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RECENT ADVANCES IN DOPING ANALYSIS (2)

M. Donike
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

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K. O. Börnsen, E. Gassmann, M. Schär, H. M. Widmer

Matrix Assisted Laser Desorption and Ionization - Mass Spectrometry: A New Analytical Tool

Corporate Analytical Research, Ciba, Basel, Switzerland

Introduction

After three years of its introduction by M. Karas et al. (1) , matrix assisted laser desorption and ionization mass spectrometry (MALDI-MS) is already a well established analytical method in chemistry and biochemistry. The high sensitivity, the broad mass range, the absence of any fragmentation and the fast sample preparation are the main reasons for this success. However, the possibility of measuring complex sample mixtures, often with minimal sample pretreatment, is one of the biggest advantages of this new method. New matrix molecules broadened the application range of MALDI-MS. Chemical classes such as polymers, additives, dyes, carbohydrates and oligonucleotides are now accessible to MALDI-MS.

Characteristics and principle of MALDI-MS

MALDI-MS is a desorption method which uses a solid matrix of small organic molecule as a medium for desorption and ionization. The analyte molecules, which are 100 - 10 000 times less in concentration than the matrix, are embedded in this matrix. The solid matrix is prepared by mixing solutions of sample and matrix. A small amount, typically 0.6 µl, is transferred to the probe tip and vacuum dried, which ensures a fast crystallization process. The sample preparation and often used matrices are summarized in Table 1.

The matrix molecules absorb the energy of a short laser puls (nitrogen laser at 337 nm with 3 ns pulse width). This leads to a disintegration of the top matrix layers at the exposed spot, dragging along the embedded analyte molecules into the gas phase. During this step, ionization of the analyte molecules takes place. The produced ions are either protonated $(M+H)^+$ or deprotonated $(M-H)^-$.

The ions are analyzed in a time of flight mass spectrometer (TOF-MS; Fig. 1). TOF-MS has the advantages, that a complete mass spectrum is obtained for each ionization event, it has a high transmission rate and an unlimited mass range. A mass resolution (full width at half maximum) $M/\Delta M$ of 75 at a mass of 65 000 Da up to 1800 Da at a mass 1348 Da is achieved in a linear TOF-MS. The accuracy of the mass determination is in the range of 0.1 to 0.01%, depending

on the purity of the sample, the resolution and the signal to noise ratio. The sensitivity lies in the pmol to fmol range.

The analysis of high molecular mass compounds can be achieved with MALDI-MS. Today, the method allows to produce and detect molecular ions of masses exceeding 200000 Da. On the lower end, matrix ions circumvent the analysis of ions with a mass smaller than 200 Da. MALDI-MS produces only low amounts of higher charged or dimeric analyte ions. Additionally, fragment ions are not observed in a linear TOF-MS. These important features allow investigating complex mixtures.

Results

The following part shows a few spectra which were recorded on a linear LDI 1700 matrix assisted laser desorption and ionization mass spectrometer from Linear Scientific Inc., Reno (now Hewlett Packard Company, Palo Alto, USA). The intention is to show with these examples the possible applications for this new mass spectrometrical method

Figure 2 shows a positive ion mass spectrum of normal Milk (bovine, 3.8% fat) as an example for a complex mixture. The sample was deluted with water 1:50. No further sample pretreatment was performed.

In Figure 3, rat plasma was diluted with water 1:100 and the mass spectrum recorded in the positive ion mode. As mentioned in Table 1, Sinapinic acid is best suited for high mass analysis. In the inset of Figure 3, Dihydroxyacetophenone was used as matrix for an improved sensitivity below 20 000 Da.

In Figure 4 a positive ion mass spectrum of starch (hydrolyzed) as an example for carbohydrates is shown. The difference of 18 mass units can be explained by branching of the carbohydrate chain. Carbohydrates are ionized by incorporation of sodium or potassium ions.

Figure 5 demonstrates the investigations of a kinetic study. The oligonucleotide $p(dT)_{20}$ was enzymatically cleaved with snake nuclease. Negative ion spectra were recorded at different times during the reaction course.

