Novel Vacuum Stable Ketone Based Matrices for High Spatial Resolution MALDI Imaging Mass Spectrometry

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ABSTRACT: We describe the use of aromatic ketones and cinnamyl ketones that have high vacuum stability for analyzing tissue sections using MALDI imaging mass spectrometry. Specifically, the matrix, (E)-4-(2,5-dihydroxyphenyl)but-3-en-2-one (2,5-cDHA) provides high sensitivity and high vacuum stability while producing small size crystals (1-2 μm). A high throughput and highly reproducible sample preparation method was developed for these matrices that first involves using an organic spray solution for small matrix crystal seeding followed by spraying of the matrix in a 30% acetonitrile/70% water solution on the tissue surface to obtain a homogeneous coating of small crystals, suitable for high spatial resolution imaging.

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Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) has become a highly effective technology to produce molecular images of compounds contained in biological tissue sections.[1,2] MALDI IMS has been used to map the localization of a broad spectrum of analytes including proteins, peptides, drugs and their metabolites as well as endogenous cell metabolites and lipids[3]. The ability to correlate potentially thousands of molecules with histological tissue features observed using microscopy and IMS is vital to our understanding of complex biological samples.

For high spatial resolution MALDI IMS (<10 μm), it is important to have a tightly focused laser beam[4-6], a sample preparation method that is capable of attaining high sensitivity and an instrument capable of fast data acquisition. The matrix coating should be homogeneous and the diameter of matrix crystals should be much smaller than that of the laser spot diameter on target. High spatial resolution generally results in long analysis time (>2 hours), and therefore, a vacuum stable MALDI matrix coating is required to ensure consistency of the result throughout the experiment. Furthermore, for high throughput applications, the sample preparation protocol must be simple, fast and highly reproducible.

The matrices commonly used for protein analyses are sinapinic acid (SA), 2',5'-dihydroxybenzoic acid (DHB), ferulic acid (FA) and α-cyano-4-hydroxycinnamic acid (CHCA)[7,8]. These matrices perform well when imaging with low spatial resolutions (>30 μm), but these compounds are not acceptable at spatial resolutions of 5-10 μm or less. We have recently used 2',5'-dihydroxyacetophenone (2,5-DHA) for the image analysis of proteins at high spatial resolutions (2.5 and 5 μm)[6,9]. This matrix provides greater sensitivity for proteins than SA, CHCA and DHB and the crystal size of 2,5-DHA using spray application is around 1-3 μm[6]. Unfortunately, 2,5-DHA is volatile and readily sublimes from the tissue under instrument vacuum conditions and as a result, can only be used for short acquisition times (<1 hour) in high vacuum ion sources. Although data processing methods such as total-ion-current normalization of spectra can be used to mitigate the fading of the ion intensities that occurs through loss of matrix, this "fix" is far from ideal and reduces detection of some chemical species.

In the work reported in the current paper, we describe a matrix that is vacuum stable over many hours, amenable for imaging analytes at high spatial resolution, and is suitable for use with robotic spraying. We evaluated matrices structurally similar to 2,5-DHA, and also tested cinnamyl ketones that are structural analogs of commonly used matrices for protein imaging.

▪ EXPERIMENTAL

Materials and methods

Ethanol, acetone, chloroform, acetonitrile (ACN), toluene, acetic acid (AcOH), activated charcoal, trifluoracetic acid (TFA) and 2',5'-dihydroxyacetophenone (2,5-DHA), 2',6'-dihydroxyacetophenone (2,6-DHA), 2',4'-dihydroxyacetophenone (2,4-DHA), (E)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (HMP), 2',6'-dihydroxybenzaldehyde, 2',5'-dihydroxybenzaldehyde, ferulic acid ((E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid, FA) and syringaldehyde were purchased from Sigma-Aldrich (St. Louis, MO); 2,5-DHA was purified by recrystallization two times with 90% ACN at 70°C. (Acetylmethylene)triphenylphosphorane was obtained from TCI America; Xylene was purchased from Acros (Morris Plains, NJ). Conductive indium tin oxide (ITO) coated microscope glass slides (2.5 x 7.5 x 1.1 mm, CG-81IN-S115) were purchased from Delta Technologies (Stillwater, MN). A sublimation apparatus was constructed with a condenser and flask from Chemglass Life Sciences (Vineland, NJ). Robotic spraying was performed using a TM-Sprayer™ (HTX Technologies, Carrboro, NC). MilliQ water was obtained from a MilliQ Advantage A10 system.

Carnoy's fluid was prepared using a 6:3:1 ratio by volume of ethanol: chloroform: acetic acid.[10] Fresh frozen mouse brains were purchased from Pel-Freez Biologicals (Rogers, AZ); rat kidney, rat testis and rat brains were purchased from Bioreclamation, LLC (Hicksville, NY).

Microscope images of matrix coated targets were acquired using a Nikon Eclipse 90i microscope. SEM pictures of matrix coated targets were acquired using a Zeiss Merlin instrument with a Gemini II Column from Vanderbilt Institute of Nanoscale Science and Engineering.

Synthesis of cinnamyl ketone matrices:

(E)-4-(2,5-dihydroxyphenyl)but-3-en-2-one (2,5-cDHA), (E)-4-(2,6-dihydroxyphenyl)but-3-en-2-one (2,6-cDHA) and (E)-4-(4-hydroxy-3,5-dimethoxyphenyl)but-3-en-2-one (HDMP) were prepared as described below. In a 500 mL round bottom flask, 11.3 mmol of the corresponding aldehyde (2,5-dihydroxybenzaldehyde for 2,5-cDHA, 2,6-dihydroxybenzaldehyde for 2,6-cDHA, or syringaldehyde for HDMP) was mixed with 10.7 mmol (8.30g) (acetylmethylene)triphenylphosphorane and 300 mL of anhydrous toluene. The mixture was agitated using a magnetic stirring bar for 48 hours at room temperature. The target products (2,5-cDHA, 2,6-cDHA or HDMP) were insoluble and precipitated from reaction solution by filtration under vacuum resulting in crude products of ~70% yield. 2,5-cDHA was recrystallized using 8% ACN (detail procedure described below). 2,6-cDHA was recrystallized with 20% ACN at 60°C and HDMP was recrystallized with 30% ACN at 70°C using common organic recrystallization procedures (briefly, the mixture is heated on a hotplate until it becomes a clear solution then is cooled to room temperature)[11,12]. NMR spectra were acquired (data in supplementary material) and confirmed with the literature values for 2,5-cDHA[13] and HDMP[14], or predicted NMR for 2,6-cDHA using Advanced Chemistry Development, Inc. (ACD/Labs) Software V11.01 from Scifinder.cas.org.

