4	1040.7601	−0.2
1042.7582	2.4	−1.3	1042.7588	0.6
1044.7562	−3.0	2.4	1044.7559	−0.3
1046.7542	−7.5	−7.9	1046.7461	−7.7

[a] Reference compounds: α-cyclodextrin (MNa^+, *m/z* 995.3067) + gramicidin S (MNa^+, *m/z* 1163.6957). [b] Deviation of average values from theoretical mass.

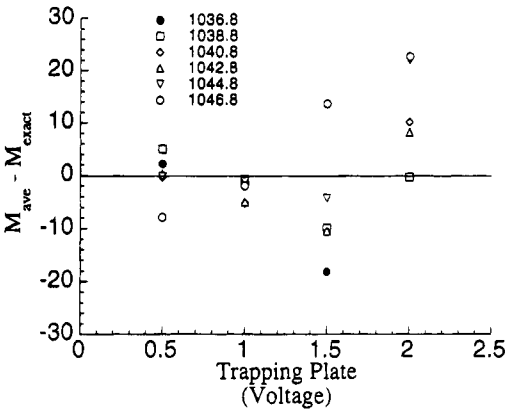

Figure 4. Plot of difference between the calculated and determined mass for ions in the quasimolecular ion region of mass spectrum for unknown 3 (later identified as bastadin-19). The smallest differences occur when the trapping voltages are set to 1.0 V or less. Large differences are observed with trapping plate voltages set to 1.5 V and greater.

Figures 2 and 3.). These signals correspond to the potassiated parent and could provide further confirmation of the assignment of the quasimolecular ions. More importantly, these species illustrate that the exact masses of mixtures can be determined simultaneously. These determinations are aided by the propensity of MALDI-FTMS to produce primarily quasimolecular ions. The determination of mixtures will greatly facilitate the speed of the analysis particularly for compounds or groups of compounds that are chemical homologues.

The values of the trapping plate voltage are important for exact mass determination and must be kept as low as possible. The observed natural frequencies of the ions are known to shift as the trapping plate voltages are increased.[12,18] Even with the internal standard, care must be taken to use low trapping voltages. Figure 4 shows the differences between the exact mass and the determined mass (in ppm) as a function of trapping plate voltage. The largest deviations occur with 2.0 V, which is also the largest voltage investigated (Table 4). The most useful trapping voltages appear to be between 0.5 and 1.0 V as these conditions exhibit the least scatter and maintain the lowest deviation between determined and actual mass for the lowest mass isotopmer. With 0.5 V trapping the largest deviation is 7.8 ppm, for the heaviest isotopmer, while with 1.0 V of trapping potential the largest deviation is 5.2 ppm. The difference between the observed mass

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Table 4. Differences between Theoretical Mass and Determined Mass as a Function of Trapping Plate Voltages for Bastadin-19[a]

theor mass (m/z)	Δ (m/z)			
	0.5 V	1.0 V	1.5 V	2.0 V
1036.7642	2.2	−1.2	−18.2	−136.5
1038.7623	5.0	−0.7	−9.9	−0.3
1040.7603	−0.3	−5.2	−10.6	10.1
1042.7582	0.3	−5.0	−10.5	8.2
1044.7562	−0.2	−1.1	−4.3	22.0
1046.7542	−7.8	−2.0	13.7	22.6

[a] Reference compounds: α-cyclodextrin (MNa^+, m/z 995.3067) + gramicidin S (MNa^+, m/z 1163.6957).

of the lightest isotopmer and the known mass is less than 3 ppm with 0.5 and 1.0 V of trapping. With 1.5 V, nearly all masses have deviations that are greater than 10 ppm. The deviations increase further with 2.0 V on the trapping plates. Below 0.5 V the ion intensities decrease significantly, but there is no observable effect on signal strength between 0.5 and 2.0 V.

MALDI-FTMS is useful for determining the exact masses of compounds. The method described is relatively simple requiring only the addition of the calibrants. All other conditions are typical for MALDI-FTMS. The method does not require modifications in either the hardware or the software to implement. The low fragmentation yield allows simple assignments of the pseudomolecular ions. More importantly, there is a real potential for performing mixture analyses and obtaining exact masses of several compounds simultaneously. Furthermore, there is little tuning required between samples as the internal calibrant helps minimize varying instrumental conditions.

ACKNOWLEDGMENT

Funds provided by the National Institute of General Medical Sciences, NIH (Grant GM49077-01) and the National Science Foundation (Grant CHE 9310092) are gratefully acknowledged. The authors also thank Prof. R. T. McIver, Jr., and Dr. Yunzhi Li for their helpful comments and suggestions.

Received for review February 17, 1995. Accepted August 2, 1995.[⊗]

AC9501811

[⊗] Abstract published in *Advance ACS Abstracts*, September 1, 1995.