High-Speed Mass Spectrometry

# Hadamard Transform Time-of-Flight Mass Spectrometry: More Signal, More of the Time

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analytical methods · Hadamard transform · mass spectrometry · signal multiplexing

*Hadamard transform time-of-flight mass spectrometry (HT-TOF MS) is a type of mass analysis that was developed to couple continuous ion sources to the inherently pulsed nature of time-of-flight measurements. Unlike conventional TOF MS, the Hadamard transform method offers a duty cycle of 50 %, with the possibility of extending it to 100 %. Because it is a multiplexing technique, the attainable signal-to-noise ratio (SNR) is also significantly higher than that of conventional TOF MS. This review covers the basic principles behind HT-TOF MS. We illustrate, through examples, the source of the high-duty cycle and the increase in SNR. These features translate to a mass spectral storage rate that is the fastest among similar instruments, which enables its use as a detector for high-speed separations.*

## 1. Introduction

A mass spectrometer is a device for weighing molecules. It converts a neutral molecule into a positively or negatively charged ion, and it subjects the ion to known electric or magnetic fields. The subsequent motion of the ion depends on its mass and charge. An analysis of the ion's motion allows its mass-to-charge ratio ($m/z$) to be determined, and hence its molecular weight. Many types of mass spectrometry exist. By far the simplest and least expensive method is time-of-flight (TOF) mass spectrometry (MS), in which ions of different masses are given the same kinetic energy but different velocities, each ion experiencing the same potential drop. After acceleration, the flight time of the ion is measured over a fixed distance between a starting point and the ion detector. It is easily shown that the square of the flight time $t$ is directly proportional to the mass-to-charge ratio of the ion $m/z$. Our review concerns a new method for carrying out TOF MS that has the advantages of high efficiency and rapid acquisition of mass spectra.

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In TOF MS, the ion source is pulsed to create packets of ions. In the conventional procedure, the system waits for all the ions in a packet to reach the detector before injecting the next packet of ions. This pulsing creates inherent complications when TOF MS is coupled to a continuous ion source. Such coupling is often accomplished by an orthogonal extraction scheme in which a packet from a continuous stream of ions is pushed at a right angle to the ion stream and directed toward the ion detector. Because of the system delay between packet extractions, the duty cycle of an orthogonal extraction instrument is usually between 5–15 %. This degree of merit means that up to 95 % of the information contained in the ion stream is lost. In contrast, we describe here an on-line modulation–demodulation scheme that allows, on average, half of the ions in the ion stream to strike the ion detector to give a duty cycle of 50 %. Moreover, because we modulate this beam at a frequency of 10 to 20 MHz, mass spectra can be collected, one after the other, at a rate of more than 250 second$^{-1}$. The advantage of such a high sampling rate and good ion-collection efficiency is that TOF MS done in this manner can be combined with some fast chromatographic or electrophoretic separation technique prior to ionization, to give a large dynamic range and a large capacity factor for what can be separated, detected, and quantified in a complex mixture.

To make our discussion more concrete, let us consider a very simple example of mass determination, namely, weighing three bananas of slightly different mass. This example might seem silly at first, but it provides deep insights into the weighing process. Suppose our weighing device is a single-pan scale (Figure 1) that takes one second to make a weight measurement and whose response has an uncertainty given by

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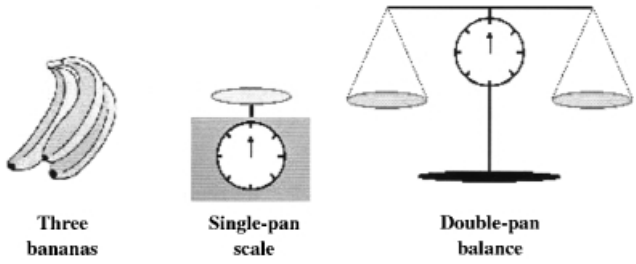


***Figure 1.*** Weighing three bananas with two types of scales.

the square root of the signal, that is, the square root of the mass. What is the optimum strategy for weighing the bananas?

Initially, the answer appears to be straightforward. Each banana is placed on the pan and weighed separately for one second. Yet, with a bit of reflection we realize that a better strategy is to weigh the bananas together as three different pairs and then subtract the weight of one pair from another to get the individual weights of the three bananas. The second procedure gives the same answer but with less uncertainty. Why? The single-pan scale spends the same time weighing more bananas. Similarly, we seek a means to multiplex signals so that the time-of-flight mass spectrometer spends more time detecting the ions contained in the ion stream.