Conclusions

MALDI-MS is an emerging analytical technique. Through its speed, broad application range and high information content of the results, MALDI-MS opened new possibilities for investigating proteins, carbohydrates and oligonucleotides. In these fields, MALDI-MS is used for unamiguous determination of molecular masses, the detection of contamination and for monitoring chemical and biochemical reactions. For other fields, like medicine and food sciences, MALDI-MS applications still need to be explored.

Acknowledgment

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References

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   M. Schär, K. O. Börnsen, G. Tschopp, E. Gassmann and H. M. Widmer; Würzburger Bertsch Verlag, 109
2. R. C. Beavis and B. T. Chait; Rapid Commun. Mass. Spectrom., **3**, 432 (1989)
3. U. Pieles, W. Zürcher, M. Schär and H. Moser; Nucleic Acids Research, 21, **14**, 3191(1993)
4. K. Strupart, M. Karas, F, Hillenkamp; Int. J. Mass Spectrom. Ion Proc., **111**, 89 (1991)

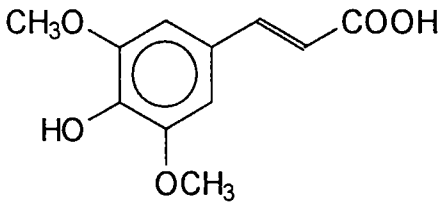
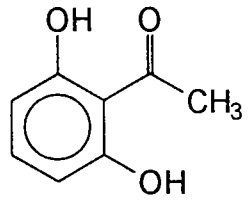
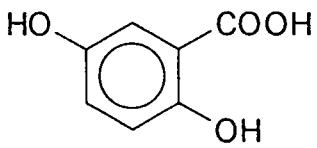
Matrix: 10^{-2} M solution (2µl) + ***Analyte:*** 10^{-3} to 10^{-7} M (2µl) ***mixed*** ***0.6 µl on probe tip*** ***vacuum dried***	***Solvents:*** H_2O, MECN, THF,Toluene, Methanol, Ethanol, DMSO ***Salt tolerance:*** up to 300 mM of Buffers and salts (Na^+, K^+)
 	***Sinapinic acid***[2], found in the early days of MALDI-MS, has the broadest application range. It gives results in nearby all application fields. Especially, strong signals in the high molecular mass range are obtained. The ability to analyze low and high mass compounds is demonstrated in Figure 3.
 Ciba Patent	***2,6-Dihydroxyacetophenone (DHAP)***[3] has also a broad application range up to a mass of about 30 kDa. Also, the pH of this matrix is close to neutral, which makes DHAP the matrix of choice for acid labile compounds. DHAP produces good resolved spectra with a high sensitivity for peptide mixtures. In combination with Diammoniumhydrogene citrate DAHC as an additve, DHAP delivers unmatched results for complex protein mixtures, such as milk (and for small oligonucleotides .
 	***2,5-Dihydroxybenzoic acid (DHB)***[4], as a water soluble matrix, is often used for small polymers and carbohydrates. Carbohydrates are always detected as their Na^+- or K^+- adducts.