Recrystallization of 2,5-cDHA:

Briefly, 1.5 g of crude 2,5-cDHA was dissolved in 17 mL of 95% acetonitrile at 70°C and 500 mg of active charcoal was added to the solution. The mixture was stirred for another 5 min and then filtered under vacuum to obtain a light-yellow solution which was poured into 200 mL of Milli-Q water under room temperature. The solution was placed at 4°C overnight for the crystallization of 2,5-cDHA. The crystals were then filtered, washed with ice cold Milli-Q water, and allowed to dry to obtain 1.1 g of purified matrix.

UV/vis spectra of matrices

Five mg of each matrix was dissolved in 1 mL of ethanol, and 10 µL of this solution was mixed with 1990 µL ethanol to obtain 200 time dilution. One mL of the diluted solution was used to measure the UV/vis absorbance from 220 nm to 420 nm at 25 °C using a SpectraMax® M2 microplate reader (Molecular Devices). The extinction coefficients was calculated using the formula: $A_{355}=\varepsilon lc$ (A_{355}, absorbance at 355 nm; ε, extinction coefficients; l, length of optical path=1 cm; c, molar concentration of compound).

For measurement of the solid film, 5 µL of each matrix solution (200 time diluted) was pipetted on one side of optical window of UV/vis cuvette and left to dry. The cuvette is a 1.5 mL semimicro methacrylate cuvette from Fisher Scientific (14-955-128). The absorbance of the solid film was acquired as the same way as for ethanol solution but using air as reference.

Evaluation of matrices using protein standards:

All the matrix solutions were prepared by dissolving 15 mg matrix in 800 µL of H_2O, 200 µL acetonitrile and 5 µL of TFA. Then 5 µL of each matrix solution was mixed with 5 µL of protein standard solution. The protein standard contains: oxidized insulin chain b from bovine pancreas (m/z: 3497), insulin from porcine pancreas (m/z: 5779), ubiquitin from bovine erythrocytes (m/z: 8561), cytochrome c from equine heart (m/z: 12385), apomyoglobin from equine skeletal muscle (m/z: 16952) and trypsinogen from bovine pancreas (m/z: 23982). The protein standard/matrix mixture (0.4 µL of each) was spotted on a Bruker MTP AnchorChip 384-well target (Bruker Daltonics, Billerica, MA). The MALDI spectra were acquired on Bruker Autoflex Speed MALDI TOF instrument (Bruker Daltonics). The spectra of four locations (100 laser shots each location) having the highest intensity were summed during acquisition and the resulting spectrum was baseline subtracted using Bruker FlexAnalysis 3.3.

Matrix stability measurements:

Ketone matrices (2,5-DHA, 2,5-cDHA, 2,6-DHA, 2,6-cDHA, HMP, HDMP) that produced high quality spectra with protein standards were coated on ITO slides by spraying using acetonitrile as the solvent (5 mg/mL) with the TM Sprayer™. The solutions were sprayed at a flow rate of 150 µL/min with nitrogen as the nebulization gas

(set to 10 psi). The spray nozzle was housed inside a heated block maintained at 30°C. The spray was deposited at a linear velocity of 1200 mm/min with an offset line spacing of 1.5 mm. Eight passes were performed. On alternating passes, the spray pattern was changed by 90° to achieve a crossed pattern. The distance of the spray nozzle from the MALDI target was approximately 40 mm.

The weights of the slides were measured using a balance (Mettler Toledo MS105DU Semi-Micro Analytical Balance) before matrix coating, after the matrix coating and after storage inside the instrument under vacuum (4.4E-7 mbar) for various time periods.

Tissue sectioning and storage:

Frozen tissue sections were sectioned at 12 µm thickness using a Leica CM3050 cryostat (Leica Microsystems GmbH, Wetzlar, Germany), thaw mounted on ITO-coated microscope slides, and dried under ambient conditions for 10 minutes. The slides were placed in a plastic slide holder box (Electron microscope science, Hatfield, PA) sealed with parafilm and stored at -80°C. When ready for analysis, the slide box was placed into a vacuum desiccator under ambient conditions for 30 minutes to allow the slides to reach room temperature while preventing water condensation on the sections which could cause delocalization of analytes.

Matrix deposition onto tissue sections by robotic spraying:

Tissue sections mounted on ITO slides were sequentially rinsed in glass petri dishes (100 x 15 mm, ThermoFisher) with a series of 10 mL of the following solutions using a modified method described previously[15]: 70% ethanol for 30 s, 100% ethanol for 30 s, Carnoy's fluid for 2 min, 100% ethanol for 30 s, 40% ethanol for 30 s and 100% ethanol for 30 s. This washing procedure removed excess salts and lipids. The slides were allowed to dry for 15 min in a hood prior to matrix application.

Spray application of 2,5-cDHA on tissue sections

Step 1: Thirty mg of 2,5-cDHA was dissolved in 5 mL ethyl acetate and 5 mL of toluene. This solution was sprayed on the slides containing the washed tissue sections with the TM Sprayer™: 95°C nozzle temperature, 600 mm/min nozzle velocity, 1.5 mm track spacing, 0.05 mL/min flow rate, and 4 passes.