Let us explore further the challenge of devising an optimum weighing strategy for the three bananas by replacing the single-pan scale with a double-pan balance (Figure 1). With two pans, the best weighing procedure is to put one banana on one side of the balance and the other two on the opposite side. This weighing is repeated twice more, and each time a different banana is chosen as the lone counterbalancing weight. The double-pan balance is superior to the single-pan scale, because with two pans all three bananas are being weighed all the time, which thus increases the quality of the information. We will discuss how a similar procedure can be implemented in time-of-flight mass spectrometry to increase the duty cycle from 50 to 100%. But first we will describe how to achieve a 50% duty cycle.

Conventional TOF MS can be compared to the weighing method in which each banana is weighed separately on a single-pan scale. The ion source is pulsed at a constant rate that allows ions of only one $m/z$ value to strike the detector in a given time bin. As with the bananas, it is also possible to modulate and detect ions in such a way that the measurement in each time bin yields information about all $m/z$ values. We accomplish this task by gating a continuous beam on and off at a rate so rapid that multiple ion packets exist in the flight chamber of the instrument at the same time. Differences in ion velocities cause the packets to overlap as they drift; thus, the detector registers multiple $m/z$ values. Interpretation of the signal requires knowledge of the gating sequence. We provide the ion gate with a sequence of on and off pulses that may be represented by "1"s and "0"s. If the sequence contains a 1, a packet of ions is launched at the detector; if the sequence contains a 0, the ions are deflected to miss the detector. The best sequence consists of about as many "1"s as "0"s. In this manner, the detector has a 50% duty cycle.

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One way to make these on and off pulses sharply defined in space and time is to use a "Bradbury–Nielsen gate" (BNG) (Figure 2).[1,2] The BNG consists of two electrically isolated sets of equally spaced wires that lie in the same plane but alternate in potential, between positive and negative. When no potential is applied to the wires, the trajectory of the charged ion beam is undeflected by the BNG. To deflect the beam, potentials of equal magnitude and opposite polarity are applied to the two wire sets, which causes the ion beam to miss the detector. To feel the force of the charged wires, ions must

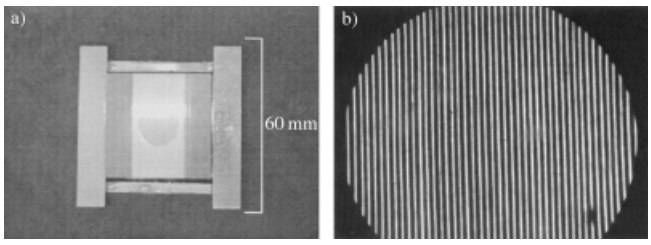


***Figure 2.*** a) Finely spaced Bradbury–Nielsen gate (front view, 100 μm spacing) and b) closer view of the optical aperture showing the two interleaved wire sets.

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be within a distance of the gate approximately equal to the wire spacing. Consequently, it is possible to modulate the ion beam on and off in a controlled manner at a sharply localized starting point.

Brock et al.[3,4] first reported the use of the BNG to achieve on-axis modulation of an ion stream by means of a well-defined pseudorandom sequence of pulses that transmits or deflects the ions. This BNG may be regarded as chopping the ion stream into packets of different lengths. The ions in these packets travel through the flight tube between the BNG and the ion detector at different velocities, with typical flight times of hundreds of microseconds. At the BNG, the temporal width of an ion packet and the time between adjacent ion packets is three orders of magnitude shorter than these flight times (ns versus μs). Consequently, as the ion packets travel the length of the flight tube, faster ions in one packet overtake slower ions in earlier packets, which causes the packets to interpenetrate and overlap. The response of the detector appears to be noise (Figure 3a), but this assessment is incorrect. Actually, the signal output of the detector is the convolution of many conventional TOF mass spectra, each shifted in time according to the pseudo-random binary modulation sequence. Hence, the TOF mass spectrum can be extracted from this data by demodulating the output signal using knowledge of the applied sequence (Figure 3b). The demodulation corresponds mathematically to an "inverse Hadamard" transform. Thus, the procedure is called Hadamard transform time-of-flight mass spectrometry (HT-TOF MS). Under ideal circumstances, in which the sequence applied to the ions is known exactly, the transform returns the mass spectrum with no distortion.