Table 1

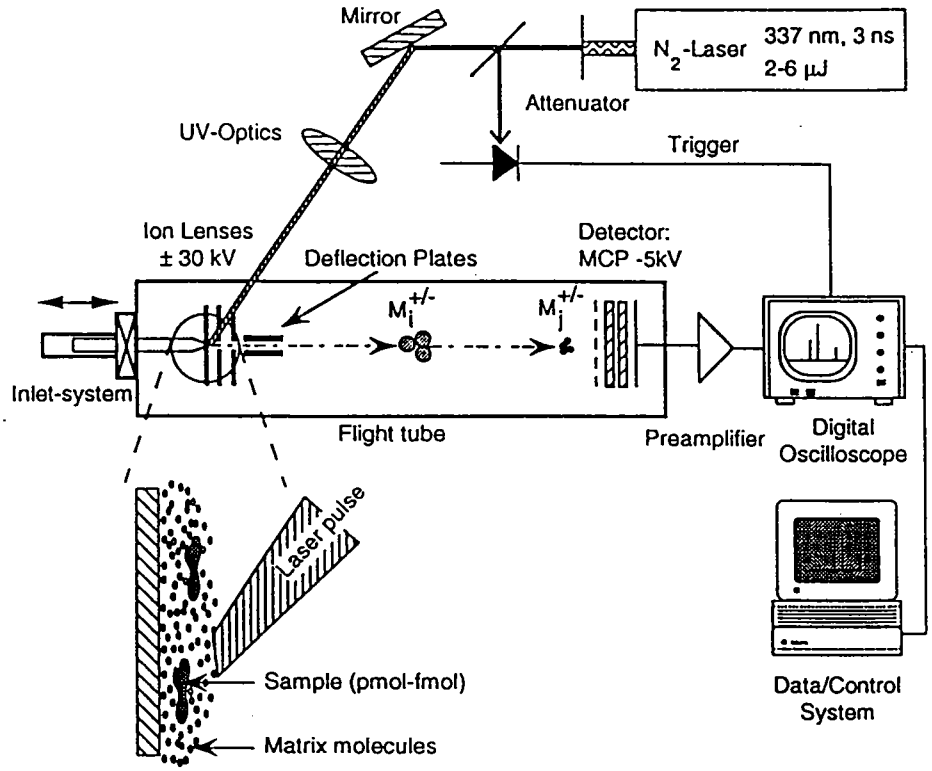


Figure 1

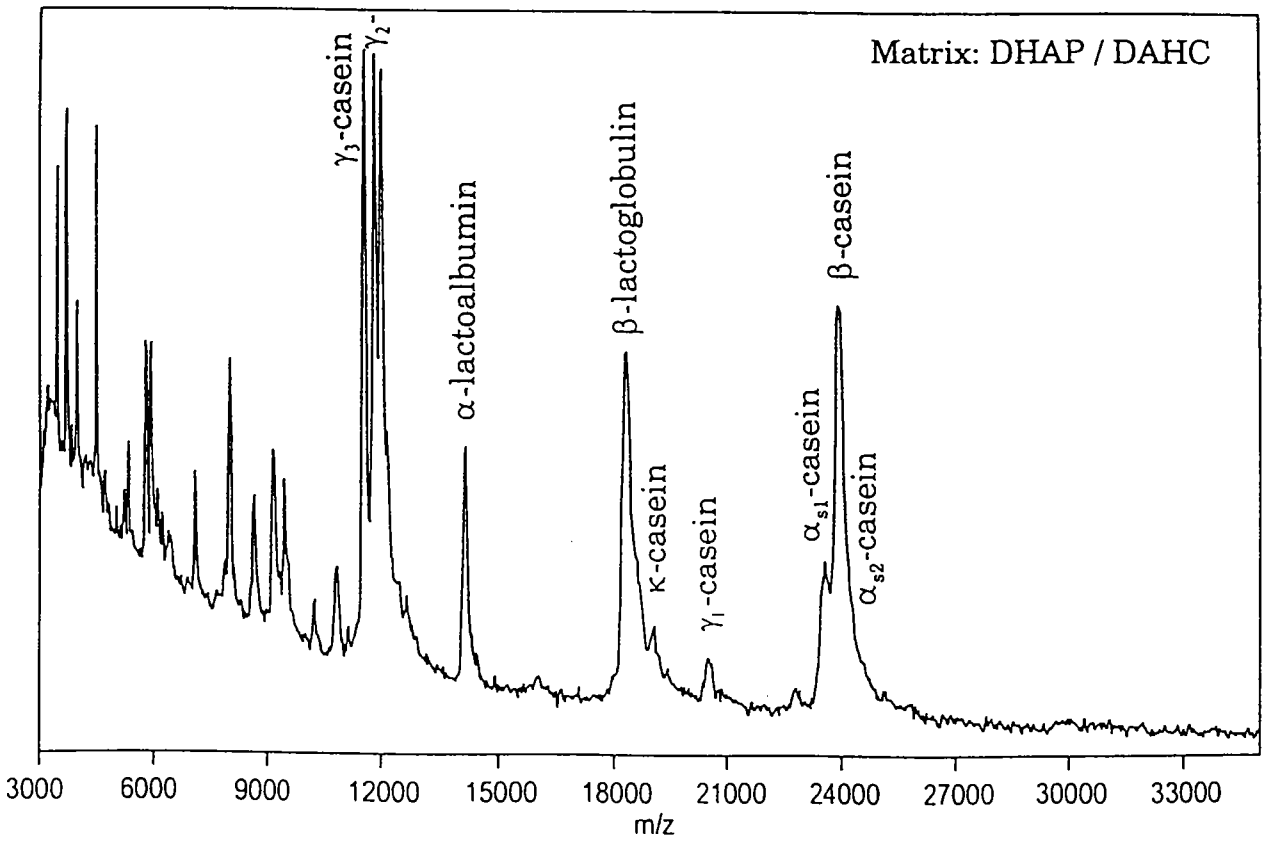


Figure 2