Step 2: Ninety mg 2,5-cDHA was dissolved in 3 mL ACN, 7 mL H_2O, 100 µL TFA and 50 µL ammonium hydroxide followed by robotic spraying of the slides from step 1: 95°C nozzle temperature, 1100 mm/min nozzle velocity, 1.5 mm track spacing, 0.05 mL/min flow rate and 8 passes.

Spray application of HMP, HDMP, and 2,6-cDHA on tissue sections

For these three matrices, the tissue was first seeded with dry powdered matrix. Briefly, 50 mg of the matrix was ground by using a mortar/pestle to obtain ~5 µm diameter crystals and the matrix powder was applied on the surface of the tissue section using a fine paint brush. The brush was loaded with matrix powder and shaken

above the surface of the tissue section to release the powder until the surface of the tissues was totally covered. The excess powder on the tissue was removed by blowing a stream of nitrogen across the surface. This step is important for obtaining a consistent coating quality in terms of crystal size (10-20 μm) and coverage of the tissue surface[16]. The dry powder "seeded" slides were then sprayed with HMP, HDMP, and 2,6-cDHA respectively by the two-step method used for 2,5-cDHA.

SA coating for the serial sections of 2,5-cDHA coated sections for the comparison:

These sections were rinsed using the same procedures as for the 2,5-cDHA samples and coated with SA using sublimation (0.15mg/cm^2), and then recrystallized with 1 mL of water and 100 μL TFA at 85 °C.[15]

Imaging Mass spectrometry

MALDI IMS experiments were performed using a Bruker Rapiflex Tissuetyper (Bruker Daltonics) equipped with a Smartbeam 3D laser at 10 k repetition rate and FlexControl 3.3 software. Protein analysis was performed in positive ion linear mode. Ion images were processed using FlexImaging 5 and spectral analysis was performed with FlexAnalysis 3.3. The laser beam dimension was set to the same value as the spatial resolution. The number of laser shots at each pixel was 600 when imaged at a 15 μm spatial resolution and 300 when imaged at 10 and 5 μm spatial resolution.

Hematoxylin and eosin stain for imaged sections.

Following MALDI imaging analysis, the slides were rinsed with ethanol and acetone to remove the matrix. The tissue sections were then subjected to a standard hematoxylin and eosin (H&E) staining procedure.[17] H&E stained sections were scanned using a Leica slide scanner (SCN 400) at 20 X magnification.

▪ RESULTS AND DISCUSSION

Ketone matrices

2,5-DHA was introduced as a matrix for protein detection two decades ago,[18] enabling highly sensitive MALDI analysis of peptides, proteins and glycoproteins.[19] This compound provides higher sensitivity for protein analyses than that of SA[6]. However, 2,5-DHA is volatile under high vacuum, limiting its usage for acquiring images for less than 1 hour. We investigated higher molecular weight compounds that have similar structures to 2,5-DHA that could provide high quality images but have better vacuum stability. For ease of use, a new matrix should be suitable for automatic spraying for high throughput applications and to achieve high reproducibility. We investigated compounds based on the structure of 2,5-DHA (MW 152) such as the cinnamyl version, 2,5-cDHA (MW 178) (Figure 1A) that exhibits markedly increased vacuum stability. We also investigated several other cinnamyl ketone containing compounds such as HDMP (based on SA), HMP (based on FA) and 2,6-cDHA (isomer of 2,5-

cDHA), respectively (Figure 1A). We also investigated four other isomers of 2,5-DHA: 2,6-DHA, 2,4-DHA, 3,5-DHA and 2-chloro-3,4-DHA. The synthetic schemes for 2,5-cDHA, 2,6-cDHA and HDMP are shown in Figure 1B.

The preparations of gram scale quantities of 2,5-cDHA, 2,6-cDHA and HDMP are straightforward using the Wittig reaction[20]. These products precipitate from toluene, greatly simplifying their purification. Further purification is obtained by recrystallization.

Performance with proteins standards and UV/vis absorbance of matrices

We tested the performance of these ketone matrices using proteins standards (Figure 2). All the matrices tested provided high quality spectra except 2,4-DHA, 3,5-DHA, and 2-Cl-3,4-DHA. We also acquired UV/vis spectra of these matrices in ethanol solution or as a solid film (supplementary Figure 1). The UV/vis spectra of these matrices are very similar, and thus the extinction coefficients at 355 nm in ethanol can be used to quickly evaluate these matrices. In this regard, spectra of 2,4-DHA, 3,5-DHA and 2-Cl-3,4-DHA corelate with their low extinction coefficients (in the order of 10^2) while all other matrices have extinction coefficients in the order of 10^3 or higher (Table 1). As a result, we focused on 2,5-DHA, 2,5-cDHA, 2,6-DHA, 2,6-cDHA, HMP and HDMP for evaluation of stability under vacuum and suitability of these matrices for imaging proteins in tissue sections.

Vacuum stability of ketone matrices

The vacuum stability of the matrices was evaluated by a gravimetric measurement after incubation under instrument high vacuum conditions at different times throughout the process. We assessed the stability in terms of the percentage weight loss under vacuum as shown in Figure 3. Under high vacuum conditions of 4.4E-7 mbar along with the heat present in some MALDI sources (~35°C for Rapiflex), 2,6-DHA is completely sublimated in 30 min and 2,5-DHA in 2.5 hours. HMP loses 50% of its original coating on the slide in 4 hours, HDMP loses about 20% in 21 hours, and 2,6-cDHA loses 15% in 24 hours. No significant change of 2,5-cDHA coating was measured with our method after 24 hours. Since 2,6-DHA and 2,5-DHA are extremely volatile under high vacuum, they were not investigated further.