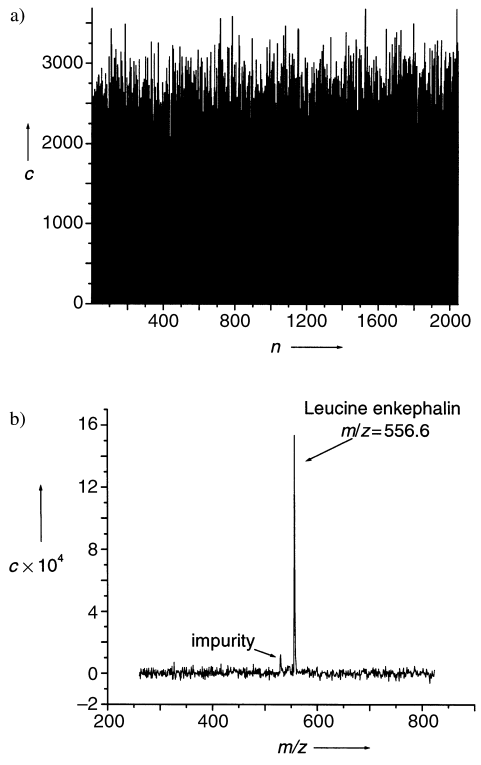


***Figure 3.*** Electrospray ionization–HT-TOF mass spectrum of 1.4 pmol of the peptide leucine enkephalin (1+) dissolved in methanol:water:-acetic acid 50:50:1. The acquisition time was 2 s. Diagram a) shows the raw (convoluted) data and b) shows the deconvoluted mass spectrum obtained in low resolution mode.[19] The pseudorandom binary sequence consisted of $(2^{11}-1) = 2047$ elements, each of 100 ns duration. $c$ = counts, $n$ = bin number.

The Hadamard transform belongs to the same family of transforms as that of the more familiar Fourier transform.[5] In the Fourier transform, the basis functions used to express the signal function in the time domain are an infinite series of sine and cosine functions. In contrast, for the Hadamard transform we use as a basis set a finite series of binary elements. As a consequence, the Hadamard transform is significantly faster to compute, and it does not suffer from aliasing effects (the process by which smooth curves and other lines become jagged because the resolution of the device or file is not high enough to represent a smooth curve (the process in which the signal becomes distorted because the sampling rate is not sufficiently high to capture the signal features). Hadamard transforms have already been used in many different chemical applications such as Raman imaging[6] capillary electrophoresis,[7,8] and Fourier-transform mass spectrometry.[9] They are applicable to any phenomenon in which a series of on and off signals are recorded. Because Hadamard transforms are rather unfamiliar, the bulk of this review is devoted to working through a simple example. We close with one recent application of HT-TOF MS to the analysis of a mixture of peptides—an application that hints at the power of combining separations with HT-TOF MS analysis.

## 2. The Basics of HT-TOF MS

We will explain the basics of HT-TOF MS with the aid of a simple example. The first step is to build a virtual TOF spectrum. Our virtual sample is an aqueous solution that contains the peptide P ($M_w = 1620$ Da) and a protein such as a protease ($M_w = 56\,000$ Da). One way to produce gas-phase ions from this sample is to use electrospray ionization (ESI). In ESI, the sample is nebulized through a needle that has a high potential relative to the mass spectrometer.[10] This electrical field causes a spray of finely divided, charged droplets. As solvent evaporates, the surface charge density of the droplets increases, which leads to a Coulombic explosion that causes the droplets to fission into yet smaller droplets. Evaporation and fission continue, which result in a beam of "naked", positively charged gas-phase ions. Basic compounds such as peptides and proteins often carry more than one charge per molecule and consequently appear in the TOF spectrum with a mass-to-charge ratio lower than their molecular weight.