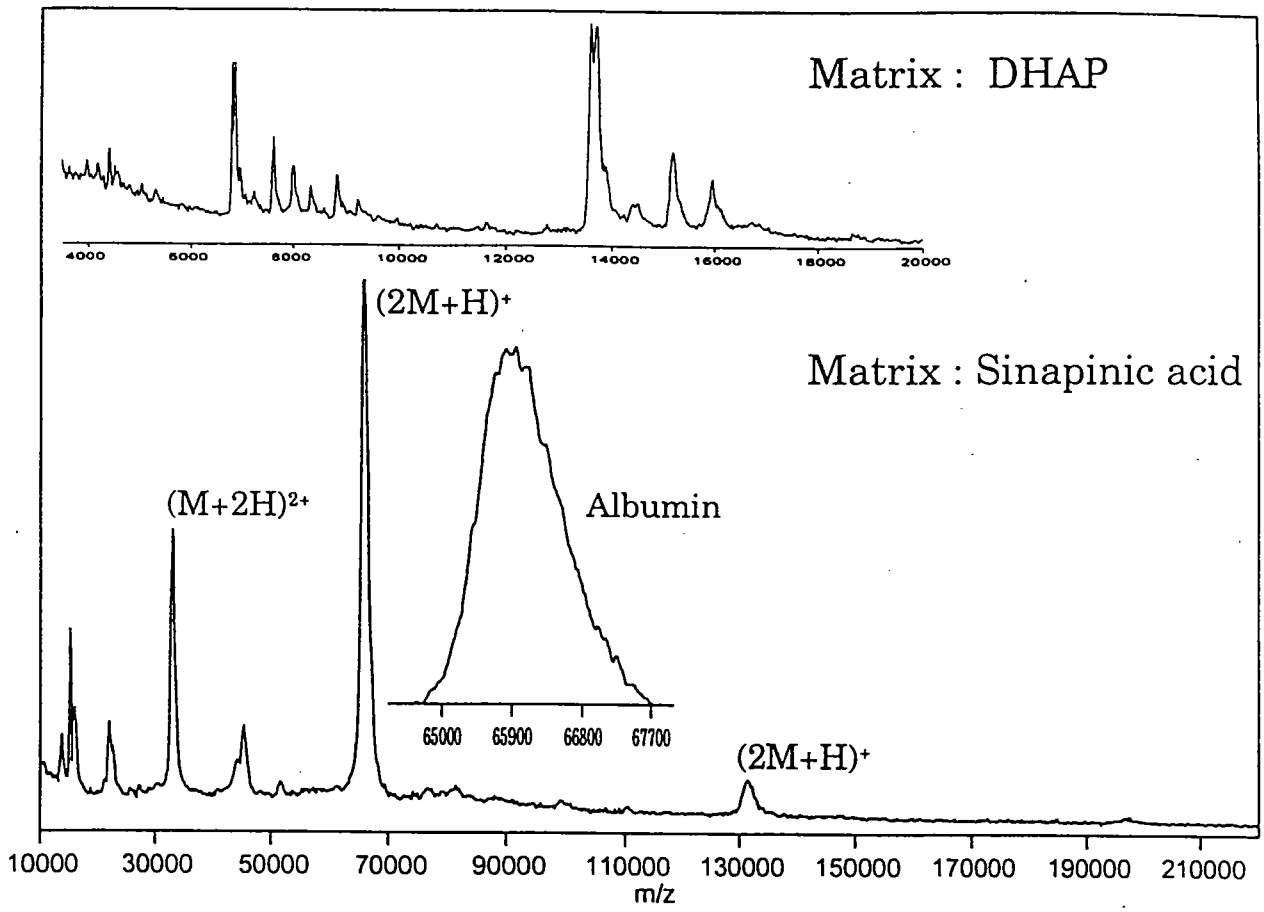


Figure 3

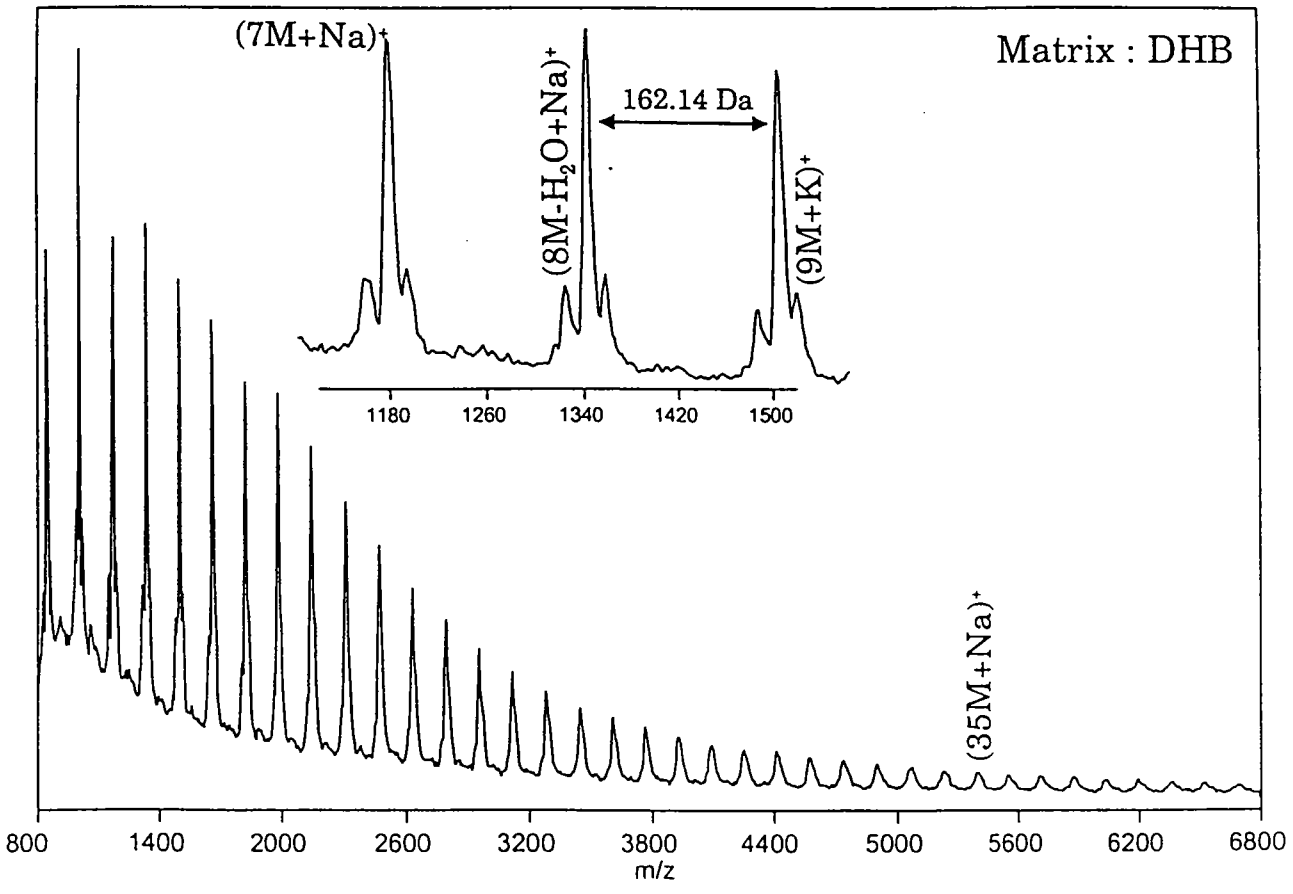


Figure 4