Ketone matrices for imaging proteins in tissue:

To image proteins in tissue sections at high spatial resolution, a uniform coating of matrix with small crystals is necessary. We observed that ketone matrices tend to form large needle-like crystals having a length ranging from 30 to 200 μm when they are sprayed onto tissue in 30-70% acetonitrile solution and an oversaturated solution accumulates on the surface in the form of liquid droplets. To obtain small matrix crystals (≤ 5 μm), we applied a "seeding" approach used in spotting matrix previously[16,21] and developed a new method for spraying. With the seeding approach, a homogeneous and consistent coating can be obtained (examples of seeded and non-seeded coatings are shown in Supplementary Figure 2). During the seeding step, either by powder application or by non-aqueous solution spraying, small crystals are formed leading to a thin layer matrix coating. The second step of spraying is made with matrix solution with 30-70% water to extract proteins. The concentration of matrix

solution is made to close to the saturation point of the matrix, so that after the solution is sprayed from the heated nozzle, the droplets of the matrix solution become saturated. We hypothesize that the aqueous component in the solution extracts proteins in the tissue and the extracted proteins co-crystallize with the matrix. It is important to have the solution in the second step solution close to the saturation point of the matrix to minimize dissolution of the seeds.

Using this seeding approach, we found that the 2,5-cDHA coating provides superior performance when compared to 2,6-cDHA, HMP and HDMP under the same conditions. The size of the matrix crystals of 2,5-cDHA ranges from 1 to 2 μm (Figure 4 A and B), while 2,6-cDHA, HMP and HDMP all provide coatings with single crystals in the 10 μm range or larger with large matrix clusters on the surface and with gaps between these matrix clusters (Figure 4 A). As a result, 2,5-cDHA is the only matrix among those investigated that provided a coating suitable for high spatial resolution imaging (<10 μm) for long acquisition times.

It should be noted that 2,5-cDHA can be coated on tissue sections without using the manual powder seeding step, but this approach gives more reproducible final images. For HMP, HDMP, and 2,6-cDHA, non-aqueous solution seeding does not provide homogenous small crystals on the surface of the tissue. For these three matrices, dry powder seeding is necessary to apply before the non-aqueous solution spraying.

2,5-cDHA was compared with SA for the image acquisition of protein IMS data. SA is the most commonly used vacuum stable matrix for imaging proteins as previously described[5,15]. Experiments were carried out by coating the serial sections of different tissues (mouse brain, rat testis, rat kidney and rat liver) with 2,5-cDHA and SA. These sections were then imaged under identical conditions. As shown in Figure 5 and supplementary Figures 3-5, 2,5-cDHA is greatly superior to SA in terms of sensitivity at spatial resolutions of 5 μm and 10 μm. Most of the peaks in the mass spectra obtained from the tissue coated with 2,5-cDHA have 4 times or higher peak intensity than that of the same peaks from the serial section coated with SA. Importantly, at the higher m/z range, 2,5-cDHA shows better performance than that of SA, e.g., proteins in the m/z 22,000 range from mouse brain, rat testis, rat kidney and rat liver were not detected in the SA coated sample. The comparison conditions were chosen with the best protocols for both matrices (spraying for 2,5-cDHA and sublimation/recrystallization for SA) because we found out that under identical conditions (both matrices are sprayed), the SA coating does not provide as many peaks at 5 μm spatial resolution (Supplemental Figure 7), and it was shown that SA performs better with the sublimation/recrystallization protocol rather than spraying/recrystallization (Supplementary Figure 8).

The performance of 2,5-cDHA as a MALDI matrix for imaging at high spatial resolution was further demonstrated by imaging a mouse brain section at 5 μm for 7 hours (Figure 6). As the previous data would suggest, the

performance of 2,5-cDHA under the demanding conditions of a high spatial resolution MALDI IMS experiment demonstrates that this matrix can be used to acquire high quality protein results while circumventing the negative impacts that poor vacuum stability has on image quality. Even during the prolonged (>7 hours) exposure to high vacuum, no change in ionization is observed.

CONCLUSIONS:

We successfully synthesized a new matrix, 2,5-cDHA, suitable for imaging proteins at high spatial resolution in biological tissues where data acquisition took place over many hours. This is the result of its high vacuum stability and propensity to form small crystals (1-2 μm) homogeneously over the tissue surface. In addition, 2,5-cDHA performs well for lower molecular weight analytes in both positive and negative ionization modes (supplementary Figure 9).

Although HMP appears to have great sensitivity for imaging protein among the matrices screened, we found it difficult to achieve the coating homogeneity that high spatial resolution imaging (<5-10 μm) demands. Furthermore, HMP is somewhat volatile under high vacuum, even though it is more stable than 2,5-DHA and 2, 6-DHA. With current spraying method, HMP may be useful for imaging at medium spatial resolution (~50 μm) for short time period acquisition (~ 2.5 hr.) (supplementary Figure 6 B).

Two of the other matrices that were evaluated, 2,6-cDHA and HDMP, gave poor result for imaging proteins in tissue sections and are not recommended using the matrix application method described here (supplementary Figure 6 A and C).

SUPPORTING INFORMATION

NMR data of 2,5-cDHA, HDMP and 2,6-cDHA; UV/vis spectra of matrices in solution or solid phase (film); difference of the 2,5-cDHA coating with/without seeding step; comparison of imaging performance of 2,5-cDHA and SA as matrix on the serial sections of a rat testis, a rat kidney, and a rat liver; Ion images from tissue sections coated by HDMP, HMP and 2,6-cDHA; lipid ion images of rat brain by 2,5-cDHA;

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Notes

The authors declare no competing financial interest.