To record the TOF spectrum of our virtual sample in a conventional TOF instrument, the BNG would be opened for a fixed period of time (a few hundreds of ns) and then closed. Ions in the packet that passed the open gate would drift through the instrument and be recorded as they arrived at the detector. Ions reaching the gate when it is energized would be deflected and miss the detector. Counts are usually collected in time bins that are a few ns wide, by using a time-to-digital converter. However, to simplify the calculations and reduce the size of the Hadamard matrices needed, we chose to use

much larger time bins in our example. If a 10 kV field were used to accelerate ions and the flight path were 50 cm long, the flight times of the components in our sample mixture would be 1.56 μs for $H_3O$ (1+), 10.24 μs for substance P (2+), and 26.94 μs for the protease (10+). To record the full mass spectrum, we chose to set up the acquisition system to acquire ion counts in seven time bins, each 5 μs wide. This arrangement is sufficient to record a maximum flight time of 35 μs, which corresponds to an upper mass cutoff of 9560 Da.

The recorded spectrum looks something like Figure 4. The intensity of each solute peak reflects the abundance of the component in solution multiplied by the ionization efficiency. We arbitrarily chose the three peaks to have the ratio 3:2:1. The peaks originating from the solvent reflect only the amount of charge carried by ionized solvent molecules[11] and do not usually carry analytical information. Notice that each peak in the TOF spectrum shown in Figure 4 has some background noise associated with it. This noise is proportional to the square root of the ion intensity.[12] In this particular case of the protease, we chose a background noise level of ten counts per bin ($\sqrt{100}=10$). In this scenario, the signal-to-noise ratio (SNR) for the protease peak is $100/10=10$. Time bins showing no peaks will have only the detector dark noise.

Let us perform the same TOF experiment using Hadamard multiplexing. A Hadamard matrix $H_n$ of order $n$ is a matrix of $+1$ and $-1$ with the property that the scalar product of any two different rows or columns is 0.[13] As we mentioned before, a mass spectrometer is used to weigh molecules. To record the $+1$ and $-1$ states in a Hadamard matrix, we would need a spectrometer furnished with two detectors, which is analogous to the double-pan balance in our example of weighing bananas. Hadamard multiplexing can also be done with only one detector if we use a type of Hadamard matrix that contains only $+1$ and 0. Such matrices, called Simplex matrices, are easily derived from Hadamard matrices.[13] Using a Simplex matrix is like weighing bananas in bunches with a single-pan scale. As in the conventional TOF example, we need to accommodate a maximum flight time of 35 μs in seven bins. Hence, we require a $7\times7$ Simplex matrix (Figure 5).

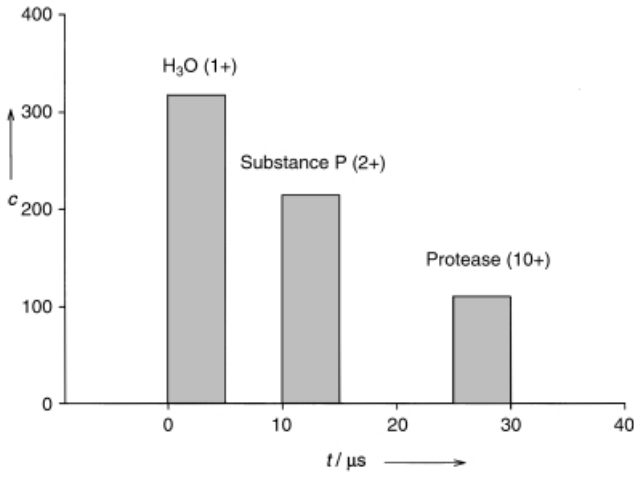


***Figure 4.*** TOF mass spectrum used in the example of the peptide model.

What is the meaning of this matrix? Its first row contains the sequence of voltage pulses that are sent to the ion gate at 5 μs intervals. A sequence of "1 1 1 0 1 0 0" means that the beam state is "on, on, on, off, on, off, off." Notice that the second ion packet is injected into the flight chamber immediately after the first. Consequently, we will record a signal much more complex than with conventional TOF. Mathematically, the spectrum acquired in HT-TOF MS is found by multiplying the TOF distribution, regarded as a seven-element column vector, by a $7\times7$ Simplex matrix and adding a seven-element column noise vector. The resulting vector shows hits from different compounds in the same time bin. For example, the last element of the convoluted spectrum is obtained by multiplying the fifth row of the Simplex matrix by the TOF spectrum. The obtained value of 449 counts accounts for hits of protease and $H_3O^+$ injected during the first and fifth time bins, respectively, and it includes the background noise. Also notice that the noise level is higher than in the conventional TOF spectrum (49 counts versus 10 counts). Because we have analyzed four ion packets, the total signal is larger, and the noise increases with increasing signal ($\sqrt{2400}\approx49$). For reasons explained later, there is still a gain in overall signal-to-noise ratio as a result of multiplexing. Figure 7 shows the result of the convolution presented in Figure 6. Figure 3a is the equivalent of Figure 7, but with an $8191\times8191$ matrix.