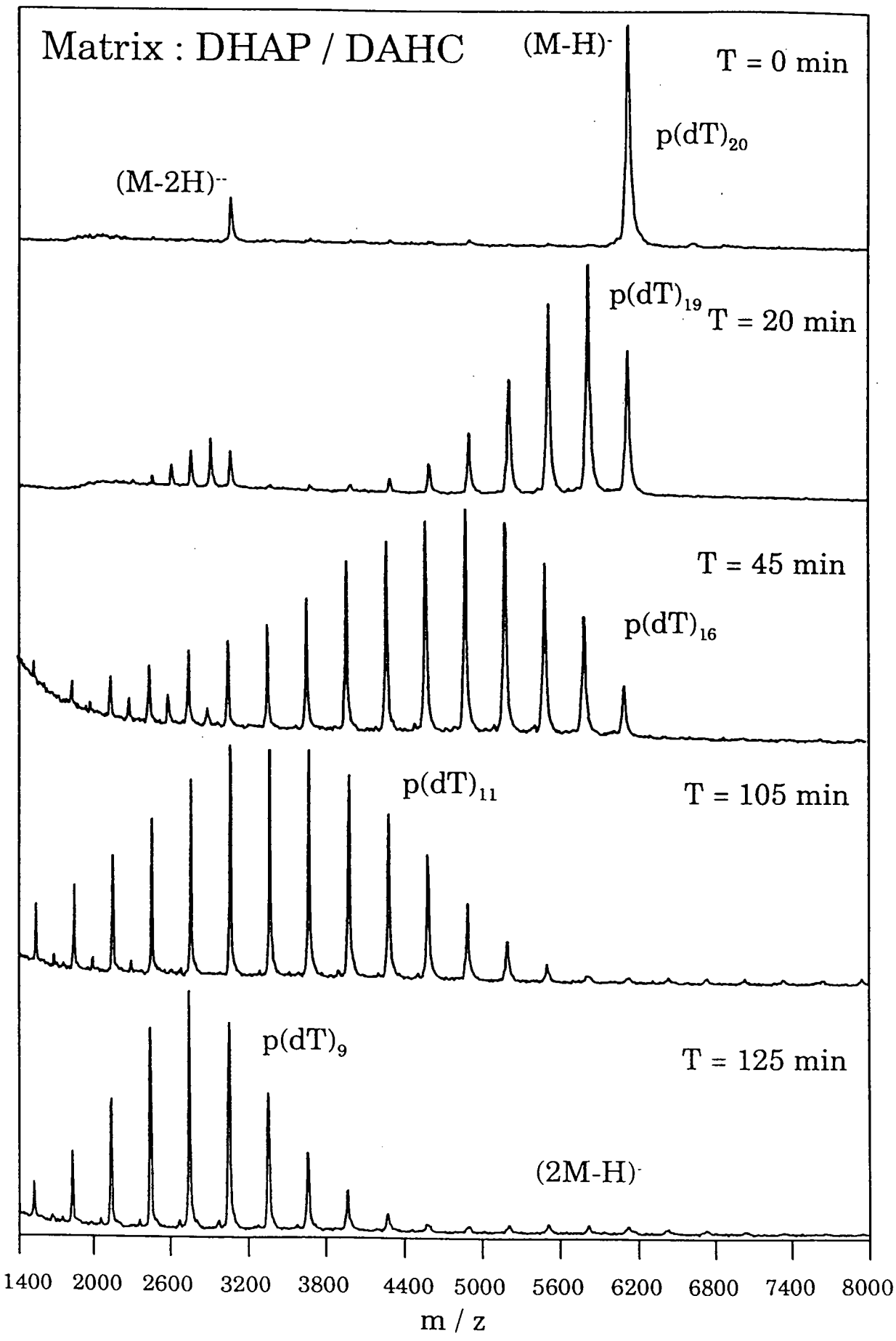

Matrix : DHAP / DAHC
(M-H)-
T = 0 min
p(dT)20
(M-2H)--
p(dT)19
T = 20 min
T = 45 min
p(dT)16
p(dT)11
T = 105 min
p(dT)9
T = 125 min
(2M-H)-
1400 2000 2600 3200 3800 4400 5000 5600 6200 6800 7400 8000
m / z

Figure 5