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Table 1 Extinction coefficients of different matrices at 355 nm in ethanol

Matrices	2,5-cDHA	2,5-DHA	2,6-cDHA	2,6-DHA	HDMP	HMP	2,4-DHA	2-Cl-3,4-DHA	3,5-DHA	SA	FA	DHB
ε	5.97E+03	3.69E+03	5.06E+03	2.36E+03	1.61E+04	1.44E+04	2.04E+02	8.46E+02	5.31E+02	5.10E+03	2.53E+03	2.02E+03
ε, extinction coefficient ($M^{-1}\cdot cm^{-1}$)												

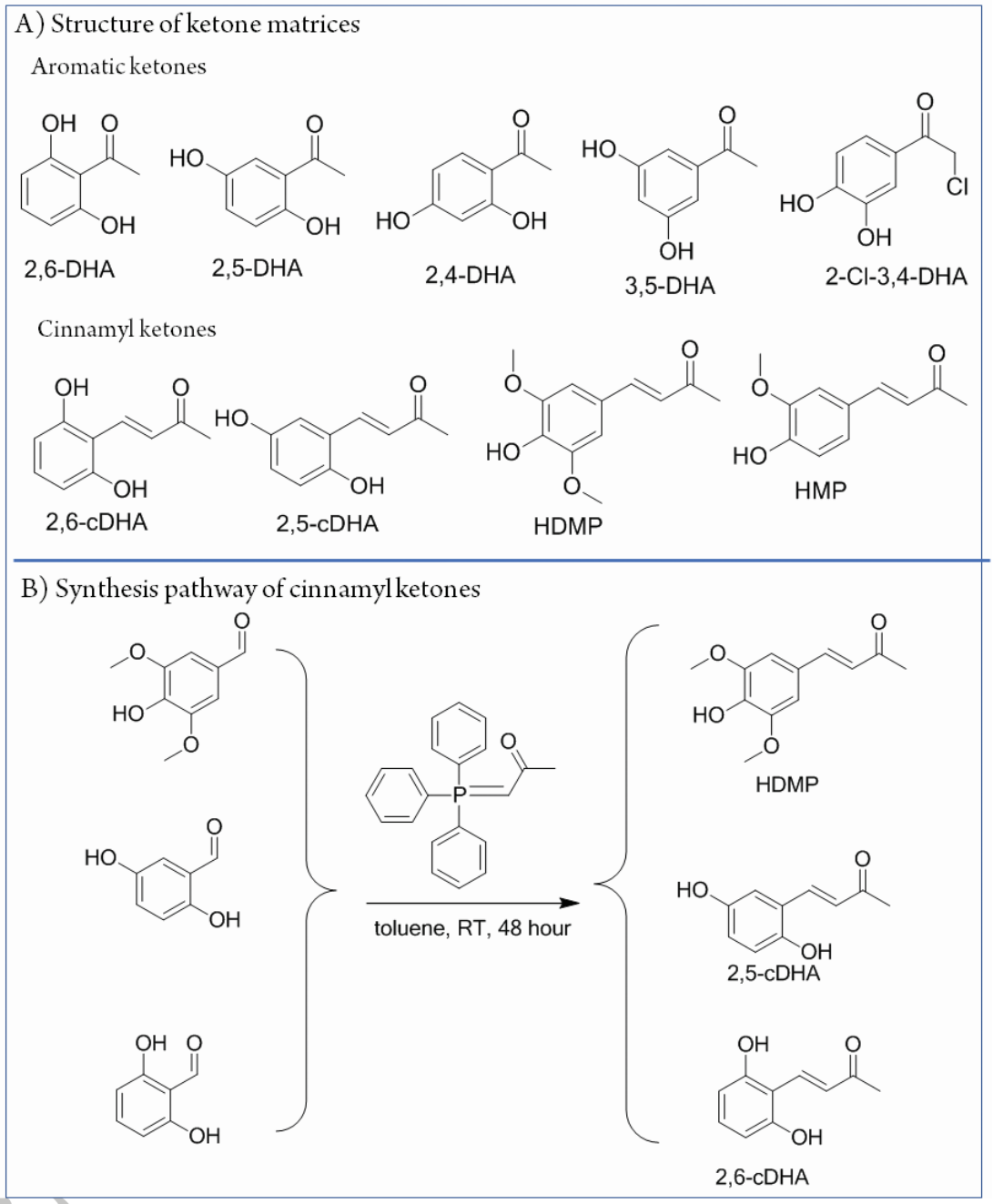

Figure 1 A) Structure of aromatic ketones: 2,4-DHA, 2,5-DHA, 2,6-DHA, 3,5-DHA and 2-Cl-3,4-DHA; cinnamyl ketones: 2,6-cDHA, 2,5-cDHA, HDMP, and HMP; B) Synthesis of cinnamyl ketones