$$S=\begin{bmatrix}1&1&1&0&1&0&0\\1&1&0&1&0&0&1\\1&0&1&0&0&1&1\\0&1&0&0&1&1&1\\1&0&0&1&1&1&0\\0&0&1&1&1&0&1\\0&1&1&1&0&1&0\end{bmatrix}$$

***Figure 5.*** Simplex matrix $7\times7$ used in the example.

The TOF spectrum is deconvoluted from this signal by multiplying the experimental vector by the inverse of the Simplex matrix (Figure 8). This multiplication can be done in real time because the matrix inverse needs to be calculated only once and is then stored in the computer. Moreover, the inversion process requires only additions and subtractions.

Is there an advantage in using Hadamard multiplexing? Figure 9 compares the outcomes of a conventional and an HT-TOF MS experiment. As we can see, the noise (12.25 counts $bin^{-1}$) in the HT-TOF MS experiment is larger than the noise (10 counts $bin^{-1}$) of the protease peak in the conventional TOF experiment but smaller than the noise (17 counts $bin^{-1}$) in the $H_3O^+$ peak. The SNR from HT-TOF MS is 8.2 compared to 10 from conventional TOF MS for the protease peak and 24.5 compared to 17.3 in the $H_3O^+$ peak, respectively. It might seem at first that Hadamard multiplexing offers no clear gain, but this conclusion fails to take into account how the SNR scales with sequence length for the same mass resolution. It has been shown that the SNR is a function of the square root of the matrix size.[12] If a much

$$\begin{bmatrix}1&1&1&0&1&0&0\\1&1&0&1&0&0&1\\1&0&1&0&0&1&1\\0&1&0&0&1&1&1\\1&0&0&1&1&1&0\\0&0&1&1&1&0&1\\0&1&1&1&0&1&0\end{bmatrix}\times\begin{bmatrix}300\\0\\200\\0\\0\\100\\0\end{bmatrix}+\begin{bmatrix}49\\49\\49\\49\\49\\49\\49\end{bmatrix}=\begin{bmatrix}549\\349\\649\\149\\449\\249\\349\end{bmatrix}$$

***Figure 6.*** Matrix form of the Hadamard convolution.

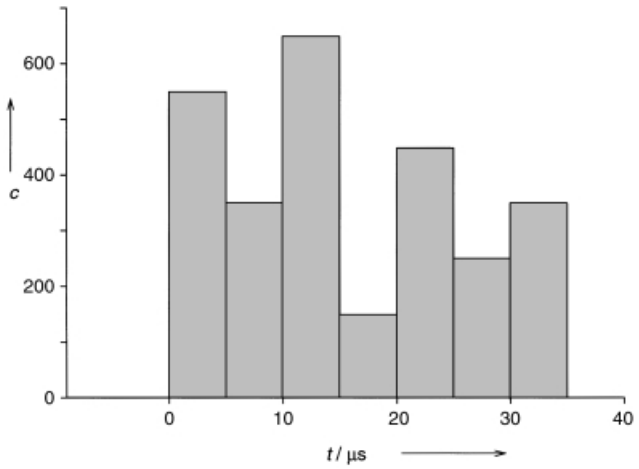


***Figure 7.*** TOF spectrum used in the example after convolution with a Simplex matrix derived from a Hadamard matrix.

$$\frac{1}{4}\times\begin{bmatrix} 1 & 1 & 1 & -1 & 1 & -1 & -1\\ 1 & 1 & -1 & 1 & -1 & -1 & 1\\ 1 & -1 & 1 & -1 & -1 & 1 & 1\\ -1 & 1 & -1 & -1 & 1 & 1 & 1\\ 1 & -1 & -1 & 1 & 1 & 1 & -1\\ -1 & -1 & 1 & 1 & 1 & -1 & 1\\ -1 & 1 & 1 & 1 & -1 & 1 & -1 \end{bmatrix}\times\left(\begin{bmatrix}500\\300\\600\\100\\400\\200\\300\end{bmatrix}+\begin{bmatrix}49\\49\\49\\49\\49\\49\\49\end{bmatrix}\right)=\begin{bmatrix}312.25\\12.25\\212.25\\12.25\\12.25\\112.25\\12.25\end{bmatrix}$$

***Figure 8.*** Hadamard deconvolution. The noise vector is shown separately for clarity.

larger matrix is used in place of the $7\times7$ matrix in the example, we can expect a significant gain in SNR.