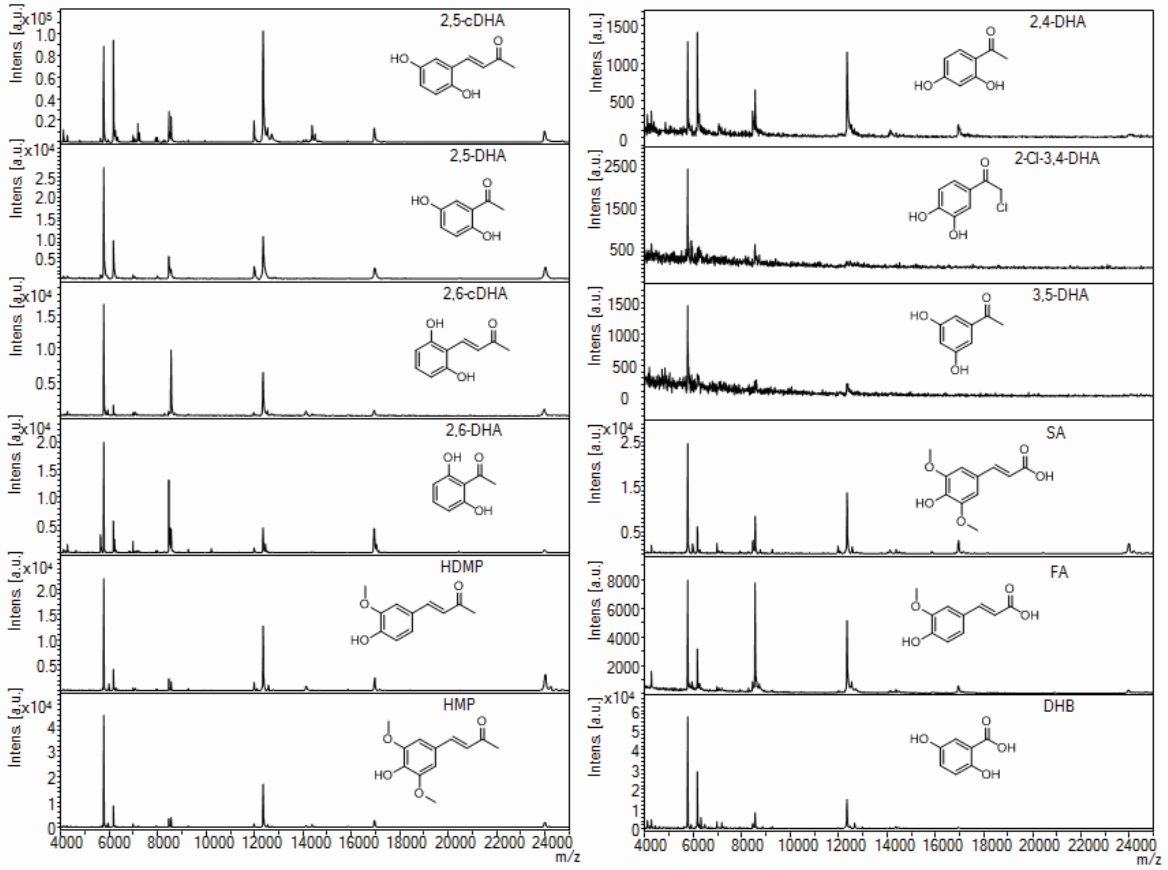

Figure 2 Performance of ketone matrices for the detection of standard proteins on Bruker Autoflex Speed in linear positive ion mode: insulin chain b oxidized from bovine pancreas (m/z: 3497), insulin from porcine pancreas (m/z: 5779), ubiquitin from bovine erythrocytes (m/z: 8561), cytochrome c from equine heart (m/z: 12385), apomyoglobin from equine skeletal muscle (m/z: 16952) and trypsinogen from bovine pancreas (m/z: 23982). For this experiment, laser energy, concentration of standards, and matrix concentration were identical for each matrix tested.

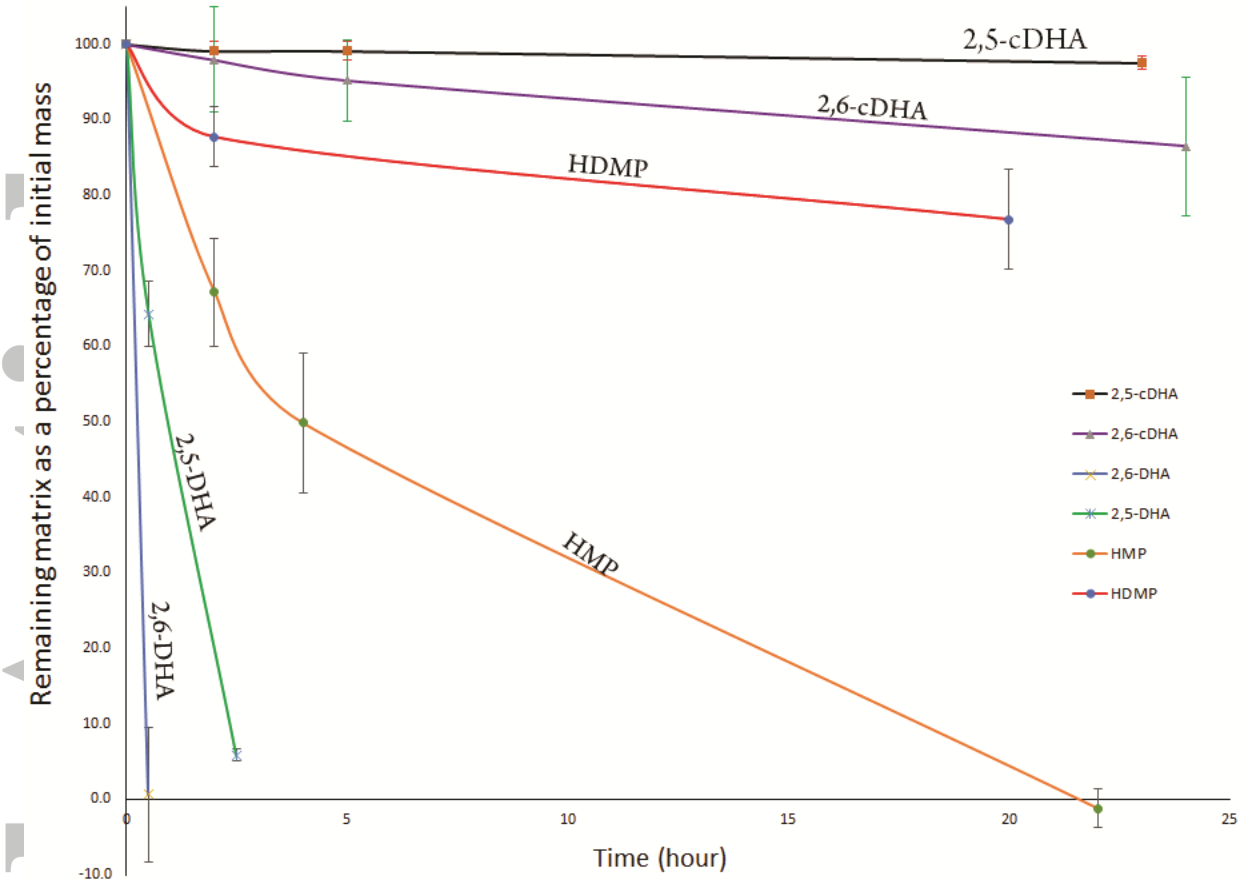

Figure 3 Stability of ketone matrices under vacuum (4.4E-7 mbar) in term of remaining matrix as a percentage of initial mass. 2,5-cDHA, 2,6-cDHA and HDMP appear to be stable more than 24 hours.

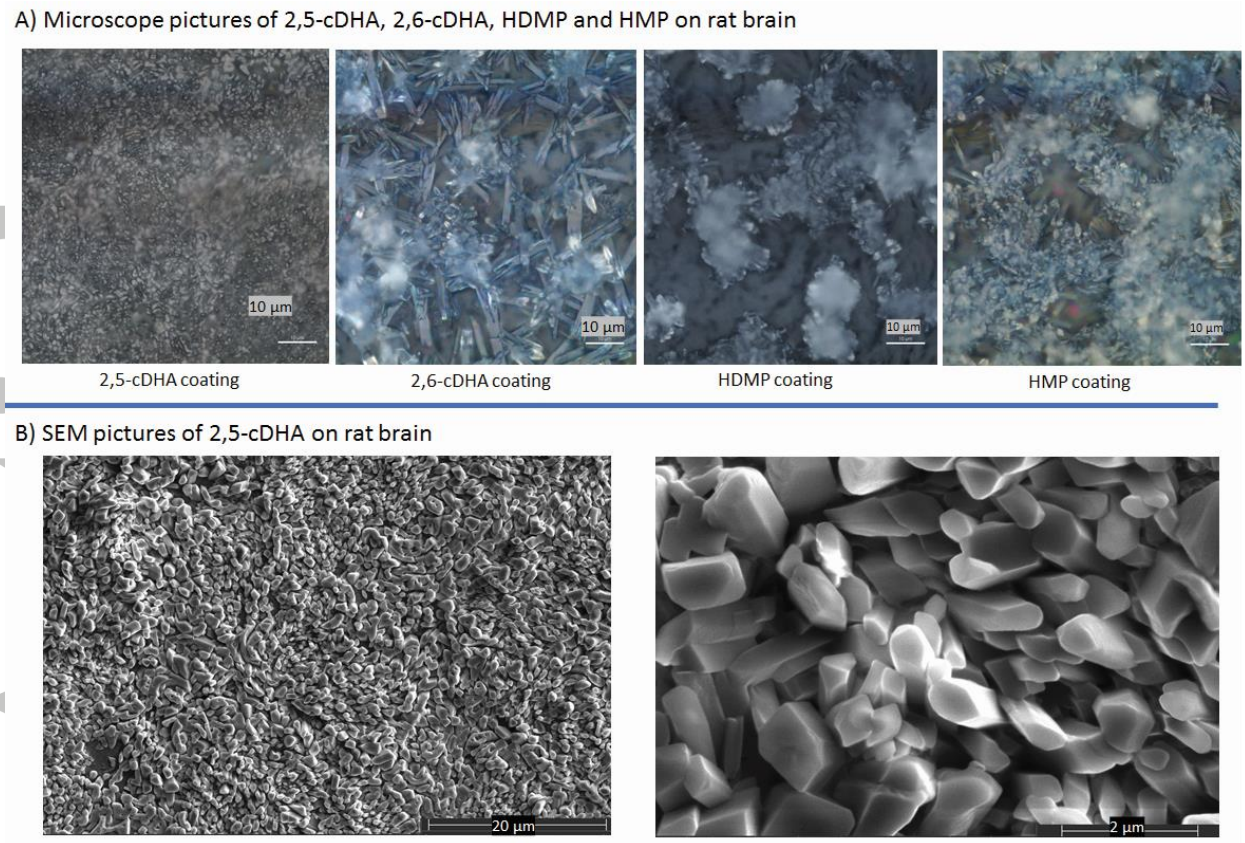

Figure 4 A) Microscope pictures of 2,5-cDHA, 2,6-cDHA, HDMP and HMP on rat brain at different zoom. 2,5-cDHA coating provides smallest crystals and homogeneity; B) SEM pictures of 2,5-cDHA on rat brain