In many experiments we use a sequence length of $N=(2^{13}-1)=8191$. According to the work by Wilhelmi and Gompf,[14] the expected maximum SNR gain for a $8191\times8191$ matrix varied between $\sqrt{(8191/2)}=64$ and $\sqrt{8191}/2=45$, depending on the background level. The SNR gain is also a function of spectrum sparsity, and the best results are obtained for spectra with very few, strong mass peaks. Experiments in our laboratory have shown that the experimental SNR gain is in accordance with these theoretical values.[12]

## 3. Applications and Outlook

The principal advantage of multiplexing in TOF MS is increased sensitivity, which leads to lower detection limits. This increased SNR enables a larger spectral storage rate. If instead of a continuous ion beam of constant composition, the output of a capillary separation technique is directly coupled to the ESI source in the HT-TOF MS, we face the challenge of analyzing an ion beam with fast, transient signals corresponding to the eluting compounds. The sensitivity of modern TOF spectrometers requires that several spectra must be averaged in order to display a single spectrum with a good SNR. This fact limits the spectral storage rate of the mass spectrometer. If the peak widths of the eluting compounds become smaller, such as in microchip separations,[15] high-speed gas chromatography,[16] or ultrafast capillary electrophoresis,[17] the MS detector may not be able to keep up with the speed of separation. As a result, peak shapes become drastically distorted.[18] Owing to its improved SNR, HT-TOF MS provides a faster storage speed than conventional TOF spectrometers, which makes it an ideal tool for capillary and other high-speed separations.

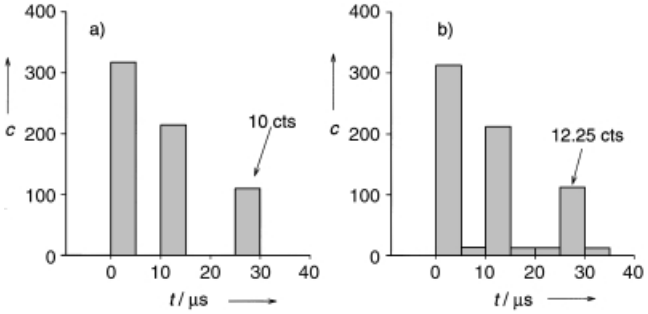


***Figure 9.*** Comparison of SNR obtained in a) the conventional TOF spectrum and b) the deconvoluted HT-TOF spectrum used in the example ($N=7$). cts = noise counts.

Recently, we demonstrated the feasibility of detecting the capillary electrophoretic separation of a mixture of nine peptides with HT-TOF MS.[19] The results are shown in Figure 10. In this same study, we acquired spectra across peaks with widths of the order of hundreds of milliseconds and obtained a maximum spectral storage rate of 277 mass spectra $s^{-1}$, nearly 3.5 times faster than the best conventional instrument operating over the same mass range.

It might be wondered whether saturation is a problem when so many ion packets are incident on the ion detector at essentially the same time. We have observed response linearity in the 10–200 micromolar range for peptides, which suggests that no saturation is occurring. Ion counts from solvent and buffer components have been suppressed by efficient desolvation in the ESI source to reduce unwanted signals and to avoid detector saturation.