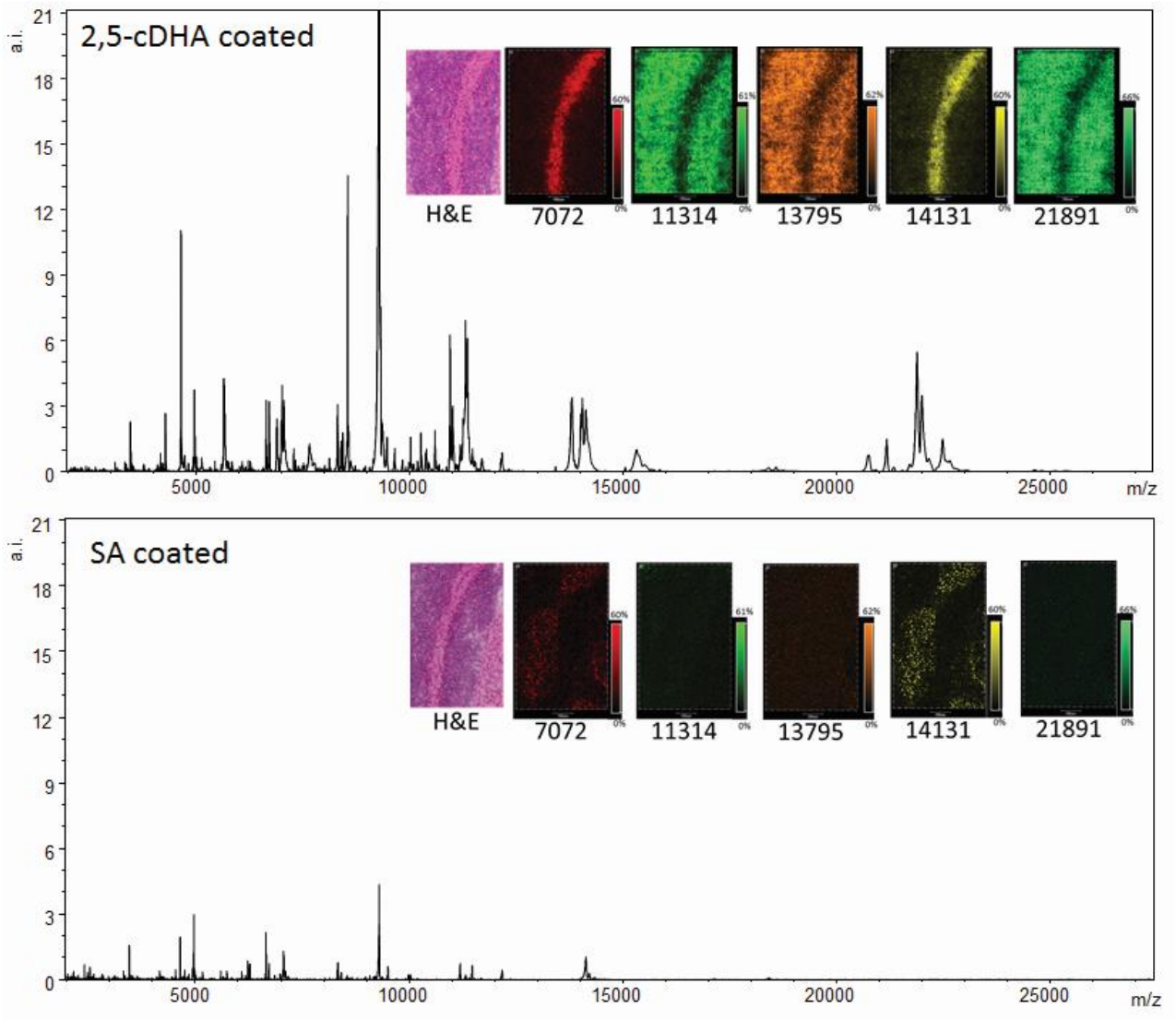

Figure 5 comparison of imaging performance of 2,5-cDHA and SA as matrix on the serial sections of a mouse brain imaged at 5 µm, scale bar is 100 µm; spectra were TIC normalized. For this experiment, laser energy settings were the same for both matrices. The numbers under each ion images are m/z for the ions.

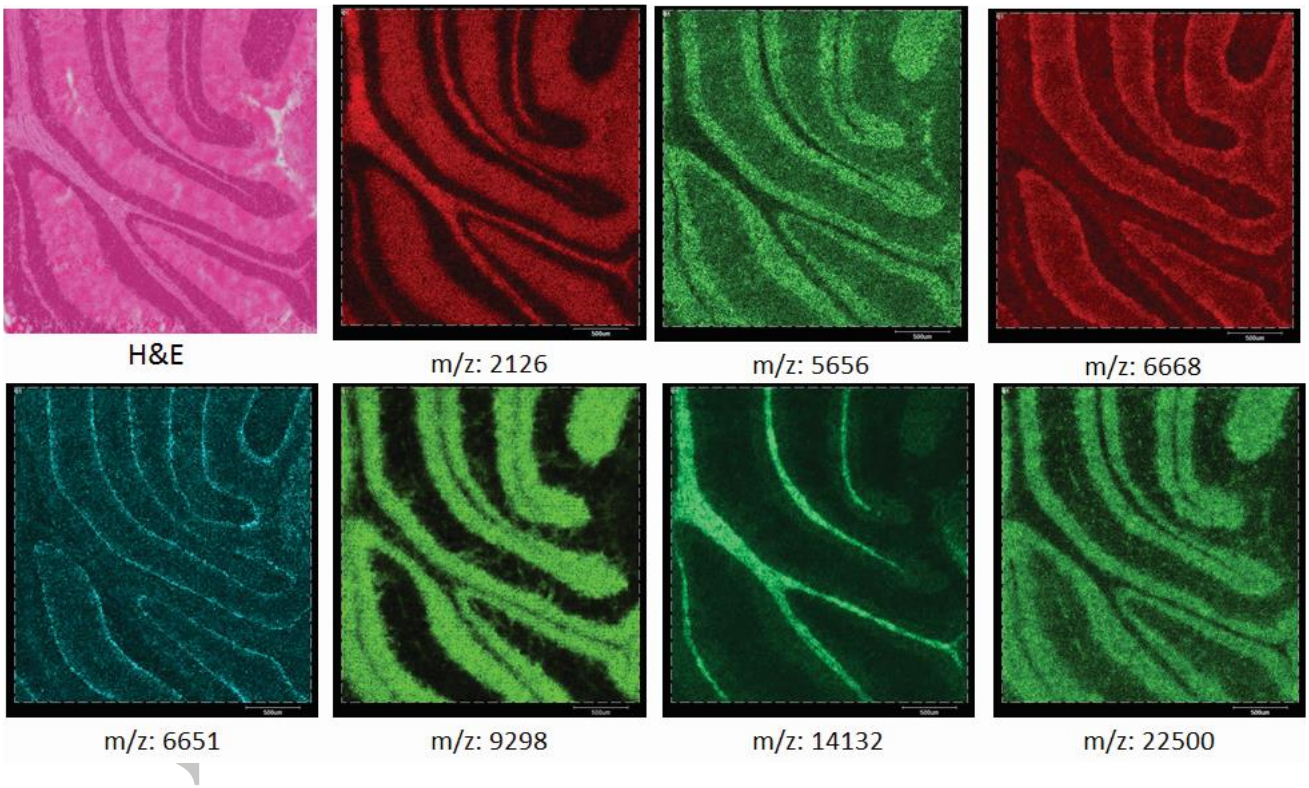

Figure 6 ion images mouse brain at 5 μm spatial resolution with acquisition time of 7 hours, scale bar is 500 μm.