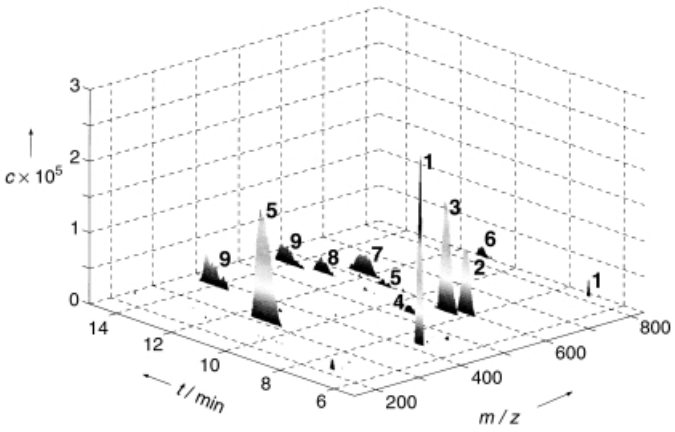


***Figure 10.*** Separation of a nine-peptide mixture by pressure-assisted capillary electrophoresis and ESI–HT-TOF MS. Peak assignments: **1**: bombesin, **2**: bradykinin (fragment 1–5), **3**: leucine enkephalin, **4**: oxytocin, **5**: methionine enkephalin, **6**: substance P, **7**: human luteinizing release hormone, **8**: Arg 8-vasopressin, and **9**: bradykinin.

Although obtaining a 50% duty cycle is a big step toward high-efficiency TOF mass spectrometry, the advantages of the Hadamard transform can be pursued even further. Installation of a position-sensitive ion detector that is able to sense both the deflected and undeflected beams in different zones of its active area would allow the use of Hadamard matrices instead of Simplex matrices in the deconvolution process.

Drawing an analogy between a two-detector experiment and weighing bananas with a double-pan balance is not exact, but, for both bananas and ions, the principal advantage of two detectors is clear: no species goes unmeasured. Moving to this mode of operation would increase the SNR by 40%,[13] raise the duty cycle to 100%, and, ultimately, increase the spectral storage rate of the MS. Although fully position-sensitive ion detectors based on resistive anode encoders are already available,[20,21] high-resolution $(x,y)$ information is not necessary for implementing a full Hadamard encoding. Instead, a conventional microchannel plate (MCP) detector assembly with a two-zone patterned anode can be used. A schematic of such a device is shown in Figure 11. A modified HT-TOF MS spectrometer that uses full Hadamard encoding is under development in our laboratory at the present time.

To appreciate what promise HT-TOF MS may hold, let us make a conservative estimate of how many different mass peaks might be determined in a chemical analysis that combines a fast capillary-format separation (GC, for example) with this type of mass spectrometric detection. Let us imagine that a peak has a full width at half maximum of 200 ms and that the separation of the mixture is completed in 400 s. During that time, between 2000 peaks (the maximum possible) and 740 (37%, based on statistical considerations[22]) might be recorded. But the HT-TOF MS can easily have a mass resolution of 1000 and record a mass range $m/z$ up to 5000,[23] which means that more than 1000 different species that co-elute with the same retention time can be distinguished. This amount also depends on the number of isotopomers and charged species produced per analyte by the electrospray ionization source, but a conservative estimate is that about one million different compounds could be detected in this two-dimensional format. Certain other forms of mass spectrometry offer higher resolution, but this gain in resolution comes at the expense of acquisition time. Notice that in order to monitor a chromatographic peak that is 100 ms wide, a spectral storage speed of 100 mass spectra $s^{-1}$ or more is needed, if it is assumed that ten points are required to define the peak.

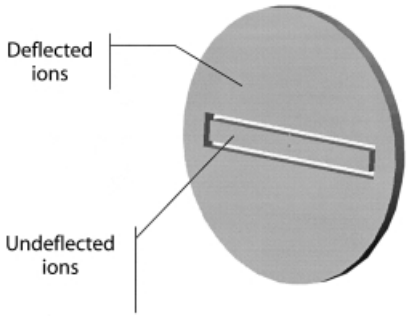


***Figure 11.*** Patterned anode of the position-sensitive MCP detector. The central square detects the undeflected portion of the ion beam and the peripheral segment detects the deflected portion.

As we move toward faster and more complex separations, the need for MS with moderate resolution and high acquisition rate becomes increasingly urgent. The HT-TOF MS technique, coupled to a fast separation technique, begins to approach two-dimensional gel electrophoresis in its resolution of different components in a mixture, but with the marked advantages of an enormous gain in sample throughput and identification power.

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[23] Resolution in HT-TOF MS is primarily governed by the frequency at which the BNG is switched. In contrast to conventional TOF MS, the detector response time and digitizing rate play a secondary role. For example, a resolution $\Delta m/m$ of 2000 at $m/z = 1000$ could be achieved at 25 MHz if the fidelity of the modulation is maintained at this frequency. The mass range of a HT-TOF mass spectrometer is a function of the sequence length and the mass cutoff of the BNG. For an in-depth discussion of these topics see ref. [12].